dome-shaped, or pedunculated papule, plaque, or nodule.[77] It may be difficult to distinguish clinically from amelanotic melanoma.

HISTOLOGY

Histologic features defining BCC are aggregates of neoplastic basaloid cells stemming from the epidermis or the epithelium of adnexal structures.[75] Aggregates are organized as lobules, islands, nests, or cords of cells that display an orderly arrangement of the basaloid cell nuclei at the periphery, a palisading array. Occasionally, central necrosis or cystic changes are seen within the tumor lobules. Individual tumor cells have uniform, hyperchromatic, round to oval nuclei. Mitotic figures are uncommon, but the presence of apoptotic cells and necrosis is frequently observed. Pleomorphic variant of BCC shows tremendous variability of nuclear size and chromatism, as well as multiple mitoses and multinucleated giant cells. A stromal retraction or clefting around the neoplastic aggregates is commonly seen in the dermis. An accumulation of mucin within and around the tumor lobules may be evident.

Histologic subtypes of BCC include nodular and micronodular, superficial, and infiltrative BCC (Fig. 92.6A,B). Nodular BCC accounts for approximately 50% of all histologic variants of BCCs and exhibits characteristic features as previously described. Superficial multifocal BCC accounts for approximately 15% of BCCs and is a broad lesion characterized by basophilic buds extending from an atrophic epidermis into the papillary dermis. In a two-dimensional view, tumor buds appear to be multifocal, but upon three-dimensional imaging analysis they form a netlike pattern. Retraction artifact is present, as is peripheral palisading within the buds. FEP, which accounts for 1% of BCCs, is characterized by a polypoid lesion in which basaloid cells grow downward from the surface in a network of anastomoses of cords of cells in loose connective tissue.

The biologic behavior of the micronodular, infiltrating, and morpheaform (sclerosing) subtypes of BCC is more aggressive than that of the nodular and superficial forms. Infiltrative histology is seen in 15% to 20% of BCCs. Individual tumor islands manifest small, irregular outlines with a spiky appearance that invade into and throughout the dermis. Palisading is characteristically absent. The stroma is less myxoid than in the nodular form. In the morpheaform variant, small groups or cords of tumor cells often only one to two cells in thickness infiltrate and dissect a dense, collagenous stroma often parallel to the skin surface. Mixed histology is frequently apparent in BCCs. Areas of follicular, sebaceous, eccrine, or apocrine differentiation may also be seen in some BCCs. BCCs may contain focal areas of squamous differentiation, ranging from individual dyskeratotic cells to keratin pearls. The term *basosquamous (metatypical) carcinoma* denotes BCC with a predominance of mature, atypical keratinizing squamous component. Biologic characteristics of a basosquamous carcinoma are more similar to those of SCC with a possibility for metastasis.

The significance of histologic subtype lies in the correlation with a tumor's biologic aggressiveness. The infiltrative and micronodular types are the most likely to be incompletely removed by conventional wide local excision (WLE). Rates of incomplete excision vary from 5% to 17%.[78] Incompletely excised infiltrative and micronodular BCCs recur at rates of 33% to 39%. Recurrences after RT show a tendency toward infiltrative histology and squamous transformation, and even recurrent BCC after excision or C&D may become metatypical. Although historical reports in the literature suggested that 60% of incompletely excised BCCs will not recur, none of these studies provided an appraisal of recurrence rates as a function of histologic subtype.[68,69] In general, incompletely excised BCCs

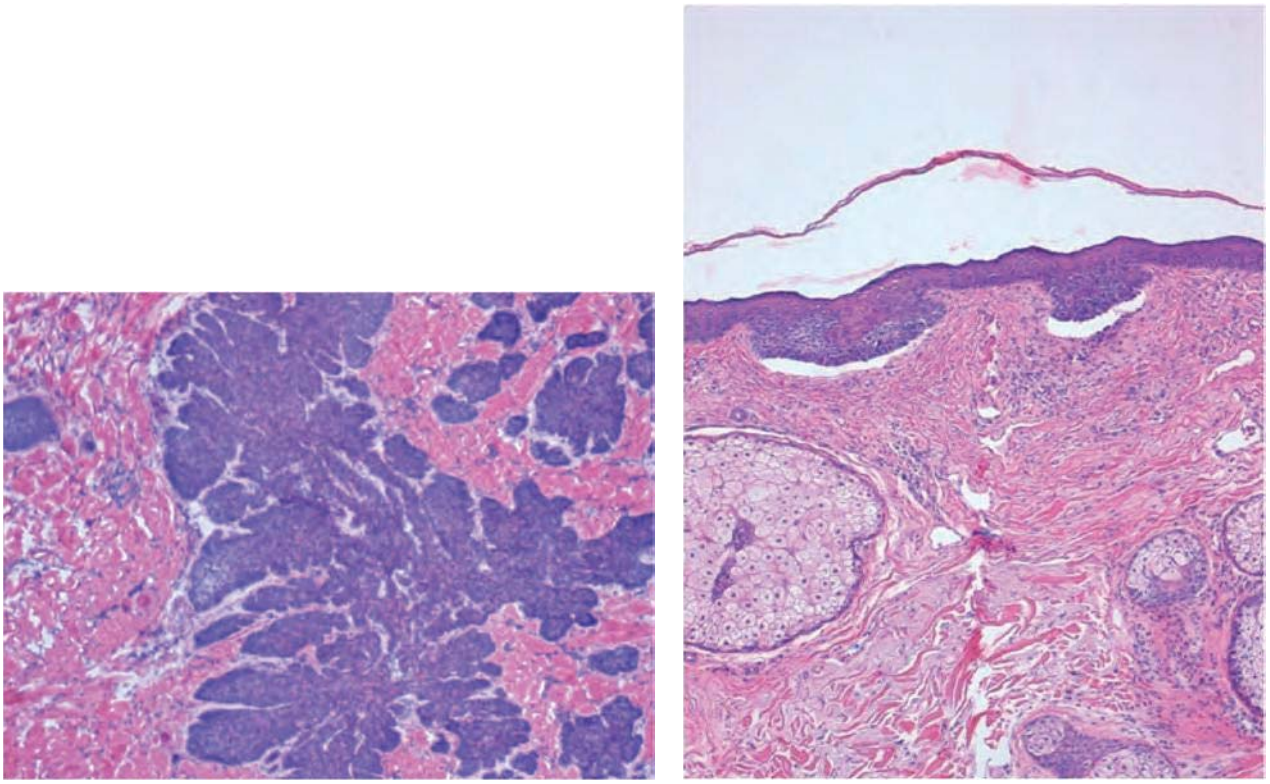

Figure 92.6 **(A)** Histology of nodular basal cell carcinoma (BCC). Nodular BCC is characterized by the presence of rounded tumor islands extending from the epidermis into the dermis. Peripheral palisading of nuclei is prominent, and surrounding retraction artifact may be present. **(B)** Histology of multifocal superficial BCC. Superficial BCC is characterized by basophilic buds extending from an atrophic epidermis into the papillary dermis. Retraction artifact is present, as is peripheral palisading within the buds.

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should be removed completely, preferably by MMS, especially if they occur in anatomically critical areas such as the central zone of the face, retroauricular sulcus, or periocular area.

TREATMENT

Excisional surgery, MMS, and C&D have all been used to treat circumscribed, noninfiltrative BCCs. MMS is the treatment method of choice for all recurrent and infiltrative BCCs, particularly if a tumor is located on the face.[6,79] RT is best suited for poor surgical candidates and patients with extensive lesions not amenable to surgery.[80,81] RT is not indicated for recurrent or morpheaform lesions and in patients with NBCCS.

C&D is frequently used by dermatologists in the treatment of primary BCC. Knox[82] noted cure rates as high as 98.3%. Kopf et al.,[83] in an earlier study, cited a significant difference in the cure rates obtained between patients treated by private practitioners (94.3%) and those treated by trainees in the New York University Skin and Cancer Unit (81.2%). This supports the premise that C&D, although simple and cost-effective, is highly dependent on operator skill. Traditionally, it was recommended that the procedure be repeated for three cycles, but histology, location, and behavior of the tumor should dictate the number of cycles. C&D should be reserved for small or superficial BCCs, not located on the midface, in patients who may not tolerate more extensive surgery.

Surgical excision offers a unique advantage of histologic evaluation of the excised specimen. It has been demonstrated that 4-mm margins are adequate for removal of BCC in 98% of cases of nonmorpheaform BCC of <2 cm in diameter.[78] Extending the excision into subcutaneous fat generally is adequate for a small primary BCC.

MMS permits superior histologic verification of complete removal, allows maximum conservation of tissue, and remains cost-effective as compared with traditional excisional surgery for NMSCs including BCCs.[4,6] In a large study of treatment of primary BCC by Rowe et al.,[84] MMS demonstrated a recurrence rate (RR) of 1% over 5 years. This was superior to all other modalities, including excision (RR = 10%), C&D (RR = 7.7%), RT (RR = 8.7%), and cryotherapy (RR = 7.5%). In a similar study of recurrent BCC, treatment with MMS demonstrated a long-term RR of 5.6%.[85] In that treatment group, MMS was superior to all other modalities, including excision (RR = 17.4%), RT (RR = 9.8%), and C&D (RR = 40%). MMS is the preferred treatment for morpheaform, recurrent, poorly delineated, high-risk, and incompletely removed BCC, and for those sites in which tissue conservation for function and cosmesis is imperative. When the surgical approach is contraindicated, RT is a valid option for management of primary BCC. RT may be indicated postoperatively if margins are ambiguous or involved. Disadvantages of RT include lack of margin control, possible poor cosmesis over time, a drawn-out course of therapy, and increased risk of future skin cancers. The RRs for primary BCC treated by RT range from 5% to 10% over 5 years. Wilder et al.[86] compared local control rates for RT among 85 patients with 115 primary or recurrent biopsy-proven BCCs. A 95% control rate was achieved for primary BCC and a 56% control rate obtained for recurrent BCC at 5 years. Considering cosmesis, RT scars tend to worsen over time, in contrast to surgical scars that tend to improve over time.

Imiquimod is approved by the FDA for the treatment of superficial BCC <2 cm in diameter on the neck, trunk, or extremities. The FDA-recommended regimen is once-daily application 5 days per week for 6 to 12 weeks. Numerous studies evaluated safety and efficacy of imiquimod for superficial BCC.[11,87] Application schedules varied from 2 days per week to twice daily, and the treatment duration ranged from 5 to 15 weeks. Clinical follow-up ranged from 6 months to 5 years. Reported histologic clearance rates ranged from 52% to 81%, albeit high-risk tumors (within 1 cm of the hairline, eyes, nose, mouth, anogenital region, hands, and feet) or tumors >2 cm in diameter regardless of their location were excluded. Imiquimod has been used off-label for the treatment of nodular and infiltrative BCC.[88,89] Although some studies have shown favorable cure rates, imiquimod treatment of these tumors is generally not recommended as subclinical disease may persist and lead to late recurrence.

The FDA-approved protocol for treating superficial BCC with topical 5-FU is twice-daily application for 3 to 6 weeks irrespective of tumor size or location. Longer treatment protocols with an average 11 weeks are reported in the peer-reviewed literature. In a study of 31 tumors treated twice daily for an average of 11 weeks, a 90% clearance rate was observed histologically 3 weeks posttreatment.[90] Topical PDT has also demonstrated efficacy in the treatment of BCC. Clearance rates for BCC using ALA or MAL PDT range from 76% to 97% for superficial to 64% to 92% for nodular BCC after one to three treatments.[26,28,91] Many studies of PDT for nodular BCC involve curettage of the lesion prior to treatment, however, and it is unclear if the response rate would be as successful without initial curettage. In a well-designed comparative trial, 601 patients with superficial BCC were randomized to treatment with MAL PDT (two treatments given 1 week apart), imiquimod, or 5-FU according to FDA-approved protocols. Complete clinical remission at 1 year was found to be 72.8% for PDT, 83.4% for imiquimod (superior to PDT), and 80.1% for 5-FU (not statistically different from the other treatments). Patients treated with imiquimod or 5-FU were more likely to report bothersome local side effects of the treatment, however.[92]

Historically, systemic therapy for BCC was limited to cytotoxic chemotherapy that was marginally effective as salvage therapy for metastatic disease. Elucidation of the critical role of abnormal hedgehog signaling in BCC, however, has led to development of a novel class of molecularly targeted small molecule inhibitors of *Smoothened*, most notably vismodegib.[74] Based on a seminal trial of 33 patients with metastatic BCC and 63 patients with locally advanced BCC not amenable to surgical therapy, vismodegib was approved (2012) for treatment of advanced BCC (either metastatic or not amenable to surgery or radiation).[73] With once daily oral dosing of 150 mg vismodegib, 30% of patients with metastatic BCC and 43% of patients with locally advanced BCC had an objective response (at least 30% decrease in size of tumors), and 21% of patients with locally advanced BCC had complete clinical resolution of tumors. None of the patients with metastatic BCC had a complete response. Adverse events in the trial were common, including serious adverse events in 25% of patients and fatal adverse events in 7% of patients. Most common adverse events in this and other trials were muscle spasms, alopecia, dysgeusia leading to weight loss, fatigue, diarrhea, and hyponatremia, and between 12% and 54% of patients discontinued therapy because of adverse effects.[73,74,93] Due to the relatively low response rate, the high incidence of adverse events, the potential for resistance with a single mutation,[94] and reports of rapid recurrence of BCC upon discontinuation of the medication,[95] *Smoothened* inhibitors remain a limited, albeit important, addition to our treatment options for BCC. Vismodegib may also be an effective suppressive therapy for select patients with NBCCS, where it was shown to decrease the incidence of new tumors by 93% during an 8-month period.[93]

It is imperative that patients with a history of BCC receive annual full-body skin examinations. Although most recurrences appear within 1 to 5 years, recurrences decades after initial treatment are reported in the literature. Rowe et al.[96,97] found that 30% of recurrences developed within the first year after therapy, 50% within 2 years, and 66% within 3 years. A separate new primary BCC can present at rates of approximately 40% within 3 years, with 20% to 30% within 1 year of treatment of the original lesion.

SQUAMOUS CELL CARCINOMA

SCC is a neoplasm of keratinizing cells that shows malignant characteristics, including anaplasia, rapid growth, local invasion, and metastatic potential. More than 200,000 cases of SCC are

diagnosed in the United States each year, making it the second most common human cancer after BCC.[98] People of Celtic descent, individuals with fair complexions, and those with poor tanning ability and predisposition to sunburn are at increased risk for developing SCC. SCC in blacks arises most often on sites of preexisting inflammatory conditions such as burn injuries, scars, or trauma.[99] Patients treated with PUVA or undergoing immunosuppressive therapy following solid-organ transplantation are at increased risk of SCC (see the following).

PATHOGENESIS OF SQUAMOUS CELL CARCINOMA

Major factors involved in the pathogenesis of SCC include cumulative exposure to UVR, genetic mutations, immunosuppression, and viral infections. UVR acts as both a tumor-initiating and a tumor-promoting factor. Both UVA and UVB (UVB more than UVA) contribute to mutagenesis of DNA by inducing UV landmark mutations (two tandem CC:GG to TT:AA and two C:G to T:A transitions at dipyrimidic sites). UV-induced mutations in TSG lead to uncontrolled cell-cycle progression and subsequent transformation of keratinocytes.[100] In addition to direct mutagenesis, exposure to UVB leads to decreased density and antigen-processing capability of Langerhans cells and may suppress production of the T-helper cell type 1 cytokines IL-2 and INF-γ.[101]

Alterations in the TSG *p53* are the most common genetic abnormality found in AK, SCCIS, and invasive SCC. Under normal conditions, UVR induces *p53* gene activity. The amount of p53 protein rapidly increases in keratinocytes after UVR, and drives the expression of downstream genes including *Mdm2*, *GADD45*, and *p21 CIP/WAF1*, leading to cellular arrest in the G1 phase. In cases of squamous dysplasia or SCC, one allele of *p53* contains a missense point mutation with UV signature, while the remaining *p53* allele is often deleted. Based on whole exome sequencing and copy number variation data obtained from cutaneous SCC, it appears that loss of both copies of *p53* is an early event in carcinogenesis, facilitating subsequent clonal expansion and accumulation of many additional point mutations.[102] In this study and in similar studies of SCC of the oropharyngeal mucosa, loss of *p53* was the most common mutation, but inactivating mutations in other TSG were also noted, including *CDKN2A* and *NOTCH1*, which encodes a membrane receptor critical for directing cell fate determination in development.[102–104] Activating mutations or gene amplifications of oncogenes have also been reported in SCC, most notably involving the epidermal growth factor receptor (EGFR) and its downstream signaling components such as *ras*.[40,62,102–105] Although these genome-wide studies highlight the mutational complexity and heterogeneity of SCC, two underlying features emerge. First, that both inactivating mutations in TSGs and activating mutations in oncogenes (often multiple) are required for malignant progression, and second, that loss of functional *p53* is a central feature of SCC pathogenesis observed in a majority of tumors.

Other agents associated with development of SCC include (1) chemical agents (e.g., petroleum, coal tar, soot, arsenic); (2) physical agents (e.g., ionizing radiation); (3) exposure to PUVA: calculated adjusted relative risk for a cumulative exposure of between 100 and 337 treatments is 8.6; (4) HPV, especially important for SCC in the anogenital and periungual regions, in the setting of immunosuppression with HIV and solid-organ transplantation, and in patients with epidermodysplasia verruciformis; and (5) smoking. Development of SCC has also been associated with chronic nonhealing wounds, burn scars, and chronic inflammatory dermatoses (discoid lupus, ulcers, osteomyelitis).[106] Certain cervicofacial regions such as the ear and the lower lip are more prone to developing SCC than BCC. Heritable conditions associated with higher incidence of SCC include xeroderma pigmentosum, dystrophic epidermolysis bullosa, and oculocutaneous albinism. HPV-16 and -18 are frequently implicated in the pathogenesis of subungual and periungual SCC and SCCIS of the digits.

Immunosuppression, including endogenous (underlying lymphoproliferative disorder) and iatrogenic immunosuppression plays a role in pathogenesis of SCC (see the following). In addition to immuosuppressive agents, other medications may also enhance the risk of SCC. Chronic use of photosensitizing drugs, such as the antifungal agent voriconazole, can facilitate actinic damage and have been implicated in accelerated SCC development, particularly in immunosuppressed patients.[107] Vemurafenib, a tyrosine kinase inhibitor recently approved by the FDA for the treatment of metastatic and unresectable melanomas harboring V600E mutations in the *BRAF* gene, appears to increase the risk of keratoacanthoma and SCC development by directly altering signaling through the Ras-Raf mitogen-activated protein kinase pathway known to be involved in SCC pathogenesis.[108]

CLINICAL FEATURES OF SQUAMOUS CELL CARCINOMA AND ITS VARIANTS

Clinically, SCCIS appears as a discrete solitary, sharply demarcated, scaly pink to red papule or thin plaque (Fig. 92.7). Erythroplasia of Queyrat (SCCIS on the glans of penis of uncircumcised male related to HPV infection) presents as a verrucous or polypoid papule or plaque, often eroded. Invasive SCC appears as a slightly raised papule plaque or nodule that is skin-colored, pink, or red (Fig. 92.8). The surface of the tumor may be smooth, keratotic, or ulcerated. The lesion may also be exophytic or indurated. Rarely, the tumor is symptomatic with pain or pruritus. Bleeding with minimal trauma is common. It can be clinically difficult to distinguish an invasive SCC from a hypertrophic AK, a benign seborrheic keratosis, or a benign inflammatory lesion. An appropriate biopsy should be performed.

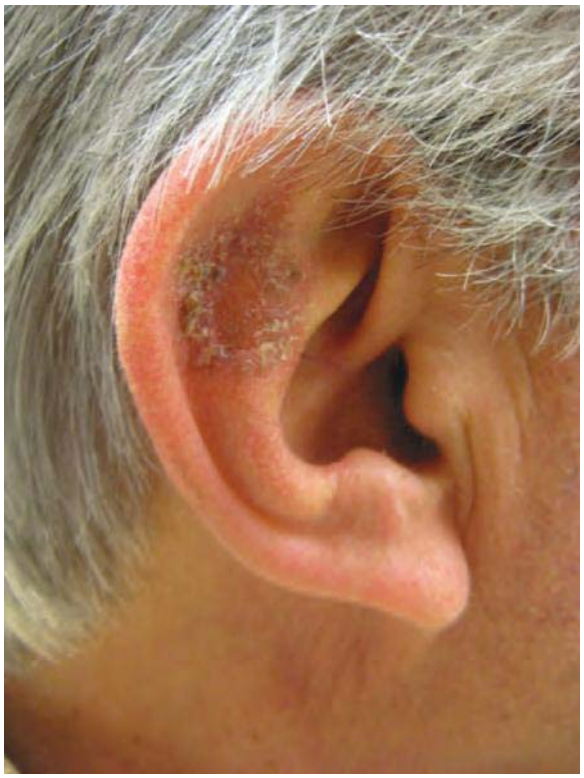

Figure 92.7 Squamous cell carcinoma in situ presents as an erythematous plaque that can be difficult to differentiate from a benign inflammatory process.

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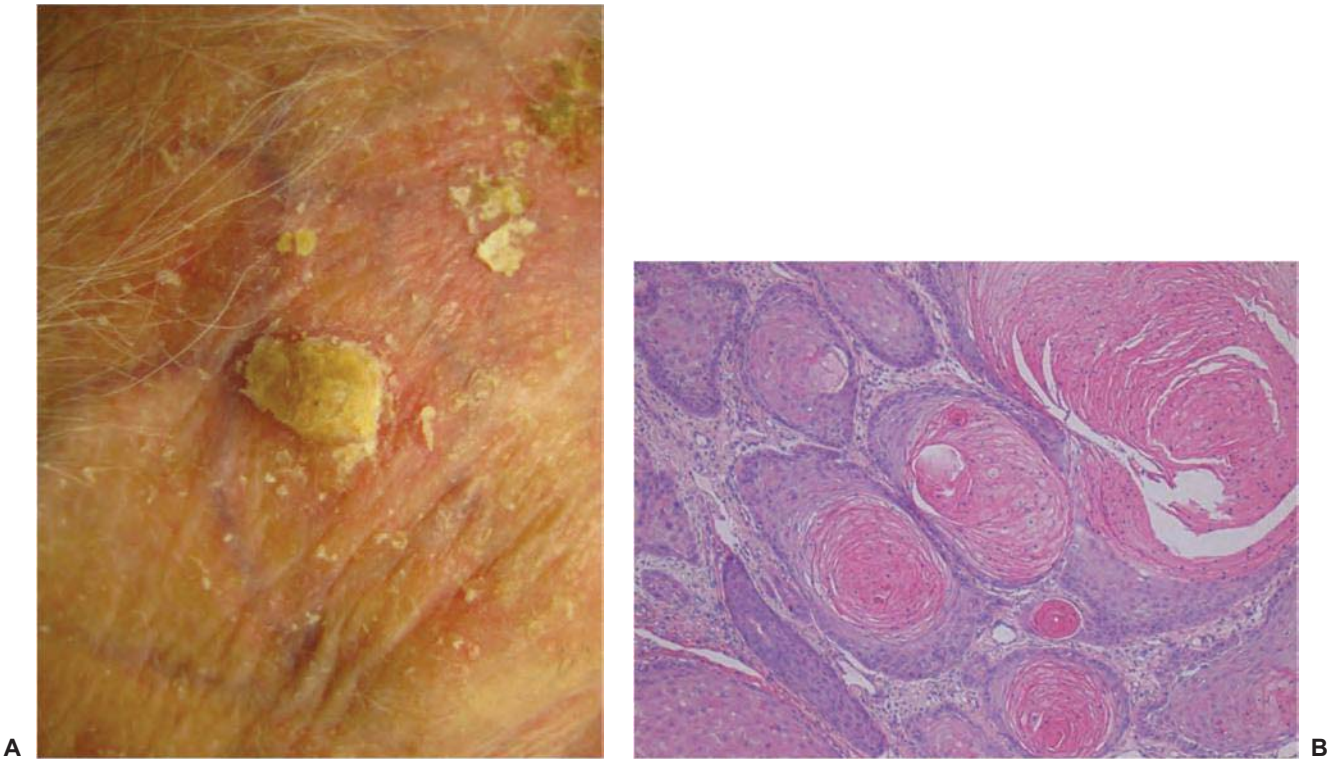

Figure 92.8 **(A)** Clinical differential diagnosis of cutaneous horn includes squamous cell carcinoma (SCC). Biopsy of the pictured lesion confirmed a clinical diagnosis of SCC. **(B)** Histology of well-differentiated SCC.

Keratoacanthoma (KA) is a variant of SCC defined by a symmetric crateriform architecture and a clinical presentation marked by rapid growth (up to several centimeters) over a period of several weeks. The tumor then typically stabilizes in size and often spontaneously regresses. Although certain KAs may thus behave in a benign fashion, it is impossible to predict which lesions will regress and which will progress. It is thus recommended to treat KAs as a subtype of SCC with appropriate surgical therapy. Verrucous carcinoma, a variant of SCC, includes oral florid papillomatosis, giant condyloma of Buschke-Lowenstein (on the genitalia), and epithelioma cuniculatum (on the plantar foot).[109] Verrucous carcinoma is considered a low-grade carcinoma. It grows slowly and rarely metastasizes, but is frequently deeply invasive into underlying tissue and therefore is difficult to eradicate. Following treatment with RT, verrucous carcinoma may become aggressive or even metastasize.

HISTOLOGY

The histologic criteria defining SCCIS include involvement of the entire thickness of epidermis with pleomorphic keratinocytes and involvement of the adnexal epithelium. The degree of keratinocytes atypia in SCCIS is variable. Marked anaplasia, nuclear crowding, loss of polarity, dysmaturation of the keratinocytes, numerous mitotic figures, including atypical and bizarre forms, and occasional dyskeratotic keratinocytes are seen giving epidermis a "windblown appearance." The epidermis may also be hyperplastic with psoriasiform appearance and broad rete ridges. A pigmented variant of SCCIS has abundant melanin accumulated within keratinocytes and scattered superficial dermal macrophages. The histologic differential diagnosis of SCCIS includes AK, bowenoid papulosis, Paget disease, extramammary Paget disease, and malignant melanoma in situ. Immunostaining may be required for proper diagnostic assessment. Bowenoid papulosis is histologically indistinguishable from SCCIS.

SCC is characterized histologically by its relatively large cellular size, nuclear hyperchromatism, lack of maturation, nuclear atypia, and the presence of mitotic figures. Presence of dermal invasion separates invasive SCC from SCCIS. In well-differentiated SCC, cytoplasmic keratinization is manifested by the presence of keratin pearls (horn cysts) and individual cell dyskeratosis. Invading keratinocytes frequently demonstrate minimal cytologic atypia. In contrast, poorly differentiated or undifferentiated SCC shows decreased evidence of keratinization, higher degree of cytologic atypia, and increased number of mitotic figures. Other histologic subtypes include spindle cell, acantholytic, desmoplastic, and adenosquamous (mucin-producing) SCC, which have all been associated with more invasive tumors and increased risk of recurrence.

CLINICAL BEHAVIOR OF SQUAMOUS CELL CARCINOMA

Cutaneous SCCIS is a full-thickness intraepidermal carcinoma. Most lesions are indolent and enlarge slowly over years, seldom progressing to invasive carcinoma. Retrospective studies suggest that the risk of progression to invasive SCC is approximately 3% to 5%. The risk of progression into invasive disease for genital erythroplasia of Queyrat is approximately 10%.[110] Bowenoid papulosis classically presents as a reddish brown verrucous papule and is associated with HPV-16 and -18. Bowenoid papulosis usually involves the genitals but may be present elsewhere.

While overall the prognosis of cutaneous SCC is good to excellent, it has been estimated that 3% and 2% of patients, respectively, develop nodal metastasis and die from SCC, leading to >4,000 deaths per year in the United States.[98] Several studies have attempted to elucidate which factors define patients at highest risk of disease progression. In a pivotal review of studies of SCCs from 1940 to 1992, Rowe et al.[96] correlated the risk for local recurrence and metastasis with treatment modality, prior treatment, location, size, depth, histologic differentiation, evidence of perineural

involvement, precipitating factors other than UVR, and immunosuppression. Tumors arising in areas of chronic inflammation and at mucocutaneous junctions had a 10% to 30% rate of progression to metastatic disease, whereas the incidence of metastasis from SCC arising on sun-exposed skin in the absence of preexisting inflammatory or degenerative conditions varied widely from 0.05% to 16.0%.[96] SCC with perineural involvement exhibited a lower 10-year survival (23% versus 88%) and a higher local RR (47% versus 7.3%) than those without perineural disease. For tumors >2 cm in diameter, RRs double from 7.4% to 15.2%, and for tumors >4 mm in depth, metastasis rates dramatically increase from 6.7% to 45.7%. It was also observed that locally recurrent SCC had an elevated metastasis rate of 30%, particularly when located on the lip and ear (31.5 and 45% metastasis rates, respectively).

Based upon these studies and other retrospective case series, the National Comprehensive Cancer Network identified key clinical and histologic risk factors for recurrence of NMSC,[111] and the American Joint Committee on Cancer (AJCC) developed staging criteria for cutaneous SCC based upon these characteristics in 2011.[112] The AJCC criteria specifically identify four high-risk features: depth >2 mm thickness or Clark level IV or greater, perineural invasion, location on ear or nonglabrous lip, and poor histologic differentiation. Tumors ≤2 cm in diameter with less than two of these high-risk features are classified as T1, whereas tumors >2 cm or any tumor with two or more high-risk features are classified as T2. Immunosuppression is not specifically included in the staging criteria, but the authors acknowledged that this feature is correlated with worse prognosis and must be considered in patient management.[112,113] Tumors with invasion of bone are classified as T3 and T4, depending on whether there is invasion of facial bones (T3) or other skeletal sites (T4).

A critique of the AJCC criteria is that while T3 and T4 are exceedingly rare, T2 tumors comprise a heterogeneous group with anywhere from intermediate to high risk of progression. As such, Schmults and colleagues,[114] based on a restrospective, multivariate analysis of 256 high-risk SCC, proposed an alternative staging system with only four risk factors: tumor diameter ≥2 cm, depth of invasion beyond subcutaneous fat, poor histologic differentiation, and perineural invasion. When tumors with one risk factor were classified as T2a, tumors with two to three risk factors were classified as T2b, and tumors with four risk factors or bone invasion were classified as T3, the authors demonstrated improved prognostication of recurrence or nodal metastasis (<1% for T1, 4% for T2a, 37% for T2b, and >75% for T3 at 5 years). While additional studies are needed to define the optimal tumor staging system, the presence of any of the risk factors discussed here should alert clinicians to the elevated risk of disease progression and the need for appropriate treatment and follow-up.

TREATMENT

Many of the treatments for BCC are also appropriate for SCC. The type of therapy should be selected on the basis of size of the lesion, anatomic location, depth of invasion, degree of cellular differentiation, history of previous treatment, and immune status of the host. There are three general approaches to treatment of SCC: (1) destruction by C&D, (2) removal by excisional surgery or MMS, and (3) radiation therapy.

Cautery/Electrodesiccation

C&D is a simple, cost-effective technique for treating low-risk SCCs. Honeycutt and Jansen[115] reported a 99% cure rate for 281 SCCs after a 4-year follow-up. In this study, two recurrences were noted in lesions <2 cm in diameter. C&D is frequently used for SCCIS; however, as with all forms of destructive therapy, final pathologic review of the tumor is not obtained and clinically unrecognized foci of invasive tumor are a concern. Although extension of SCCIS down hair follicles has been reported, this appears to be an uncommon phenomenon that has not been systematically assessed. Nevertheless, C&D is generally not indicated for thicker plaques of SCCIS, for tumors in dense hair-bearing areas, or when tumor extends into the subcutaneous layer.

SCCIS may be treated by cryotherapy. As with BCC, two freeze–thaw cycles with a tissue temperature of −50°C are required to destroy the tumor sufficiently. A margin of normal skin also should be frozen to ensure eradication of subclinical disease. Complications include hypertrophic scarring and postinflammatory pigmentary changes, both hypo- and hyperpigmentation. Concealment of recurrence within dense scar tissue presents a danger. Imiquimod has demonstrated efficacy in the treatment of SCCIS, but it is currently not approved by the FDA for the treatment of this neoplasm.[11]

Surgical Modalities

Surgical excision is a well-accepted treatment modality for SCC. Brodland and Zitelli[116] have demonstrated that lesions of <2 cm in diameter can be safely treated by excision, with a 95% confidence interval using margins of 4 mm and 6 mm for low-risk and high-risk tumors, respectively. These investigators defined high risk as a size of ≥2 cm, histologic grade higher than 2, invasion of the subcutaneous tissue, and location in high-risk areas (primarily periorifical central face). Carcinomas of the penis, vulva, and anus are usually treated by excision or MMS. Surgical excision is the treatment of choice for verrucous carcinoma.

MMS is indicated for high-risk SCCs including invasive, poorly differentiated SCCs, and lesions occurring in high-risk anatomic sites or sites in which conservation of normal tissue is essential for preservation of function and/or cosmesis. Recurrence rates with MMS are superior to those obtained with traditional excisional surgery in primary SCC of the ear (3.1% versus 10.9%), primary SCC of the lip (5.8% versus 18.7%), recurrent SCC (10% versus 23.3%), SCC with PNI (0% versus 47%), SCC >2 cm (25.2% versus 41.7%), and poorly differentiated SCC (32.6% versus 53.6%).[109] MMS has proven useful in SCC involving the nail unit and has been used as a limb-sparing procedure in cases of SCC arising in osteomyelitis.

Radiation Therapy

Indications for RT for patients with SCC are similar to those for patients with BCC. The likelihood of cure for early stage lesions is similar for both surgery and RT. Therefore, the decision on which modality to employ depends on other factors, including a patient's underlying medical status, age, expected posttreatment cosmesis, cost, and treatment availability.

In young patients, surgical treatment is a preferable because the late effects of radiation progress gradually with time and, with long-term follow-up, may be associated with a suboptimal cosmetic result compared with surgical resection and reconstruction. In special sites such as lower lip with advanced (over 30% to 50% of the lip involved) SCC, RT allows for excellent maintenance of oral competency with cure rates similar to those of surgical modalities.[33]

Advanced cutaneous cancers may be treated with surgery and adjuvant RT. Adjuvant postoperative RT is added in situations in which the possibility of residual disease is high. In a retrospective study of patients with SCC of the lip, Babington et al.[117] reported a 53% local RR in patients who underwent surgery alone (37% of whom had positive, borderline, or unreported surgical margins) compared with a 6% local RR in the minority of patients treated with surgery and adjuvant RT. In the setting of documented clear surgical margins (such as with MMS), no studies to date have shown a benefit of adjuvant RT,[118] although it has been used anecdotally for tumors deemed particularly high risk. Indications

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for postsurgical RT include positive margins, PNI (especially if symptomatic), multiple recurrences, and underlying tissue invasion. Advanced unresectable cancers, such as those with marked PNI or with gross disease in the cavernous sinus, may be treated with RT alone.

Management of Regional Lymph Node Metastases

Treatment of nodal disease may involve local RT, lymph node dissection, or a combination of both. Skin cancer metastatic to the parotid nodes is commonly managed with superficial or surgical total parotidectomy followed by adjuvant RT (60 Gy in 30 fractions). Extreme care should be taken to preserve the function of the facial nerve. Nonetheless, in certain cases resection is necessary to achieve a gross total resection. With surgery and adjuvant RT, 5-year disease-free survival ranges from 70% to 75%. Although the risk of subclinical disease in the clinically negative nodes is ≥20%, the ipsilateral neck may be electively irradiated when the parotid is treated postoperatively. RT alone is used for patients with unresectable disease and for those who are medically inoperable. The likelihood of cure is lower with RT-only treatment compared with RT plus surgery, but nodal regression and good palliation are commonly seen. Treatment and palliative doses should be at least 60 to 66 Gy and 40 Gy, respectively.[33]

Cervical node metastases may be managed with neck dissection in patients with a solitary node with no extracapsular extension, and with surgery plus adjuvant RT in patients with more advanced disease.[119] Similar to parotid node metastasis, surgery with adjuvant RT has shown improved 5-year disease-free survival compared to surgery alone (74% versus 34%).[120] Depending on the location of the primary tumor, the probability of subclinical disease in the clinically negative parotid may be high and the parotid nodes should be considered for elective treatment.

Medical Therapy for Advanced Subcutaneous Cell Carcinoma

Apart from surgical therapy or RT, treatment options for SCC are limited. Local, intralesional chemotherapy with methotrexate (one to three injections of 5 to 40 mg each) and 5-FU (up to eight injections of 10 to 150 mg each) have been reported to be up to 100% effective for treatment of KA-type SCC in small case series, although these studies were uncontrolled and this is not a commonly utilized therapy.[121] For typical SCC, intralesional treatment with IFN has been found to be an effective treatment, and may be particularly useful as salvage therapy for advanced or multiply recurrent disease.[122] The largest study to date treated 27 invasive SCC and 7 SCCIS with intralesional IFN-alpha-2b, 1.5 million units three times a week for 3 weeks, and found a 97% cure rate at 3 months, although long-term follow-up was not reported.[123]

The long-term prognosis for metastatic SCC is extremely poor. Treatment of metastatic disease may include systemic chemotherapy or treatment with biologic response modifiers. Although the efficacy of these methods has not been established, the recent development of targeted inhibitors of the EGFR holds promise for treatment of advanced SCC, particularly in light of the known aberrations in EGFR signaling in SCC. Cetuximab is a chimeric monoclonal antibody directed against EGFR that inhibits EGFR signaling; it is approved for treatment of head and neck (mucosal) SCC and has been used off label for cutaneous SCC.[124] The first phase 2 study of monotherapy with weekly cetuximab infusions for unresectable or metastatic cutaneous SCC showed a complete response rate of 6% and a partial response (at least 30% decrease in tumor size) of 22%.[125] Subsequent case reports have shown improved response rates (up to 50% complete response) when cetuximab was combined with adjuvant RT.[124] Although the efficacy of these investigational treatments is clearly inferior to excisional surgery, it compares favorably to the historical prognosis of advanced SCC, in which 10-year survival rates are <20% for patients with regional lymph node involvement and <10% for patients with distant metastases.[126] While surgical therapy remains the cornerstone of SCC treatment, it is likely that additional molecularly targeted therapeutics will be incorporated as adjuvant or salvage therapy for advanced SCC.

FOLLOW-UP

Invasive SCC can be a potentially lethal neoplasm and warrants close follow-up. A critical review and meta-analysis has found that for people with fewer than three previous NMSCs, the risk of developing another NMSC within the following 3 years is 38%. In people with three to nine previous NMSCs, this risk rises to 93%.[127] In another study, approximately 30% of patients with SCC developed a subsequent SCC, with more than half of these occurring within the first year of follow-up.[128] Thus, it is recommended that patients with SCC be examined every 3 to 6 months during the first 2 years after treatment and at least annually thereafter. Evaluation should include total-body cutaneous examination and palpation of draining lymph nodes. Currently, radiography, magnetic resonance imaging, and computed tomography (CT) play no role in the routine workup of uncomplicated cutaneous SCC.

IMMUNOSUPPRESSION AND NONMELANOMA SKIN CANCER

The role of the immune system in the pathogenesis of skin cancer is still not completely understood. Immunosuppressed patients with lymphoma or leukemia and patients with depressed cellular immunity secondary to HIV infection develop NMSC at a significantly younger age and show a higher frequency of NMSC than the general population.[129] An Italian study of HIV patients found a three-fold increase in the incidence of NMSC over the general population.[130] Incidence of clinically aggressive HPV-related anal SCC is significantly increased in this population, requiring serial examinations and anal cytologies for surveillance.[131]

Solid-organ transplant recipients (e.g., heart, kidney) have a three- to four-fold increased risk over the general population of developing any cancer.[22] These patients experience a marked increase in the incidence of SCCs (40- to 250-fold increase) and BCCs (5- to 10-fold increase). Skin cancers in immunosuppressed patients appear primarily on sun-exposed sites. Incidence of NMSC in renal transplant recipients in Australia increases exponentially over time: 3% within the first year, 25% at 5 years, and 44% at 9 or more years posttransplant.[132] Similar results were observed in heart transplant patients, with an inverted SCC:BCC ratio of 3:1.[133] Furthermore, the SCCs in organ transplant patients occur at a younger age and tend to be more aggressive. There is an increased risk of local recurrence, regional and distant metastasis, and mortality. In case series of renal transplant patients from the United States and Australian heart transplant recipients, SCC-related mortality rates were 5% and 27%, respectively.[133] Although organ transplant recipients have an increased incidence of viral warts, HPV infection does not appear to be the primary cause of skin cancer in this population.[134] Patients who receive hematopoietic transplants do not experience marked increased in skin cancer incidence, presumably because of the shorter duration of immunosuppression.

Cumulative UVR is the primary pathogenic factor for the development of NMSC in solid-organ transplant recipients, but degree, type, duration of immunosuppression, and age at transplantation are also significant.[133] Sirolimus (rapamycin), a bacterial macrolide and antitumor agent, is a newer immunosuppressive agent that shows promise in decreasing incidence and severity of

posttransplant NMSCs.[135] Changing immunosuppressive therapy to sirolimus from a standard regimen of calcineurin inhibitors was shown to be effective for secondary prevention of SCC in renal transplant recipients in a randomized trial, decreasing the risk of subsequent SCC by 44%.[136] However, because 23% of patients in the sirolimus group discontinued the medication due to adverse effects (including edema, aphthous ulcers, and pneumonitis), sirolimus is often reserved for use in selected patients with particularly elevated risk of SCC complications. As discussed previously, preventative therapy with systemic retinoids is another viable option for minimizing morbidity from SCC in transplant recipients.[23] Prevention, patient education, aggressive sun protection, and timely and aggressive management of skin cancers as well as altering the degree or type of immunosuppression whenever possible are crucial to reduce the significant risk of NMSC complications in this population.

ANGIOSARCOMA

Angiosarcoma (AS; synonyms are malignant hemangioendothelioma, hemangiosarcoma, and lymphangiosarcoma) is an uncommon, aggressive, usually fatal neoplasm of vascular endothelium origin accounting for <2% of all sarcomas.[137] The overall incidence of this tumor is approximately 0.1 per million per year. Four variants of cutaneous AS currently are recognized and include AS of the "head and neck" (also known as idiopathic AS) accounting for 50% to 60% of all cases, AS in the context of lymphedema (lymphedema-associated AS [LAS]; Stewart-Treves syndrome), radiation-induced AS, and epithelioid AS. Although these variants differ in presentation, they share key features, including clinical appearance of primary lesions, a biologically aggressive nature, and, ultimately, poor outcome.

PATHOGENESIS

Pathogenesis of AS is poorly understood. Approximately 50% of ASs express markers of lymphatic differentiation in addition to vascular endothelium-associated antigens. More recently, AS was found to coexpress podoplanin and podocalyxin, markers of lymphatic and vascular endothelium, respectively.[138] Human herpesvirus-8 etiologic factor in Kaposi sarcoma appears not to be associated with AS. Cumulative sun exposure has not been shown to be a predisposing factor.

CLINICAL PRESENTATION AND PROGNOSIS

Cutaneous AS of the head and scalp usually affects older adults. Approximately 70% of AS occurs in patients over the age of 40 years and the highest incidence of the disease is reported in those over 70 years of age.[137] Men are more commonly affected than women with 1.6 to 3:1 ratio.

Clinically, cutaneous AS presents as a violaceous to red, ill-defined patch on the central face, forehead, or scalp, often initially resembling a bruise.[137] Facial swelling and edema may be present. Differential diagnosis at initial presentation may include benign vascular tumor, hematoma secondary to trauma, or even an inflammatory dermatosis. More advanced lesions are violaceous elevated nodules with propensity to bleed easily. Ulceration may also be present. Satellite lesions are common.

The prognosis of cutaneous AS is poor, with a mortality rate of 50% at 15 months after diagnosis, and the survival rates ranging from 10% to 30% over a 5-year period, with median survival 18 to 28 months.[139,140] Metastatic potential of AS is high. Metastases to lung, liver, lymph nodes, spleen, and brain are common. Prognosis for metastatic disease is poor. Although prognosis does not correlate with degree of cellular differentiation, there appears to be a correlation with lesion size at presentation; increased survival has been demonstrated in lesions <5 cm at time of diagnosis. In a clinical univariate analysis of 69 cases, older age, anatomic site, necrosis, and epithelioid features directly correlated with increased mortality.[141] Other prognostic factors proposed in the literature include depth of invasion >3 mm, mitotic rate, Ki-67 staining, positive surgical margins, and local recurrence.[142]

LAS accounts for about 10% of all cutaneous AS and was first reported by Stewart and Treves[143] in six patients with postmastectomy lymphedema. In each case, AS developed in the ipsilateral arm and occurred several years after mastectomy. Subsequently, LAS was reported after axillary node dissection for melanoma and in the context of congenital lymphedema, filarial lymphedema, and chronic idiopathic lymphedema. The risk for developing LAS 5 years after mastectomy is approximately 5%. The most common site is the medial aspect of the upper arm.

LAS presents as a firm, coalescing violaceous plaque or nodule superimposed on brawny, nonpitting edema. Ulceration may develop rapidly. The duration of lymphedema prior to appearance of AS ranges from 4 to 27 years. The pathogenesis of LAS is incompletely understood and may be related to imbalances in local immune regulation or angiogenesis, leading to proliferation of neoplastic cells. The prognosis is poor, and survival rates are comparable to AS involving the scalp and face. Long-term survival has been reported in isolated cases after amputation of the affected limb.

Radiation-induced AS has been reported to occur after RT for benign or malignant conditions.[137] AS may occur from 4 to 40 years after RT for benign conditions (acne and eczema), or from 4 to 25 years after RT for malignancies. Overall prognosis is poor and comparable to that observed in other forms of AS.

Epithelioid AS is a rare, recently described variant of AS.[144] It tends to involve the lower extremities. On microscopic examination, the tumor may mimic an epithelial neoplasm, with sheets of rounded, epithelioid cells intermingled with irregularly lined vascular channels. Epithelioid AS results in widespread metastases within 1 year of presentation. Prognosis is poor.

HISTOLOGY

Histology of AS, although highly variable in the degree of cellular endothelial differentiation between and within individual tumors, does not vary between individual subtypes.[137] In well-differentiated lesions, an anastomosing network of sinusoidal irregularly dilated vascular channels lined by a single layer of flattened endothelial cells with mild to moderate nuclear atypia is commonly seen. These exhibit a highly infiltrative pattern, splitting collagen bundles and subcutaneous adipose tissue. Less-differentiated tumors show proliferation of atypical, polygonal, or spindle-shaped, pleomorphic endothelial cells with increased mitotic activity and anastomosing vascular channels. In poorly differentiated AS, luminal formation may be no longer apparent and mitotic activity is high. Poorly differentiated AS may mimic other high-grade sarcomas, carcinoma, or even melanoma. The state of cellular differentiation, however, has not been shown to correlate with prognosis.[139] Immunohistochemical analysis may be of value in diagnosis of AS, as cells stain positively for *Ulex europaeus* I lectin and factor VIII–related antigen. *Ulex* I is considered to be more sensitive marker for AS. In addition, AS cells express stem cell antigen CD34 and endothelial cell surface antigen CD31. The majority of AS cases stain positively for vimetin, D2-40, and VEGFR-3.

TREATMENT

Because of the clinical aggressiveness, treatment options for AS are limited. Surgical excision with wide margins is the treatment of choice. Nonetheless, the RRs and possibility of metastatic disease

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are high even with histologically negative margins and may reflect the tendency for multifocality.[145] Amputation with shoulder disarticulation or hemipelvectomy is recommended for tumors involving the extremities. Because AS tends to extend far beyond clinically appreciated margins, complete surgical removal may be challenging. Several cases of AS have been treated by MMS in an attempt to control margins; however, the difference between AS and normal vasculature may be difficult to interpret on frozen sections, even with the use of immunohistochemical stains.[146] RT and electron beam should be considered postoperatively in an effort to enhance local control.

Patients with isolated lymphatic spread treated with taxol-based chemotherapeutic regimens have a favorable outcome. Both chemotherapy and radical RT are palliative only for metastatic disease and do not improve overall survival.

DERMATOFIBROSARCOMA PROTUBERANS

DFSP is a rare soft tissue sarcoma with aggressive local but low metastatic potential with an annual incidence of approximately 4 per million. DFSP constitutes approximately 1% of all sarcomas and <0.1% of all malignancies.[147] The vast majority, approximately 90% of DFSPs, are low-grade sarcomas, whereas the remainder are classified as intermediate or high grade because of the presence of a high-grade fibrosarcomatous component (DFSP-FS).[148]

DFSP most commonly affects patients in their mid- to late 30s; however, the disease can occur at any age. Childhood and congenital cases of DFSP have been reported.[149] Blacks have slightly higher incidence than whites. Both men and women are equally affected.[150]

PATHOGENESIS

The pathogenesis of DFSP is incompletely understood but may involve factors as diverse as aberrant TSG or a history of local trauma/scarring.[151] More than 90% of DFSP feature a translocation between chromosomes 17 and 22, resulting in the fusion between the collagen type Iα1 gene (*COL1A1*) and the platelet-derived growth factor (PDGF) β-chain gene (*PDGFB*). Thus, the growth of DFSP is a result of the deregulation of PDGF β-chain expression and activation of PDGF receptor (*PDGFR*) protein tyrosine kinase.[151,152]

DFSP classically presents as a solitary, frequently asymptomatic, plaque with violaceous to blue hue. The tumor exhibits slow growth. Most commonly affected sides include trunk and, less frequently, the extremities, head, and neck, but it may occur anywhere.[152] The Bednar tumor is a rare pigmented variant of DFSP.[153] Clinically, it may be difficult to differentiate from a dermatofibroma or a keloid.

HISTOLOGY

Histologically, DFSP arises in the dermis and is composed of monomorphous, dense spindle cells arranged in a storiform pattern and embedded in a sparse to moderately dense fibrous stroma.[154] Irregular projections (tentaclelike) of the tumor are common and may account for the high incidence of local recurrence after excision. The distinction between deep penetrating dermatofibroma (DPDF), which involves the subcutis, and DFSP may be challenging. In most instances, attention to the cytologic constituency of the lesions and the overall architecture is sufficient for differentiation. DPDF is typified by cellular heterogeneity. DPDF includes giant cells and lipidized histiocytes and extends deeply, using the interlobular subcuticular fibrous septa as scaffolds, or is in the form of broad fronts. In contrast, DFSP tends to be monomorphous, surrounding adipocytes diffusely or extending in stratified horizontal plates. This infiltration is characteristically eccentric, often with long, thin extensions in one direction and not another. Immunostaining for factor XIIIa, CD34, and stromelysin 3 may be helpful in distinguishing DPDF from DFSP. Characteristically, DPDF is diffusely factor XIIIa+, CD34−, and stromelysin 3+, whereas DFSP is factor XIIIa−, CD34+, and stromelysin 3−.[155]

TREATMENT

Treatment options for DFSP include WLE and MMS. Most authors advocate WLE with a minimal margin of at least 3 cm of surrounding skin, including the underlying fascia, without elective lymph node dissection.[156] The likelihood of local recurrence is directly proportional to the adequacy of surgical margins. Conservative resection can lead to RRs of 33% to 60%, whereas wider excision margins (≥2.5 cm) have been reported to reduce the RR to 10%.[157] For well-defined tumors located on trunk or extremities, WLE is likely to achieve tumor clearance with satisfactory cosmetic and functional result. However, extirpation of tumor by MMS, using frozen sections with or without confirmation by examination of paraffin-embedded sections, may be beneficial in sites where maximum conservation of normal tissue is required. Utility of MMS versus WLE was examined in a retrospective review of 48 primary DFSP cases treated at a single institution.[158] Twenty-eight patients underwent WLE and twenty patients underwent MMS. Median WLE margin width was 2 cm. For MMS, the median number of layers required to clear the tumor was two. Positive margins were present in 21.4% (6 of 28) WLE versus 0% MMS. At a median follow-up of 49.9 months for WLE and 40.4 months for MMS, local RRs were 3.6% (1 of 28) and 0%, respectively. The authors concluded that although positive margin resection was more common with WLE, local control was ultimately similar for the two surgical modalities. In a different study, Paradisi et al.[159] compared literature-reported observational data on 41 patients who underwent MMS and 38 who underwent WLE. Recurrence rates were 13.2% and 0% for WLE and MMS at 4.8 and 5.4 years follow-up, respectively. The relative risk of recurrence for WLE versus MMS was 15.9.

In the cases of congenital DFSP treated with MMS, the reported clearance rate was 100% during an average follow-up period of 4.3 years.[160] The clearance rate seen with WLE was 89% with an average follow-up period of 1.9 years. The average margins taken during MMS (1.7 cm) were smaller than those taken with WLE (2.8 cm). Based on superior cure rates and smaller surgical margins, MMS was proposed as first-line treatment for congenital DFSP.

Alternative treatment options for DFSP include RT and chemotherapy. RT was used selectively in a number of cases if surgical resection was not possible or would result in major cosmetic or functional deficit, with good local response.[161,162] A PDGF receptor inhibitor, imatinib, has been used with clinical success in advanced disease.[163,164] In a case series of 10 patients with locally advanced or metastatic disease treated with imatinib, 9 patients showed therapy response.[165] Limited clinical data are available on the use of chemotherapeutic agents such as vinblastine and methotrexate.[161]

RECURRENCE AND METASTATIC POTENTIAL

Patients with DFSP should be followed closely for evidence of local or regional recurrence or metastatic disease. DFSP has a tendency to recur locally. The average time for recurrence is within the first 3 years. DFSP of the head and neck has been reported to have a higher local RR (50% to 75%) than DFSP in other locations and might be related to smaller surgical margins used in cosmetically sensitive areas.[166] Although metastases are rare, multiple local recurrences appear to predispose to distant metastases.[167]

Lymph node metastases occur in approximately 1% of cases, and distant metastases, principally to lung, occur in approximately 4% of DFSP cases. DFSP-associated mortality is low. In a series of 218 patients, the 5- and 10-year mortality rates were 1.5% and 2.8%, respectively.[161]

A fibrosarcomatous variant, DFSP-FS, represents an uncommon form of DFSP that tends to follow a more aggressive clinical course.[168] In a series of 41 patients with DFSP-FS, a mean follow-up period of 90 months revealed a local RR of 58%. Metastatic rate was 14.7%.

MERKEL CELL CARCINOMA

MCC is a rare and aggressive tumor of neuroendocrine cell origin with an estimated annual incidence in the United States of 3 per million people.[169] Incidence of MCC is estimated in men at more than twice that in women, and whites are more than 20 times more likely to develop disease than blacks. The average age at diagnosis is 70 years.[170]

Merkel cells derive from the neural crest and differentiate as a part of the amine precursor uptake and decarboxylation system. Merkel cells function as slowly adapting type I mechanoreceptors.[170]

PATHOGENESIS

The pathogenesis of MCC is incompletely characterized. Given the increased incidence of MCC with increasing age, it is likely that accumulation of oncogenic events plays a role. UVR has been indirectly implicated in the development of MCC. The risk is higher among whites of European ancestry, incidence is inversely related to latitude, and the majority of tumors present on the face (36%), head, extremities, and trunk.[170]

The risk of MCC is particularly high with prior PUVA treatment. A multicenter study of 1,380 patients with psoriasis who were treated with PUVA showed that the incidence of MCC was 100 times higher than expected in the general population.[171] Immunosuppression, whether through iatrogenic means, HIV infection, or neoplasia, may play a role in the development of MCC. Patients with MCC have increased incidence of multiple myeloma, non-Hodgkin lymphoma, and in particular chronic lymphocytic leukemia.[172,173] Rapid progression has been reported in the setting of immunosuppressive therapy after organ transplantation.[174] Numerous chromosomal abnormalities have been described in MCC, but a definite causal relationship has not been established.

Recently, a double-stranded DNA virus, Merkel cell polyomavirus (MCPyV) was elegantly implicated in the pathogenesis of MCC.[175–177] Viral genome was detected in 8 of 10 MCCs and at low levels in 16% of unaffected skin and 8% of tissues from other body sites of patients without MCC. Within MCC substantial variation in the relative number of MCPyV, DNA was noted. Virus-positive MCCs contain between 1 viral DNA copy per 10,000 tumor cells to 10 viral DNA copies per tumor cell. MCPyV was integrated at various locations in the MCC tumor genome in a clonal pattern, suggesting that infection of the cells occurred before their clonal expansion.[177] Increased detection techniques reveal that virtually all MCC contain MCPyV[178] and that both CD4+ and CD8+ antiviral T cells are detectable within tumors and the blood of patients with MCC.[179]

CLINICAL PRESENTATION

Clinically, MCC usually presents as a rapidly growing, firm, flesh-colored or red-violaceous, dome-shaped papule or plaque on sun-exposed skin. Most lesions are <2 cm in diameter at the time of diagnosis. Clinical differential diagnosis often includes leukemia cutis, amelanotic melanoma, metastatic carcinoma, pyogenic granuloma, BCC, and SCC. Regional lymph nodes are involved in up to 30% of patients, and approximately 50% of patients develop systemic disease. Secondary sites of MCC spread include skin (28%), lymph nodes (27%), liver (13%), lung (10%), bone (10%), and brain (6%).[147,180]

HISTOLOGY

Histologic examination of MCC reveals sheets and cords of atypical cells in the dermis extending to the subcutaneous layer that sometimes form an interlacing trabecular or pseudoglandular pattern (Fig. 92.9). Three histologic subtypes have been described: intermediate, trabecular, and small cell. No clinically significant differences between subtypes have been described. A grenz zone separating tumor from epidermis is often present. Individual cell membranes often are indistinct, giving a syncytial appearance. Cells are round to oval and generally noncohesive. Cytoplasm tends to be scant, with round to oval nuclei containing two to three nucleoli. Special stains may prove useful in the histological evaluation of MCC. Cytokeratin-20 staining gives a characteristic parinuclear dot pattern. MCC also stains positively for chromogranin neuron–specific enolase and synaptophysin, and may be weakly

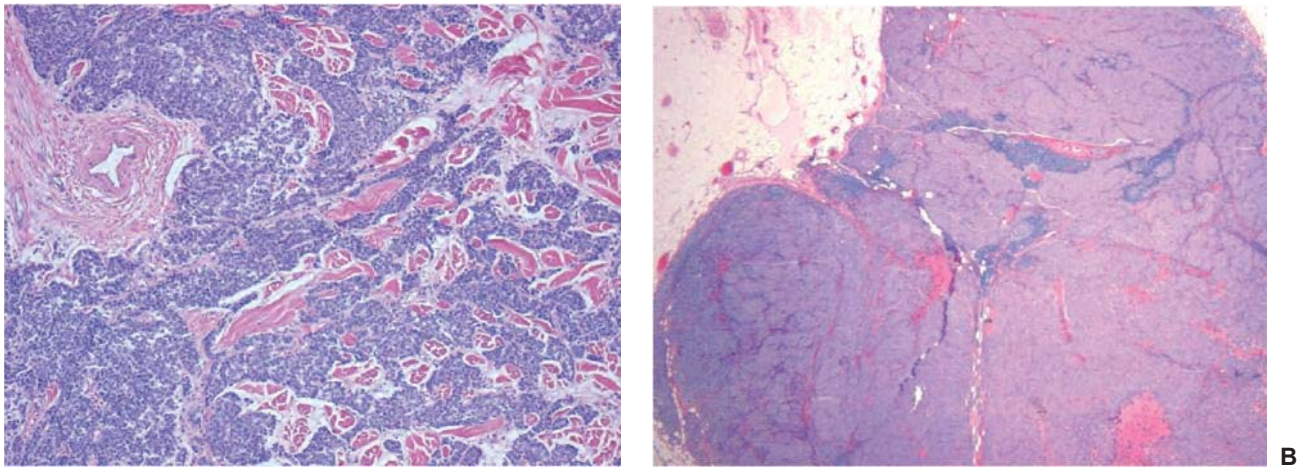

Figure 92.9 **(A and B)** Histologic examination of cutaneous Merkel cell carcinoma reveals sheets and cords of atypical cells in the dermis extending to the subcutaneous layer that sometimes form an interlacing trabecular or pseudoglandular pattern. Cells are round to oval and generally noncohesive. Cytoplasm tends to be scant, with round to oval nuclei containing two to three nucleoli.

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positive for S100 protein.[181] In 2002, it was shown that MCC also stains for the KIT receptor tyrosine kinase (CD117), and perhaps abnormal functioning of the KIT receptor could be involved in the malignant transformation of this tumor.[182]

Histologic differential diagnosis of MCC includes lymphoma, BCC, metastatic oat cell carcinoma, or noncutaneous neuroendocrine tumors. In contrast to MCC, lymphoma cells are CD45-positive and cytokeratin-20 negative. Melanoma can be differentiated from MCC by strong S100 positivity of melanocytes.

TREATMENT

MCC warrants aggressive therapy. It has a high propensity for local recurrence (20% to 75%), regional (31% to 80%), and distant metastases (26% to 75%). Approximately one-third of patients with MCC eventually die of the disease. Age older than 65 years, male sex, size of primary lesion >2 cm, truncal site, nodal/distant disease at presentation, and duration of disease before presentation (≤3 months) appear to be poor prognostic factors. All patients with histologically confirmed MCC should undergo imaging and laboratory examination to evaluate the full extent of disease. Evaluation must include full-body skin examination with lymph node evaluation, a complete blood cell count, and liver function tests. CT scanning of the chest, pelvis, and abdomen may be indicated to detect distal metastasis.[183] CT scanning of the head and neck may prove valuable in detection of nodal disease. Octreotide scans may be more sensitive than CT scans in diagnosing primary and metastatic MCC.[184] Perianal and vulvar sites have the worst prognosis of all primary sites. Metastases have been noted most commonly in skin and lymph nodes but also in the lung, liver, brain, intestine, bladder, stomach, and abdominal wall.

STAGING

Five competing staging systems have been used to describe MCC. However, these staging systems are highly inconsistent. To address these concerns, a new MCC-specific consensus staging system was developed by the AJCC. Patients with primary MCC with no evidence of regional or distant metastases (either clinically or pathologically) are divided into two stages: stage I for primary tumors no >2 cm in size and stage II for primary tumors >2 cm in size. Stage I and stage II are further divided into A and B substages based on method of nodal evaluation. Patients who have pathologically proven node-negative disease (by microscopic evaluation of their draining lymph nodes) have improved survival (substaged as IIA) compared with those who are evaluated only clinically (substaged as IIB). Stage II has an additional substage (IIC) for tumors with extracutaneous invasion (T4) and negative node status, regardless of whether the negative node status was established microscopically or clinically. Stage III includes patient with nodal disease, either microscopically positive and clinically occult nodes (IIIA) or macroscopic nodes (IIIB). Distant metastases are classified as stage IV MCC.[185]

Before the new AJCC consensus staging system was published, the recent Memorial Sloan Kettering Cancer Center (MSKCC) four-stage system was favored as it was based on the largest number of patients and was the best validated. The stages in the MSKCC system include stage I (primary tumor alone, <2 cm in diameter), stage II (primary tumor alone, >2 cm in diameter), stage III (regional nodal disease), and stage IV (distant metastatic disease).[186]

RECURRENCE AND METASTATIC RISK

MCC has a propensity to recur locally (sometimes with satellite lesions and/or in-transit metastases) following surgical excision. In a review of 18 case series, 279 of 926 patients (30.1%) developed local recurrence during follow-up. These recurrences have been typically attributed to inadequate surgical margins or possibly a lack of adjuvant radiation therapy.[187] WLE to reduce the risk of local recurrence has been recommended for patients with clinical stage I or stage II disease.

Recommendations about the optimal minimum width and depth of normal tissue margin that should be excised around the primary tumor differ among the various retrospective case series, but this question has not been studied systematically.[180,186,188] No definitive data suggest that extremely wide margins improve overall survival, although some reports suggest that wider margins appear to improve local control.[189]

Recommended management has usually been WLE with 1- to 3-cm margins; however, treatment guidelines are not well defined, owing to the rarity of the tumor, which precludes randomized clinical trials. Recurrence rates after primary therapy for MCC with surgery alone are reported to be within the range of 0% to 50% to 70%. In a single-institution case series of 95 patients with early-stage MCC, a total of 45 (47%) patients relapsed, with 80% of the recurrences occurring within 2 years and 96% within 5 years.[190] Patients with MCC in the head and neck region had a 5-year local-recurrence cumulative incidence of 19% and no distant recurrences, and patients with MCC in the extremity and trunk region had a 5-year local-recurrence cumulative incidence of 2% and a 5-year distant-recurrence cumulative incidence of 22%.[190] MMS has been proposed as being more successful in controlling local disease than WLE, especially in cosmetically sensitive anatomic locations. The relapse rate has been reported to be similar to or better than that of wide excision, but comparatively few cases have been treated in this manner and none in randomized, controlled trials.[188,191,192] In a retrospective review of 38 consecutive patients with MCC of the extremities, WLE and MMS showed similar local RR.[188]

MCC spreads to regional lymph nodes within 2 years in up to 70% of cases. Because of the propensity for early nodal spread and the significant negative impact that nodal disease has on outcome, regional lymph node dissection or sentinel lymph node (SLN) dissection may be advisable. Surgical nodal staging in clinically negative patients has identified positive nodes in at least 25% to 35% of patients.[186] At present, it is questionable whether lymph node dissection has an impact on survival, but it seems to benefit local and regional control. Clinically or radiographically positive nodes should be resected but it is unclear whether elective lymph node dissection provides benefit.

SLN biopsy is a preferred initial alternative to complete elective lymph node dissection for the proper staging of MCC. SLN biopsy is associated with lower morbidity, and in the sites with indeterminate lymphatic drainage, SNL technique can be used to identify the pertinent lymphatic basins. Several reports support the use of SLN biopsy techniques in MCC staging and management.[193–195] One meta-analysis of 10 case series found that SLN positivity strongly predicted a high short-term risk of recurrence and that subsequent therapeutic lymph node dissection was effective in preventing short-term regional nodal recurrence.[195] Another meta-analysis of 12 retrospective case series found that (1) SLN biopsy detected MCC spread in one-third of patients whose tumors would have otherwise been clinically and radiologically understaged, (2) the RR was three times higher in patients with a positive SLN biopsy than in those with a negative SLN biopsy, and (3) the relapse-free survival rate in patients with positive SLN biopsy who did or did not receive additional treatment to the nodes was 51% and 0% at 3 years, respectively.[196] Whether complete dissection of regional nodes following positive SLN biopsy improves definitively survival remains unresolved, however.

Radiation to the primary site has been considered for patients with larger (>2 cm) tumors and locally unresectable tumors, while adjuvant nodal radiation is considered for those with positive regional nodes (stage II).[169,192] Several small retrospective series have shown that radiation plus adequate surgery improves

local-regional control compared with surgery alone,[180,196] whereas other series did not show similar results.[186,191] Adjuvant RT offers a substantial benefit in both time to recurrence and disease-free survival, but a survival benefit is yet to be proven.[197] The controversy regarding the utility of adjuvant RT following excision remains.

Chemotherapy is used for nodal, metastatic, and recurrent MCC, but an optimal treatment regimen is yet to be established. From 1997 to 2001, the Trans-Tasman Radiation Oncology Group performed a phase 2 evaluation of 53 patients with MCC with high-risk, local-regional disease. Given the heterogeneity of the population and the nonstandardized surgery, it is difficult to infer a clear treatment benefit of the chemotherapy.[198] Regimens are similar to those used for small cell lung carcinoma. The most commonly used agents are cyclophosphamide, anthracyclines, and cisplatin. In a study by Voog et al.,[199] overall response to first-line chemotherapy for MCC was 61%, with a 57% response in metastatic disease and a 69% response in locally advanced disease. Reported 3-year survival rate was 17% in metastatic disease and 35% in locally advanced disease. Forty-two different regimens were used to treat these 107 reported cases.

PROGNOSIS

The prognosis of MCC is directly correlated with the stage of disease. Reported 5-year survival according to MSKCC classification is 81% for stage I, 67% for stage II, 52% for stage III, and 11% for stage IV.[147] More than 50% of patients experience recurrence with the median time to recurrence of 9 months (range, 2 to 70 months). Ninety-one percent of recurrences occurred within 2 years of diagnosis.[186] Overall survival of head and neck MCC at 5 years postoperatively ranges between 40% and 68%.[200]

MICROCYSTIC ADNEXAL CARCINOMA

Microcystic adnexal carcinoma (MAC) was first described as a distinct entity in 1982 by Goldstein et al.[201] Synonyms quoted in the literature to describe MAC include sclerosing sweat duct carcinoma, malignant syringoma, sweat gland carcinoma with syringomatous features, aggressive trichofolliculoma, and combined adnexal tumor of the skin. MAC originates from pluripotent adnexal keratinocytes capable of both eccrine and follicular differentiation. The pathogenesis of MAC is not completely understood but may involve exposure to ionizing and UVR that may precede development of MAC by as long as 40 years.[202] MAC is an aggressive, locally destructive cutaneous appendageal neoplasm with a high rate of local recurrence but low rate of metastasis. It primarily affects white, middle-aged individuals, although it has been reported in children and blacks. Unlike the other primary cutaneous malignancies, MAC has slight female predominance.

CLINICAL AND HISTOLOGIC FEATURES

MAC classically presents as a smooth-surfaced, nonulcerated, flesh-colored to yellowish asymptomatic nodule, papule, or plaque. When symptomatic, common findings include numbness, tenderness, anesthesia, paresthesia, burning, discomfort, and/or rarely pruritus of the affected site. These symptoms can relate to the frequent PNI of the tumor. MAC is locally aggressive with common perineural invasion and extension to muscle, vascular adventitia, perichondrium, periosteum, and bone marrow. MAC has a clear predilection for the head and neck (86% to 88%), particularly the central face (73%). Other sites include eyelid, scalp, breast/chest, axillae, buttocks, vulva, extremities, and tongue. This tumor is often misdiagnosed clinically and histologically.[202,203]

Histologically, MAC is a tumor of pilar and eccrine differentiation. It may be misdiagnosed as a benign adnexal process. The tumor frequently exhibits a stratified appearance with larger keratin horn cysts and epithelial nests, strands, or cords in the superficial dermis and desmoplastic features in the deeper dermis with smaller cysts and more pronounced ductal structures (Fig. 92.10). Ducts may be well differentiated, with two rows of cuboidal cells, or less differentiated, with single strands without lumina. Mitotic figures and cytologic atypia are rare. Histologic differential diagnosis of MAC includes desmoplastic trichoepithelioma, benign syringoma, papillary eccrine adenoma, morpheaform BCC, SCC, and metastatic breast carcinoma. Adequately deep biopsy is crucial for correct diagnostic assessment.

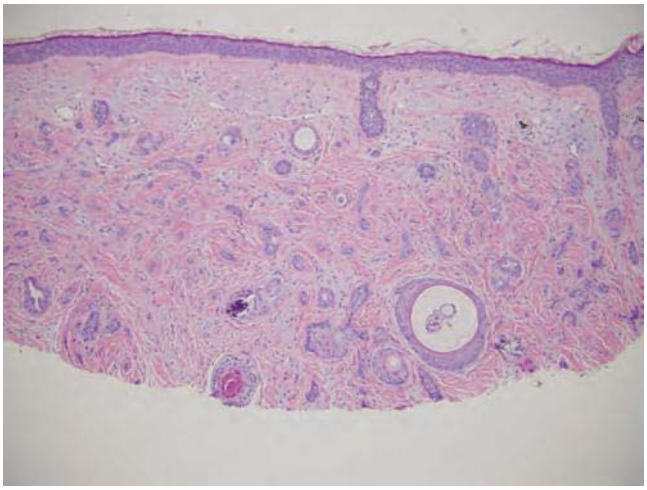

Figure 92.10 Histology of microcystic adnexal carcinoma. The tumor frequently exhibits a stratified appearance with larger keratin horn cysts and epithelial nests, strands, or cords in the superficial dermis and desmoplastic deeper dermis with smaller cysts and more pronounced ductal structures. Horn cysts may contain laminated keratin and/or small vellus hairs. Cysts may be also calcified. Ducts may be well differentiated, with two rows of cuboidal cells, or less differentiated, with single strands without lumina.

TREATMENT AND PROGNOSIS

Current standard of care for MAC is to surgically remove the tumor in its entirety whenever feasible. This task can be challenging in clinical practice because the tumor often extends microscopically centimeters beyond the clinically apparent margins. Margins reported in the literature for WLE vary from a few millimeters to 3 to 5 cm. Extirpation of tumor by MMS may prove beneficial in the management of MAC. Recurrence rates vary significantly between the two surgical techniques with rates after WLE and MMS ranging from 40% to 60%[204] and 0 to 12%,[205] respectively. To date, seven cases of metastases have been reported for a cumulative metastatic rate of <2.1%, although this is likely an overestimate. RT has been used as mono- or adjuvant therapy for MAC with reported success in only one of six case reports, highlighting the tumor's resistance to RT. Patients with MAC should have ongoing examination of the skin and lymph nodes for the remainder of their lifespan, given the potential for late recurrence decades after initial presentation.

SEBACEOUS CARCINOMA

Sebaceous carcinoma (SC) is a malignant adnexal tumor with variable sites of origin, histologic growth patterns, and clinical presentations. About 75% of SCs are periocular in location.[206] Periocular SC may arise from Meibomian glands and, less frequently, from the glands of Zeis. The upper eyelids are most frequently involved.

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Approximately 25% of cases of SC involve extraocular sites, which may include head and neck, trunk, salivary glands, and external genitalia.[207] Worldwide, SC affects all races, but Asians are particularly prone to the disease. Women are affected more commonly than men, at a ratio of approximately 2:1. SC classically presents in the seventh to ninth decades.[208] SC is associated with sebaceous adenomas, radiation exposure, and Muir Torre syndrome. Because of the strong association of SC with Muir Torre syndrome, patients presenting with SC should be referred for colonoscopy to assess for occult colon cancer. Routine genetic screening for Muir Torre in the absence of colon lesions is not currently indicated.

CLINICAL AND HISTOLOGIC FEATURES

The most frequent clinical presentation is a slowly growing, painless, subcutaneous nodule. Other presentations include diffuse thickening of the skin, pedunculated papules, or an irregular subcutaneous mass. On the eyelid, SC is most often misdiagnosed as chalazion. It may present as chronic diffuse blepharoconjunctivitis or keratoconjunctivitis, particularly with pagetoid or intraepithelial spread of tumor onto the conjunctival epithelium.[209] SC is the second most common eyelid malignancy after BCC and is the second most lethal after melanoma. Histologically, SCs are classified as well, moderately, or poorly differentiated. Most commonly, lesions have an irregular lobular growth pattern with sebaceous and undifferentiated cells. SC cells exhibit varying degrees of differentiation, nuclear pleomorphism, hyperchromatism, basaloid appearance, and high mitotic activity. Local infiltration of the surrounding tissues and neurovascular spaces can be seen. A known feature of the SC is pagetoid spread, the spread of tumor cells into the overlying epithelium. Special stains, including lipid stains as Oil-Red-O or Sudan IV for fresh tissue, and immunohistochemical stains such as EMA or LeuM1 are also helpful.[210]

TREATMENT AND PROGNOSIS

Treatment options for SC include WLE with 5- to 6-mm margins and extirpation by MMS. The local RR after WLE has been reported to be as high as 36% at 5 years and associated 5-year mortality rate of 18%.[211] In one study of 14 cases of SC excised with frozen-section margin control, five recurrences were observed in cases with surgical margins of 1 to 3 mm, whereas no recurrences were seen with margins of 5 mm.[212] Potential difficulties arise because tumors are often multicentric with discontinuous foci of tumor, and pagetoid spread is difficult to determine even on high-quality, paraffin-embedded sections. Extirpation of SC by MMS has compared favorably to WLE, with local RRs of ≤12% in reported series.[213] A series of poorly differentiated SC successfully treated with RT has also been reported.[214]

SCs have high rates of local recurrence and metastasis, particularly when occurring on the eyelid. SC can spread by lymphatic or hematogenous routes or by direct extension. Distant metastases are reported in up to 20% of cases and may involve the lungs, liver, brain, bones, and lymph nodes. Mortality of SC ranges from 9% to 50%. Extraocular SC has a reported local RR of 29% and metastatic rate of 21%, although there appears to be a reporting bias in the literature for more advanced disease. Small primary lesions <1 cm in diameter may exhibit a more favorable prognosis.

EXTRAMAMMARY PAGET DISEASE

Extramammary Paget disease (EMPD) is a rare cutaneous malignancy of older adults, often on the genitalia, with a mean age of onset of approximately 70 years. While there is a slight female predominance in Caucasian patients, there is a strong 4:1 male predominance in Asians.[215] Although histologically similar to Paget disease of the breast, EMPD is a separate entity with a distinct prognosis and is not related to breast cancer. EMPD is most often a primary intraepidermal malignancy thought to derive from eccrine or apocrine glands. EMPD has been associated with internal malignancy in 15% to 30% of cases, usually colon, bladder, or prostate cancer.[216] These cases of secondary EMPD have a worse prognosis than primary EMPD.

CLINICAL AND HISTOLOGIC FEATURES

EMPD most often presents as a pink to bright red plaque of the genitalia. Scaling, erosion, and maceration are common features. It is usually asymmetric and may extend to the perianal region, inguinal fold, suprapubic region, and medial thigh, but it is classically seen on the scrotum of men or the labia majora of women. Rare cases of primary EMPD have also been described in the axillae.[217] While most cases present as large flat plaques, nodular presentations have been described that portend a worse prognosis, as does clinical lymphadenopathy. Because of its innocuous appearance, EMPD is often misdiagnosed as benign inflammatory conditions such as psoriasis, dermatitis, or superficial fungal infections, and clinicians should have a low threshold for biopsy of suspected inflammatory conditions that do not respond to routine treatment.

Biopsy of EMPD reveals acanthosis with an intraepidermal proliferation of large, pale staining cells with prominent vesicular nuclei. These cells are spread throughout all layers of the epidermis, often forming large clusters just above the basal layer. Mitotic figures may be present. A minority of patients may have extension of EMPD into the superficial or deep dermis, which is associated with decreased survival.[217] Immunohistochemical stains may be helpful in the diagnosis of EMPD. Most cases of EMPD stain with CK7 and GCDFP-15, while CK20 staining may be more common in secondary EMPD.[218]

TREATMENT AND PROGNOSIS

To rule out internal malignancy and secondary EMPD, patients should undergo a colonoscopy and appropriate screening for genitourinary malignancy prior to initiating treatment. Complete surgical excision of EMPD has classically been the standard of care, although local RRs may be as high as 30% to 40%. MMS may be a superior option, with local RRs of 12% to 18% reported in small case series.[215,219] Because of its primarily superficial nature, nonsurgical treatment of primary EMPD has been proposed, with several case reports documenting success (and a few reporting failure) with topical imiquimod therapy.[220,221] In the authors' experience, topical therapy with imiquimod at a variable frequency for up to 12 weeks, titrated to the local inflammatory response, is an effective first-line therapy for primary EMPD. Surgical treatment may be employed for recalcitrant cases, and radiation therapy has also been reported for EMPD with some success.[222] Overall, the prognosis for primary EMPD is good, with a reported 85% overall survival at 5 years.[217] For the majority of patients that present with in situ disease, the prognosis is excellent, while the 14% of patients presenting with deep dermal invasion exhibit a 20-fold increased risk of disease-specific death.

ATYPICAL FIBROXANTHOMA AND MALIGNANT FIBROUS HISTIOCYTOMA

Until recently, atypical fibroxanthoma (AFX) and malignant fibrous histiocytoma were thought to be two distinct presentations of the same malignancy. However, following reclassification of soft tissue sarcomas by the World Health Organization in 2002 that mandated identification of cell line origin in classification of tumors, most cases of malignant fibrous histiocytoma, as previously considered,

were found to be merely a morphologic pattern rather than a defined pathologic entity.[223] In a majority of cases, ultrastructural and immunohistochemical examination allowed for reclassification into defined histologic subtypes of sarcomas. Under the new classification, the term *malignant fibrous histiocytoma* is a synonym for undifferentiated pleomorphic sarcoma (UPS) not otherwise specified. UPS is a deep-seated subcutaneous nodule rarely encountered in the skin; it is most often seen on the limbs of elderly patients. UPS is an aggressive tumor with a poor prognosis. Although complete surgical excision is the preferred treatment (often with adjuvant RT), up to 50% of patients may have distant metastasis at the time of initial presentation, with the lung being the most common site.

ATYPICAL FIBROXANTHOMA

AFX is a spindle cell tumor that occurs on the head and neck of sun-exposed individuals and on the trunk and extremities of younger patients. Tumors of the head and neck characteristically present during the eighth decade, whereas tumors involving the extremities often present during the fourth decade. The ratio of affected men to women appears to be equal. A few cases have been reported in children with xeroderma pigmentosum. The pathogenesis of AFX involves exposure to UVR, ionizing radiation, and/or aberrant immune host response. In a series of 10 cases of AFX, 7 cases showed mutation in TSG *p*53, often with UVR signature mutations.[224] Tumors may occur 10 to 15 years after local ionizing radiation. An increased incidence of AFX has been observed in renal transplant patients, and metastatic AFX has been reported in a patient with chronic lymphocytic leukemia.

CLINICAL AND HISTOLOGIC FEATURES

AFX usually presents as an asymptomatic, often rapidly growing, dome-shaped papule or nodule covered by thin epidermis on actinically damaged skin of individuals with a fair complexion. Average size at presentation is 1 to 2 cm. Secondary changes such as serosanguinous crust or ulceration may be present. The clinical appearance is not distinctive, and the clinical differential diagnosis of the lesion often includes pyogenic granuloma, SCC, BCC, amelanotic melanoma, MCC, and cutaneous metastasis. AFX may be found in the setting of other NMSCs.

On microscopic examination, AFX is a dermal or partially exophytic nodule composed of a proliferation of atypical spindle-shaped cells with moderate amounts of cytoplasm and large histiocyte-like atypical cells with abundant pale-staining vacuolated cytoplasm arranged in haphazard fashion in a collagenous or occasionally myxoid stroma.[225] The neoplastic cells have large, pleomorphic, and heterochromatic "bizarre-looking" nuclei, and some of them are multinucleated. There are numerous typical and atypical mitotic figures. Some cells may contain droplets of lipid. The epidermis overlying the dermal proliferation is commonly ulcerated. Both the spindle-shaped and the histiocyte-like cells stain positively for vimentin, while CD68 and CD10 are often, but not universally, positive in AFX. Stains for HMB-45 and S100, as well as cytokeratin stains, are negative, distinguishing this lesion from spindle cell melanoma and SCC, respectively.[225,226]

TREATMENT AND PROGNOSIS

Treatment of AFX is surgical removal by WLE or MMS. In a large retrospective series of 45 patients comparing WLE with MMS, recurrences were observed during a mean follow-up period of 73.6 months in 12% of 25 cases treated by WLE.[227] Metastatic involvement of the parotid gland occurred in one of these patients, for an overall regional metastatic rate of 4%. In contrast, no recurrences or metastases were observed over a mean follow-up period of 29.6 months in patients treated by MMS. Others have reported similarly favorable outcomes after treatment of AFX by MMS.[228,229] The authors favor the use of MMS for AFX because of the superior margin control and conservation of normal tissue.

Although AFX rarely metastasizes, it is a locally aggressive tumor with metastatic potential. Metastases to the parotid gland, lymph nodes, and lung have been reported. In a series of eight cases of metastatic AFX, poor prognostic indicators included vascular invasion, recurrence, deep-tissue penetration, necrosis, and impaired host resistance.[229,230] Because AFX is often found in the setting of diffuse actinic damage and other NMSCs, close follow-up after complete tumor extirpation is critical.

CARCINOMA METASTATIC TO SKIN

The most frequently observed cutaneous metastatic cancers are breast, colon, and melanoma in women, and lung, colon, and melanoma in men. Cutaneous involvement is also seen in the leukemias, with a wide variation in the morphology of lesions. The scalp is a common site for cutaneous metastatic disease. Immunohistochemical stains may be helpful in determining the site of the primary tumor. The discovery of cutaneous metastatic disease should prompt consultation with an oncologist for staging and management.

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93 Molecular Biology of Cutaneous Melanoma

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INTRODUCTION

The most common forms of skin cancer are basal cell carcinoma, squamous cell carcinoma, and melanoma. While melanoma represents <5% of the cases diagnosed, it is the cause of >70% of the deaths attributable to skin cancer each year. In 2013, an estimated 76,690 new cases of melanoma will be diagnosed, and 9,840 patients will die from this disease.[1] Although the annual incidence and mortality for most major cancers (i.e., lung, colorectal, breast, prostate) are decreasing, the public health burden of melanoma continues to rise. The annual incidence of melanoma has risen steadily at a rate of ~3% per year over the past 25 years.[2–4] As melanoma often strikes individuals who are young and otherwise healthy, it is also a significant financial burden, with an annual estimated cost of $3.5 billion in lost productivity in the United States due to melanoma mortality.[5]

Cutaneous melanoma arises from pigment-producing epidermal melanocytes. Clinically, melanomas are staged using the guidelines established by the American Joint Committee on Cancer.[6] For patients who present with primary tumors only (stage I, stage II), the vertical tumor (Breslow) thickness (in millimeters) and ulceration status are the most powerful indicators of prognosis. Melanoma usually spreads first regionally to draining lymph nodes. For patients with regional disease (stage III), the prognosis is determined by the number of lymph nodes involved, the size of the lymph node metastases (micrometastases versus macrometastases), the pattern of lymph node involvement (lymph nodes, in-transit metastases, or both), and the ulceration status of the primary tumor. Melanoma is also infamous for its ability to metastasize to virtually any distant organ. For patients with distant metastases (stage IV), prognosis is defined by the organ sites involved and the presence or absence of elevated serum lactate dehydrogenase levels. For patients who present with thin primary tumors without high-risk features (stage I), the long-term disease-specific survival is >95%. In contrast, for patients with distant metastases (stage IV), the median survival is 6 to 8 months, and <10% of patients are alive 5 years after diagnosis.

A number of epidemiologic studies have identified a strong link between the risk of cutaneous melanoma and exposure to ultraviolet (UV) radiation.[7,8] This initial insight into the molecular basis of this aggressive disease has expanded tremendously over the last two decades through focused molecular analyses and mechanistic studies in preclinical models. More recently, the development of high-throughput sequencing approaches that allow for exome- and genome-wide assessment of molecular changes have led to a rapid increase in the understanding of the molecular heterogeneity and pathogenesis of melanomas. Notably, these studies have demonstrated that melanomas have one of the highest rates of somatic mutations of all solid tumors (Fig. 93.1). The preponderance of the observed mutations consist of CT or GA substitutions, which are strongly associated with UV radiation–induced DNA damage, and thus confirm at the molecular level the important role of this environmental exposure in this disease.[9] The patterns of somatic aberrations have also identified a number of key functional pathways that likely contribute to the pathogenesis of this disease. Importantly, many of these findings are rapidly being translated into molecular tests and therapies that are impacting the clinical management and outcomes of patients with this highly aggressive disease.

THE RAS-RAF-MAP KINASE PATHWAY

The RAS-RAF–mitogen-activated protein kinase (MAPK) signaling pathway is a cascade of molecules that is activated by multiple cellular signals and pathways (Fig. 93.2). Signaling through RAS and RAF leads to activation of the ERK1/2 kinases, which regulate a variety of proteins through serine-threonine phosphorylation events and ultimately the transcription of many genes governing cell proliferation, survival, and other critical cellular processes. Examples of transcription factors operant in melanocytes that are regulated by ERK signaling include the microphthalmia-associated transcription factor (MITF, described in detail in the following), various ETS transcription factors, the FOS and JUN immediate early genes, among others.

Extensive genetic and mechanistic studies have unearthed a prevalence of activating MAPK pathway mutations across many tumor types. In particular, activation of this pathway appears to be one of the most frequent and important molecular events in cutaneous melanoma (Fig. 93.3). Toward this end, several MAPK signaling proteins (e.g., RAS and RAF isoforms) are encoded by "classic" oncogenes, and key transcriptional effectors downstream of MAPK also undergo oncogenic dysregulation in melanoma and other cancers. Key MAPK effectors have also been shown to regulate differentiation and senescence in non-transformed melanocytes.

RAS Family GTPases

RAS proteins (H-, K-, and NRAS) are small GTPases that comprise an initial signaling node of the RAS-RAF-MAPK cascade. The discovery of activating mutations in HRAS and KRAS led to investigations that identified mutations in this gene family in multiple cancer types, and thus the significance of this pathway in the pathogenesis of cancer. Activating mutations in NRAS are detected in 20% to 25% of cutaneous melanomas (see Fig. 93.3).[10–12] NRAS mutations are also detected in nevi, particularly congenital nevi.[13] HRAS mutations are uncommon in cutaneous melanomas, but they are detected in Spitz nevi, which are rare, benign lesions most often diagnosed in children and young adults.[14] Despite their high incidence in other cancer types, KRAS mutations are extremely rare in melanocytic lesions.

In mouse models, overexpression of activated HRAS or NRAS on an Ink4a/Arf-null background results in spontaneous melanoma formation.[15,16] However, while HRAS-induced melanomas rarely, if ever, metastasize, NRAS tumors frequently metastasize to draining lymph nodes and distal organs, in line with the apparent selection for NRAS over HRAS mutations in human melanomas.

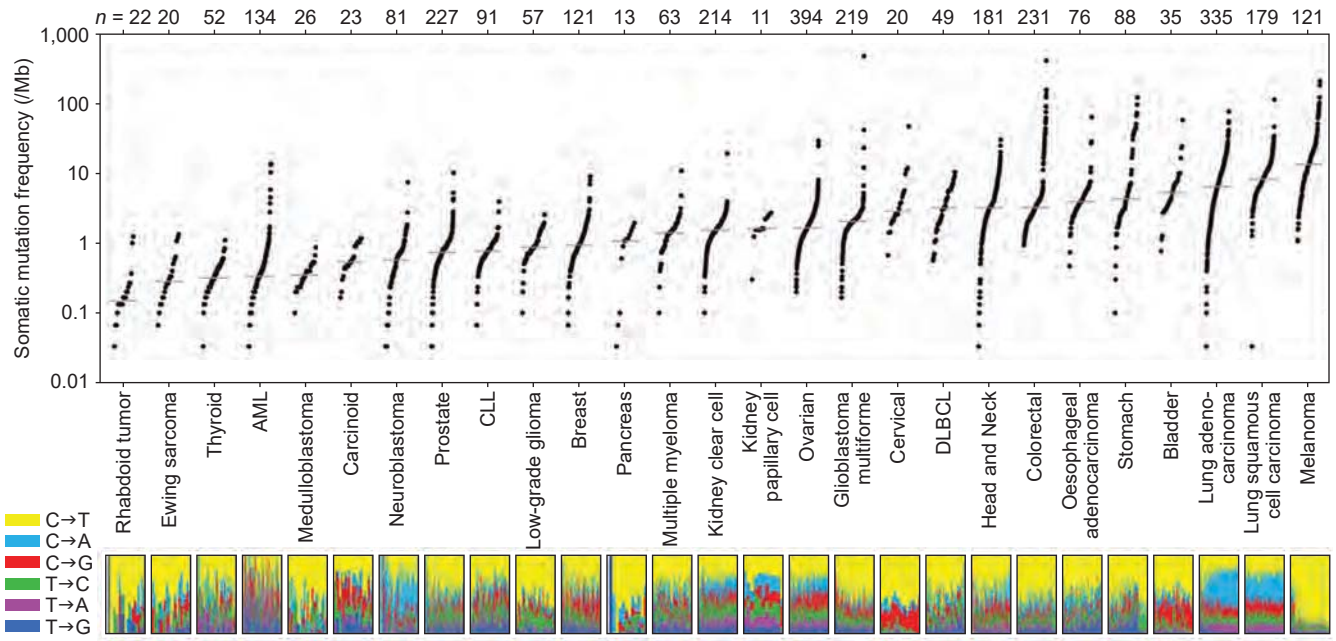

Figure 93.1 The rate of somatic mutations in whole exome sequencing analysis of various types of cancer. Each dot represents the total frequency of mutations (in mutations/Mb) in each exome of the indicated tumor types. Among the 27 tumor types analyzed, melanomas demonstrate the highest median frequency of somatic mutations. AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma. (Reprinted from Lawrence MS, Stojanov P, Polak P, et al. *Nature*. Vol. 499. London: Nature Publishing Group; 2013:215, with permission.)

Knockdown of *NRAS* in human melanoma cell lines inhibits their viability, indicating dependency on this oncogene for tumorigenicity.[17] Furthermore, shutting off transgene expression in an inducible NRAS model caused regression of melanomas that arose following transgene induction, thereby confirming the RAS oncogene dependency in these tumors.[18]

RAF Kinases

The RAF proteins (ARAF, BRAF, and CRAF) are serine-threonine kinases that comprise critical signaling effectors through the RAS-RAF-MAPK pathway (see Fig. 93.2). While each of these proteins likely plays a role in physiologic signaling, BRAF has a central role in the pathogenesis of melanoma. Somatic hotspot mutations in *BRAF* are detected in 40% to 45% of cutaneous melanoma, making them the most common oncogenic aberration detected in this disease to date.[10] Approximately 95% of these mutations result in substitutions of the valine at the 600 position in the protein. The most common mutation (~70% of *BRAF* mutations) is a T→A transversion, resulting in a valine to glutamate amino acid substitution (V600E). Although the T→A transversion is not classically associated with UV-induced damage, BRAF V600E mutations appear are more common in melanomas arising at sites with intermittent exposure to UV.[19] Other substitutions, particularly the V600K mutation that represents ~20% of *BRAF* mutations in melanoma, are more common in melanomas with evidence of chronic sun damage (CSD), although the overall rate of *BRAF* mutations in those melanomas is lower compared to tumors without CSD.[19–22] BRAF V600 mutations are an early event in melanomas, as they are also present in the majority (~80%) of benign and dysplastic nevi.[23] In addition to mutations affecting V600, somatic events affecting >20 other sites in BRAF have been detected in patients, but overall they are quite rare (total ~5% prevalence).[24]

BRAF is an immediate downstream target of RAS (see Fig. 93.3) in the MAPK pathway. The BRAF(V600E) mutation and other substitutions at the V600 site confer more than 200-fold induction of kinase activity in vitro.[25] Mutations affecting other sites in BRAF can have high, intermediate, or low catalytic activity. However, all of these mutations cause increased activation of MEK and ERK signaling. This likely occurs in low-activity mutants due to conformational changes that promote heterodimer formation with other RAF isoforms, such as CRAF, in a multiprotein complex with RAS proteins.[24] Notably, while BRAF(V600) mutations and *NRAS* mutations are mutually exclusive in newly diagnosed melanomas, frequent co-occurrence of *NRAS* mutations with nonactivating *BRAF* mutations has been observed.[26,27]

Extensive data suggest that wild-type BRAF operates on a senescence pathway in benign human nevi. Transgenic expression of BRAF(V600E) targeted to melanocytes in zebrafish produced benign nevus-like lesions, whereas invasive melanomas were produced (after extended latency) when crossed into p53-deficient zebrafish.[28] Inducible expression of BRAF(V600E) alone in murine melanocytes resulted in excessive skin pigmentation and the appearance of nevi containing hallmarks of senescence.[29] Human congenital nevi with activating BRAF mutations were shown to express senescence-associated acidic β-galactosidase, the classical senescence-associated marker.[30] This implied that activated BRAF alone is insufficient to induce tumor progression beyond the nevus stage in patients. Interestingly, immunohistochemical staining of nevoid tissues found heterogeneous patterns of INK4A that only partially overlapped with senescence-associated acidic β-galactosidase, suggesting the presence of INK4A-independent pathway(s) operative in oncogene-induced senescence. Expression of BRAF(V600E) in murine melanocytes, in the setting of inactivation of INK4A, caused melanocyte hyperplasia, but no invasive lesions.[31] However, concurrent loss of phosphatase and tensin homolog (PTEN), a negative regulator of the phosphatidylinositol kinase (PI3K) signaling pathway, resulted in 100% penetrance of invasive melanomas that formed spontaneous metastases.

The significance of *BRAF* mutations is now also supported by the functional and clinical effects of inhibiting this target. Early experiments demonstrated that RNAi knockdown of BRAF in human melanoma cells with BRAF(V600E) mutations inhibits ERK activation, induces cell cycle arrest and/or apoptosis, and blunts cell growth.[32,33] These initial results led to the development and testing of potent and selective inhibitors of the BRAF(V600E) protein. Two of these agents (vemurafenib, dabrafenib) have dem-

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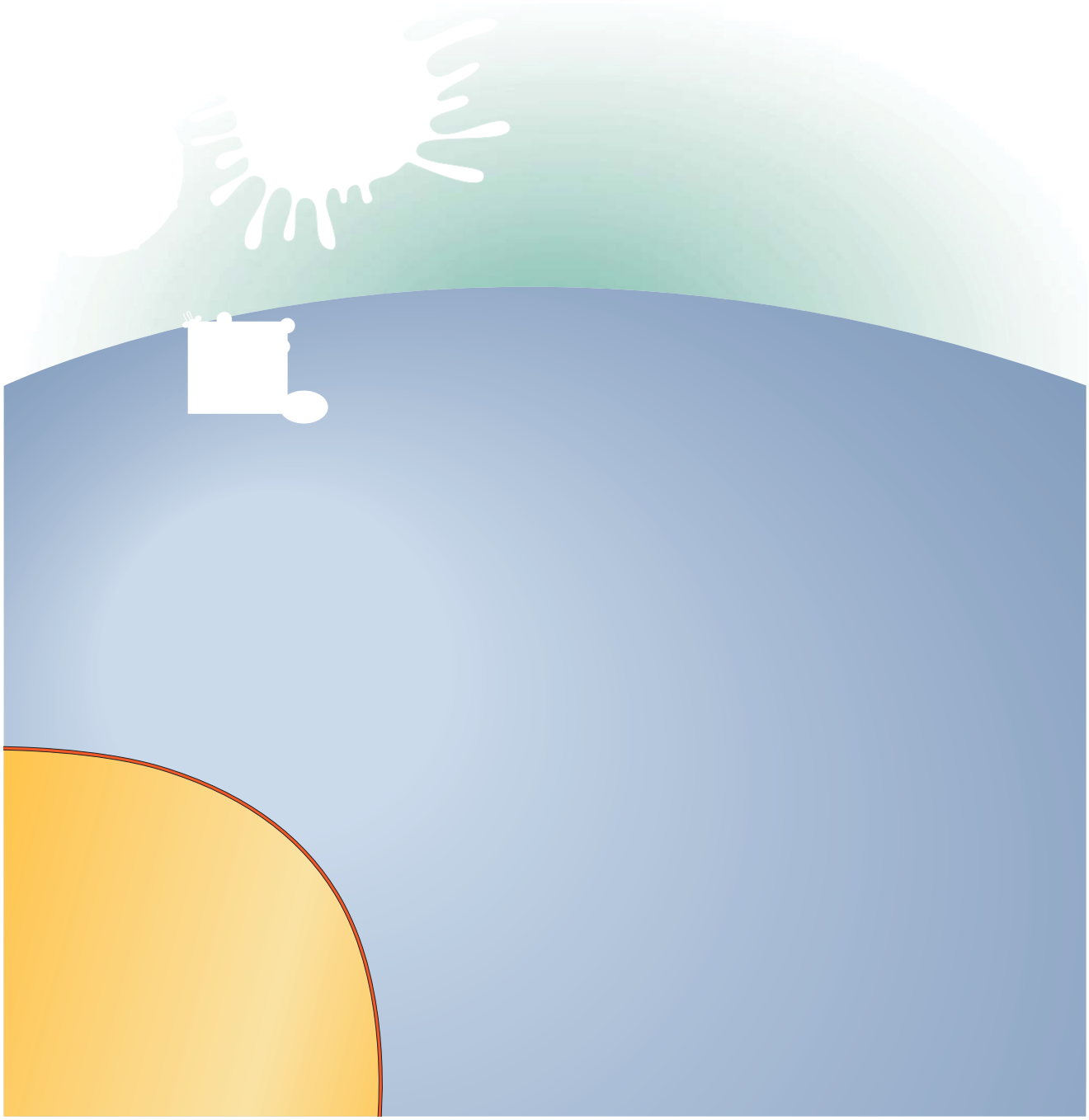

Figure 93.2 Molecular signaling pathways in melanoma. (Reprinted from Sullivan RJ, Lorusso PM, Flaherty KT. *Clinical Cancer Research*. Vol. 19. Philadelphia, PA: American Association for Cancer Research; 2013:5286, with permission.)

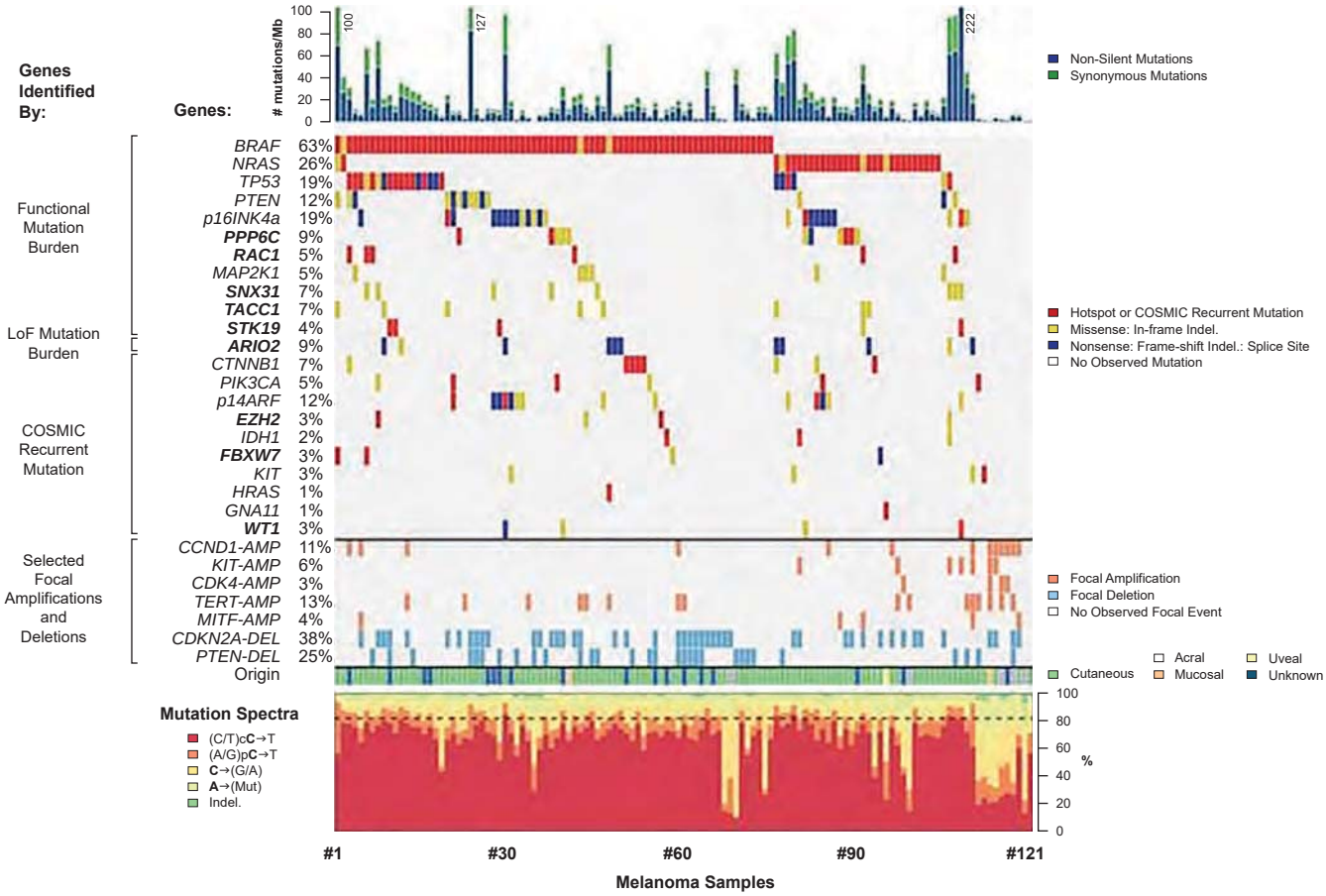

Figure 93.3 A landscape of driver mutations in melanoma. Each *row* indicates the prevalence of somatic mutations, amplifications, or deletions in each indicated gene in the cohort of 121 melanomas. Each *column* indicates the somatic events present in that tumor. The mutation spectra for each tumor is illustrated at the bottom of the figure. (Modified from Hodis E, Watson IR, Kryukov GV, et al. *Cell*. Vol. 150. Cambridge, MA: Cell Press; 2012:259, with permission.)

pathway activation through heterodimer formation with nonactivating BRAF mutations, and it may be a therapeutic target in such tumors.[40] Increased expression and/or signaling by CRAF has also been implicated as a mechanism of resistance to mutant-selective BRAF inhibitors in melanomas with BRAF(V600E) mutations.[41–43]

MEK1/2

Because the MEK1/2 serine-threonine kinases transmit the critical MAPK signal downstream of RAS and RAF, considerable interest has also emerged regarding these kinases in melanoma biology and therapeutics. MEK1/2 mutations are rare in melanoma, and in cancer in general, in contrast to *RAS* and *BRAF* mutations.[11,12,44] Nonetheless, pharmacologic MEK inhibition presents another possible therapeutic strategy for *BRAF*- or *NRAS*-mutant melanomas. Robust preclinical evidence favoring this notion derived from a genetic and pharmacologic analysis showing that various MEK inhibitory compounds demonstrated markedly enhanced potency against BRAF(V600E) cancer cells compared to cell lines lacking oncogenic MAPK pathway mutations.[45] Treatment with the MEK1/2 inhibitor trametinib as a single agent in patients with metastatic melanoma with BRAF(V600E) or BRAF(V600K) mutations resulted in significant improvements in clinical response rates, progression-free survival, and overall survival compared to chemotherapy, leading to its regulatory approval in 2013.[46] In addition, combined treatment with selective RAF inhibitors (i.e., dabrafenib) and MEK inhibitors (i.e., trametinib) significantly improves both the rate and duration of clinical responses.[47] This pivotal observation led to the approval of combined RAF/MEK inhibition for use in BRAF(V600E)-mutant melanoma. Interestingly, this combination also has less cutaneous toxicity than is observed with single-agent BRAF inhibitors, likely due to blockade of the paradoxical activation of RAS-RAF-MAPK pathway signaling in keratinocytes with RAS mutations.[39]

While mutant-selective BRAF inhibitors are contraindicated for patients with activating *NRAS* mutations, preclinical studies have demonstrated that at least some NRAS-mutant melanoma cells were also sensitive to MEK inhibitors.[45] Recently the MEK1/2 inhibitor MEK162 has demonstrated promising results in patients with metastatic melanoma with NRAS mutations, leading to randomized clinical studies versus chemotherapy.[48] MEK inhibitors have also demonstrated efficacy in NRAS-mutant melanomas as a part of combinatorial strategies (i.e., with PI3K inhibitors, CDK4 inhibitors, etc.). The safety and efficacy of several of these combinations in patients are currently being investigated in clinical trials.[49]

NF1

A small percentage of melanomas lack BRAF or NRAS mutations; however, MAPK signaling is often still operant in this setting. Recent genome characterization efforts have revealed that the tumor suppressor gene *NF1* undergoes inactivating mutations in a substantial proportion of "BRAF/NRAS wild-type" melanomas.[11,12] *NF1* encodes neurofibromin, a so-called RAS-GAP whose normal physiologic role involves negative regulation of RAS signaling effected through cleavage of the RAS-GTP. Consequently, loss of *NF1* leads to dysregualted RAS signaling. *NF1* loss is sufficient to drive melanoma genesis together with other known cancer genes in genetically engineered mouse models of melanoma.[50] More-

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over, *NF1* loss has been observed in BRAF-mutant clinical specimens that exhibit resistance to RAF inhibition.[50,51]

THE MEPK PATHWAY AND THERAPEUTIC RESISTANCE

Unfortunately, the impressive clinical effects of RAF and MEK inhibition in BRAF(V600)-mutant melanoma are transient: the vast majority of patients will experience disease progression within one year of treatment (although a subset of patients may enjoy prolonged clinical benefit). Multiple mechanisms of resistance to RAF/MEK inhibition have been described, most of which restore MEK/ERK signaling as the key downstream mechanism. Resistance mechanisms such as *NRAS* mutations[52] or *NF1* inactivation[51] accomplish this by signaling through C-RAF, which is normally inactive in BRAF-mutant melanoma but can be engaged through homo- or heterodimerization in the setting of upstream RAS signaling. An alternative splice isoform of BRAF also acts through RAF-mediated dimerization.[43] Certain receptor tyrosine kinase–driven resistance mechanisms may also work in part by augmenting A- and/or C-RAF activity in a RAS-dependent manner.[42,53] Other resistance effectors may bypass RAF proteins altogether yet still converge on MEK/ERK activation—the kinase COT (encoded by the *MAP3K8* gene) comprises an example of this mechanism.[54] Alternatively, MEK/ERK signaling may be restored through activating somatic mutations in *MAP2K1* or *MAP2K2*, which encode MEK1 and MEK2 kinases, respectively.[55,56] Interestingly, NRAS and MAPK2 mutations have been observed in the setting of resistance to both single-agent RAF inhibition and combined RAF/MEK inhibition,[57] underscoring the importance of sustained ERK signaling as a resistance mechanism to this therapeutic modality.

Although MEK/ERK-independent resistance mechanisms are less well understood, recent data suggest that such effectors may also prove important. For example, a large-scale functional screen for open reading frames whose overexpression produces resistance to MAPK inhibitors identified several dozen genes that can confer resistance to RAF, MEK, and ERK inhibition.[58] Several of these genes encode proteins that may bypass MEK/ERK signaling altogether, whereas others encode transcription factors known to operate downstream of ERK (such as MITF) or that can otherwise substitute for the ERK-driven transcriptional output. As combinatorial MAPK-directed therapy gains traction in BRAF(V600)-mutant melanoma, such ERK-independent resistance mechanisms are likely to become increasingly manifest.

Cell Cycle Regulators

The RB signaling pathway regulates the entry in and progression through the cell cycle. A significant role for this pathway in melanoma was initially implicated by the finding that germline mutations in this pathway (*CDKN2A*, *CDK4*) are the most frequently detected events in familial melanomas (more than three affected family members). Subsequent studies have demonstrated that somatic aberrations in this pathway are ubiquitous in cutaneous melanoma. Functional studies support that dysregulation of cell cycle entry and progression is critical to the pathogenesis of this disease, and potentially contributes to resistance to RAS-RAF-MAPK pathway inhibitors.

The *CDKN2A* Locus

Germline deletions and activating mutations in the *CDKN2A* locus on chromosome 9p21 are the most common event (~40%) prevalent in familial melanoma. Somatic mutations and deletions, or epigenetic silencing, are detected in as many as 70% of cutaneous melanomas.[59,60] Thus, disruption of CDKN2A function likely plays a central role in melanoma pathogenesis. The CDKN2A locus contains an unusual gene organization, which allows for two separate transcripts and corresponding tumor suppressor gene products to be produced: $p16^{INK4A}$ and $p19^{ARF}$ (see Fig. 93.2). Loss of $p16^{INK4A}$ results in the suppression of retinoblastoma (RB) tumor suppressor activity via increased activation of the CDK4/6-cyclin D1 complex; loss of ARF ($p14^{ARF}$ in human and $p19^{ARF}$ in mouse) downmodulates p53 activity through increased activation of MDM2. Thus, deletion of the entire locus accomplishes the inactivation of two critical tumor suppressor pathways: RB and p53. Homozygous deletion of exons 2 and 3 of the mouse *Cdkn2a* homolog predisposed to a high incidence of melanomas when combined with an activated HRAS transgene in melanocytes.[15] Thus, *CDKN2A* lesions may "prime" melanocytic tissue for neoplasia.

INK4A

The specific significance of INK4A function is supported by the clinical identification of intragenic mutations of *INK4A* that do not affect the *ARF* coding region that sensitize germline carriers to the development of melanomas.[61] In addition, in familial melanomas that lack *CDKN2A* aberrations, the most commonly identified genetic event is a point mutation in CDK4 that disrupts the interaction of that protein with INK4A.[62] In a mouse model engineered to be deficient only for Ink4a (with intact ARF), melanoma formation was observed in cooperation with an oncogenic initiating event (activated HRAS), albeit with a longer latency than in mice with deletions affecting the entire locus.[63] Notably, the tumors in these mice were also found to harbor either deletion of ARF or mutation of p53. Therefore, while INK4A is a bona fide tumor suppressor, additional genetic dysregulation of the p53 pathway seems obligatory for melanoma genesis, at least in the mouse.

CDK4

CDK4 is a direct target of inhibition by $p16^{INK4A}$ (see Fig. 93.2) and is a primary regulator of RB activation. As noted previously, germline mutations of *CDK4* that render the protein insensitive to inhibition by INK4A (e.g., Arg24Cys) have been identified in a melanoma-prone kindreds.[61] These tumors retain wild-type INK4A function, suggesting that INK4A is epistatic to CDK4 and that RB pathway deregulation is central to melanoma genesis. Somatic focal amplifications of *CDK4* are also observed (albeit rarely) in sporadic melanomas.[64] Carcinogen treatment induced melanomas in the animals without somatic Ink4a inactivation, similar to the mutual exclusivity observed in familial melanoma.[65] CDK4 interacts with cyclin D proteins (see the following) to drive progression through the G1/S cell cycle checkpoint. Recently, small molecule inhibitors of CDK4 and other CDKs have entered clinical trials in several tumor types, including melanomas. In particular, both tumor genetic data and recent results from genetically engineered mouse models provide a rational basis for combining CDK and MAPK pathway inhibition in NRAS-mutant melanoma.[18]

CCND1

CCND1 encodes the CyclinD1 kinase, which forms a complex with CDK4 or CDK6 to inactivate RB1 (see Fig. 93.2). Amplification of the *CCND1* locus has been identified in relatively rare event in cutaneous melanomas (5% to 10%).[11,66] However, this molecular event is enriched in cutaneous melanomas that do not have mutations in BRAF or NRAS.[11,19] Although it is rare in melanomas with BRAF(V600) mutations, amplification of CyclinD1 has been implicated as predictor of resistance to BRAF inhibitors in preclinical models, and increased copy number of CCND1 correlated with shorter progression-free survival in one study of patients with metastatic melanoma treated with the BRAF inhibitor dabrafenib.[67,68]

RB1

Germline mutations in *RB1* confer predisposition to melanoma in patients who have survived bilateral RB.[69] These melanomas exhibit loss of heterozygosity of the remaining wild-type RB1 allele. In such patients, estimates of increased lifetime risk of melanoma range from 4- to 80-fold. The *RB1* gene locus has been found deleted in some primary cutaneous melanomas,[13] and *RB1* may also be subject to genomic rearrangement in rare instances.[14]

The p53 Pathway

The p53 pathway is critical for maintenance of the normal genome by regulating a multiplicity of mechanisms, including cell cycle checkpoints, DNA damage repair activation, and the appropriate induction of apoptosis. Mutations in the *TP53* gene occur in >50% of all tumors. While initial studies suggested that the *TP53* locus is rarely mutated in human melanomas, whole exome studies have identified mutations in ~20% of tumors, generally in tumors without mutations or deletions affecting *CDKN2A* and $P14^{ARF}$.[11] Amplification of *MDM2*, which inhibits P53 function, has also been detected in melanomas with intact *CDKN2A*.[64] Functionally, loss of p53 cooperates with activated BRAF in zebrafish, and with activated HRAS in mice, to induce melanomas.[28,70] Thus, while *TP53* is rarely deleted in human melanomas, inactivation of its pathway appears critical for melanomagenesis.

The Phosphatidylinositol 3-Kinase Pathway

The PI3K-AKT pathway is affected by activating oncogenic events more frequently than any other pathway in cancer.[71] PI3K phosphorylates lipids in the cell membrane, causing the recruitment of proteins that have a pleckstrin homology domain. One of the key proteins regulated by PI3K is the serine-threonine kinase AKT. AKT is phosphorylated at two key residues (Ser473 and Thr308) at the cell membrane, activating its catalytic activity. Activated AKT phosphorylates multiple effector proteins, including GSK3, P70S6K, PRAS40, BAD, and more, which regulate cellular processes including proliferation, survival, motility, angiogenesis, and metabolism (see Fig. 93.3). The pathway can be activated genetically in cancer both by activating mutations (i.e., in *PIK3CA*, *AKT1*) and loss of function events (i.e., *PTEN*, *TSC2*) in components of the pathway. The PI3K-AKT pathway is also a critical effector pathway of RAS proteins and many growth factors and their receptors, which are also frequently aberrant in cancer.

As described previously, activation of the RAS-RAF-MAPK pathway is a nearly ubiquitous event in cutaneous melanoma. Overall, genetic events in the PI3K-AKT pathway are less common.[11] However, multiple lines of evidence support that PI3K-AKT signaling functionally complements RAS-RAF-MAPK activation in at least a subset of melanomas. Moreover, recent data indicates that PI3K pathway mutations may arise in the setting of resistance to RAF inhibition.[56,72]

Phosphatase and Tensin Homolog

Of the PI3K pathway mutations that do occur, losses of chromosome 10q encompassing PTEN tumor suppressor is the most frequent, the caveat being that additional tumor suppressor(s) may reside in this region (see the following). PTEN normally downregulates phosphorylated AKT via suppression of the second messenger PIP_3 (see Fig. 93.3). Loss of PTEN has been shown to result in increased AKT activity in multiple cancer types, including melanoma. In melanoma, somatic point mutations and homozygous deletions of PTEN are relatively rare. However, although allelic loss of PTEN is observed only in about 20% of melanoma, loss of expression of PTEN is reported to be in the range of 30% to 40% of melanoma tumors.[27,73] In multiple studies, loss of PTEN has shown to occur in melanomas with activating BRAF mutations and in melanomas with wild-type BRAF and NRAS, but it is extremely rare in tumors with NRAS mutations.[74]

As mentioned previously, in a mouse model the presence of simultaneous PTEN loss and oncogenic BRAF induction in melanocytes resulted in 100% penetrance of invasive, metastatic melanomas.[31] Notably, loss of PTEN alone did not cause a melanocytic phenotype. While this complementation of BRAF and PTEN supports the overlap of these alterations that is observed clinically, preclinical models have also demonstrated that loss of PTEN can promote melanoma motility, invasion, and metastasis in the setting of NRAS mutations.[75] However, the significance of this finding is unclear due to the very low prevalence of this occurring naturally. The functional significance of PTEN loss in BRAF/NRAS wild-type melanomas also remains to be elucidated. Functionally, ectopic expression of PTEN in PTEN-deficient melanoma cells can abolish phospho-AKT activity, induce apoptosis, and suppress growth, tumorigenicity, and metastasis.[76] Many different inhibitors of the PI3K-AKT pathway have been developed and are undergoing clinical testing. Previous data suggest that the presence of different molecular mechanisms of PI3K-AKT pathway activation may correlate with activation and functional dependence on different pathway effectors.[77] Preclinical studies support that AKT and PI3K are attractive targets for melanomas with loss of PTEN.[78]

AKT

Loss of PTEN in melanoma tumors and cell lines correlates with markedly increased expression of phospho-AKT (indicative of activation).[73] In addition to loss of PTEN, AKT can be activated by point mutations that affect the pleckstrin homology domain of the proteins. Such mutations of *AKT1* have been detected in multiple tumor types.[79] Analysis of melanoma tumors and cell lines identified the same mutation as a rare event in melanoma (~1% prevalence), but also discovered the analagous mutation in *AKT3*, which has not been reported in other cancers.[80] Each tumor with a mutation in AKT1/3 had a concurrent BRAF(V600) mutation. Copy number gain of the *AKT3* locus has also been detected in melanomas, and functional studies support that metastatic melanomas specifically demonstrate phosphorylation of and functional dependence upon that AKT isoform.[76,81]

Phosphatidylinositol Kinase

Hotspot mutations in PIK3CA, which encodes the predominant catalytic subunit of PI3K, are common in multiple cancer types, including breast, colon, and lung tumors.[82] Mutations in PIK3CA are very rare (<2%) in melanomas, and often affect residues of unknown functional significance.[83] Despite this clinical observation, preclinical studies have shown that the presence of simultaneous activating PI3K and BRAF mutations in melanocytes can induce melanomas in mouse models.[84]

Receptor Tyrosine Kinases

Receptor tyrosine kinsases (RTK) are a diverse family of transmembrane kinases that have been implicated in many neoplasms. Several RTKs map to known regions of recurrent melanoma DNA copy number gain or amplification, with corresponding alterations in their expression levels.

The RTK c-KIT and its ligand (stem cell factor) were both initially shown to play essential roles in melanocyte development. Mutation of either KIT or RTK results in pigmentation deficiencies, and injection of c-Kit blocking antibody in mice was used to identify the presence of melanocyte stem cells within hair follicles.[85] However, numerous immunohistochemical studies linked progres-

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sive loss of c-KIT expression with the transition from benign to primary and metastatic melanomas.[86] Thus, at first glance, KIT appeared to be inactivated during melanoma genesis and progression. However, more recently activating mutations and amplification of the *KIT* gene have been identified in cutaneous melanomas with evidence of CSD or that arise on acral surfaces (palms, soles, nail beds).[87] The point mutations in the KIT gene generally occur in the same regions affected in gastrointestinal stromal tumors, where the functional significance of these mutations has been proven by the clinical efficacy of KIT inhibitors for that disease.[86] Functional studies support that KIT mutations can activate multiple signaling pathways, particularly the PI3K-AKT pathway.[88] Inhibition of KIT in melanoma cell lines with recurrent point mutations results in growth inhibition and/or apoptosis.[89] In patients, initial clinical trials of the KIT inhibitor imatinib in populations of patients with KIT mutations and/or amplifications have reported clinical response rates of 10% to 30%.[90,91] This is a much higher response rate than was observed in three previous clinical trials of imatinib in patients with unselected melanoma (~1% response rate), but is much lower than the activity observed in patients with gastrointestinal stromal tumor (>70%).[86] Thus, although many of the clinical responses in patients with KIT mutations have been dramatic and durable, research is ongoing to further understand the significance of KIT mutations, and to develop more effective clinical strategies for patients with melanoma patients with KIT mutations.

Overexpression of the RTK c-MET and its ligand, HGF, is correlated with melanoma progression. Copy gains involving the c-MET locus at 7q33-qter are associated with invasive and metastatic cancers in humans,[92] and elevated MET/HGF expression is correlated with metastatic ability in murine melanoma explants.[47] HGF/SF overexpression in a transgenic mouse model triggered spontaneous melanoma formation after a long latency (up to 2 years); however, time to tumor onset was greatly reduced by exposure to UVB or Ink4a/Arf deficiency.[93] More recently, independent groups of investigators demonstrated that c-MET may be activated by HGF produced by supporting cells in the tumor microenvironment of melanomas.[53,94] This paracrine effect resulted in activation of the PI3K-AKT pathway in the tumor cells and caused resistance to MAPK pathway inhibitors. The clinical utility of c-MET inhibition in melanoma is being tested in ongoing clinical trials.

A sequencing-based study of the tyrosine kinome in melanoma found that *ERBB4* mutations may affect as many as 20% of melanomas.[95] Unlike other well-known oncogene mutations, the *ERBB4* mutations identified in this study were mostly nonrecurrent (e.g., the same amino acid or conserved region was rarely affected). However, *ERBB4* mutations produced increased activation of the receptor itself and PI3K-AKT pathway signaling, as well as dependence upon the corresponding protein for viability. Despite these promising initial findings, the clinical significance of ERBB4 inhibitors in melanoma remains unclear.

MELANIN SYNTHESIS PATHWAY

MITF

MITF encodes a lineage transcription factor whose function is critical to the survival of normal melanocytes. The identification of *MITF* amplification in melanoma defined this transcription factor as a central modifier of melanoma.[96] In so doing, this discovery identified a novel class of oncogenes termed *lineage survival* oncogenes.[97] That is, a tumor may "hijack" extant lineage survival mechanisms in the presence of selective pressures to ensure its own propagation. The elucidation of *MITF* as an oncogene took a cross-tissue approach, wherein the NCI-60 cell line panel representing nine tumor types was subjected to both gene expression and high-density single-nucleotide polymorphism array analysis.[96] A recurrent gain of 3p13–14 significantly segregated melanoma from other tumor classes, with MITF as the only gene in the region showing maximal amplification and overexpression. MITF amplification was subsequently detected in 10% of primary cutaneous and 15% to 20% of metastatic melanomas by fluorescence in situ hybridization, correlating with decreased survival in Kaplan-Meier analyses of 5-year patient survival. Exogenous MITF showed transforming capabilities in immortalized primary human melanocytes in combination with activated BRAF. Additionally, inhibition of MITF in cell lines showing 3p13–14 amplification reduced growth and survival and conferred sensitivity to certain anticancer drugs. MITF gene disruption leads to coat color defects in mice and pigmentation defects in humans, due to diminished viability of melanocytes. This suggested that MITF was essential for the lineage survival of melanocytes, supporting the contention that it is also critical for the survival of melanomas. More recently, a germline variant (E318K) of MITF has been identified that confers an increased risk of developing melanoma.[98,99]

The downstream elements of the MITF pathway include both pigment enzyme genes as well as genes involved in proliferation, survival, and metabolism.[100,101] MITF intersects with a number of established melanoma pathways, including the transcriptional activation of INK4A, c-Met, and CDK2.[102] Moreover, MITF is regulated by both MAPK signaling and c-KIT.[100,103] Recently, the ETS transcription factor ETV1 was found to positively regulate MITF expression in melanoma, and *ETV1* may function as an amplified melanoma oncogene in its own right.[104] Collectively, these observations place MITF in a central role of melanoma signal integration.

The MC1R Pathway

Pigmentation exerts a major influence on skin tumor susceptibility, as it is well documented that fair skin is more sensitive to UV radiation and melanoma genesis. The mechanism underlying this observation is partially explained by the protective effects of melanin, which is produced by melanocytes and distributed to interfollicular keratinocytes. Genetically, the red hair color/pale skin (RHC) phenotype is linked to variant alleles of the melanocyte-specific melanocortin 1 receptor gene (MC1R), which is central to melanin synthesis.[105] The ligand for the G-protein–coupled MC1R is the MSH peptide, which activates downstream signaling consisting of a cAMP-CREB/ATF1 cascade, culminating in the induced expression of MITF. Not all individuals carrying RHC alleles have identical melanin production, yet increased risk for melanoma genesis remains notable regardless,[106] implying that melanin-independent mechanisms might impact the susceptibility of RHC carriers. One possible node is cAMP, the MC1R as second messenger, which may activate pathways incompletely understood at present, such as MAPK and PI3K.[107]

Experiments have implicated the MSH/MC1R pathway in the normal UV pigmentation (tanning) response in skin, a response that is linked to skin cancer (and melanoma) risk in humans. A "redhead" mouse model (frameshift mutation in *MC1R*) was used to demonstrate that the UV tanning response is dependent on MC1R signaling, because keratinocytes respond to UV by strongly upregulating expression of MSH. The "fairskin" phenotype was rescued by topical administration of a small molecule cAMP agonist.[108] The resulting dark pigmentation in genetically redhead mice was protective against UV-induced skin carcinogenesis. Subsequent analyses revealed that the p53 tumor suppressor protein may function as a "UV sensor" in keratinocytes, translating UV damage into direct transcriptional stimulation of MSH expression.[109]

RAC1

RAC1 is a member of the Rho family of small GTPases that are regulators of cytoskeletal reorganization and cell motility. Re-

cently, two whole exome sequencing studies of >100 melanomas each identified hotspot mutations in *RAC1* that result in a P29S substitution.[11,12] Overall, the mutation was detected in 5% to 10% of samples, making it the third most common gene, after *BRAF* and *NRAS*, to be affected by hotspot mutations in the coding region. Initial characterization of the mutation confirmed that it conferred increased activity to the RAC1 protein, and promoted cell proliferation and motility in vitro. The overall clinical significance of the RAC1 P29S mutation awaits studies determining its clinical associations and functional evaluation of its inhibition.

Telomerase

Telomere stabilization through telomerase dysregulation has long been recognized as a hallmark of carcinogenesis in many cancers. However, the molecular basis for altered telomerase regulation in cancer has remained obscure. Analysis of whole genome sequencing data from a collection of melanoma tumors led to the unexpected discovery of two highly recurrent mutations affecting the promoter of *TERT*, which encodes a key catalytic component of the telomerase enzyme complex.[110,111] Both mutations generate consensus ETS transcription factor binding motifs in the setting of an identical 11-nucleotide stretch, suggesting a gain-of-function effect. Since this index observation, multiple studies have confirmed the high frequency of *TERT* promoter mutations in melanoma and other tumor types, and that the presence of these mutations is associated with enhanced *TERT* expression. Thus, melanoma genetic studies produced the first example of a highly recurrent functional mutation that falls within the regulatory region of a gene.

MOLECULAR GENETICS OF MELANOMA: LOOKING AHEAD

The genetic and molecular understanding of melanoma, and its impact on therapy, is currently in the midst of a transformative era. The last decade witnessed an almost exponential increase in our understanding of the pathogenesis of this disease. Massively parallel sequencing technology has made it possible to obtain the complete sequence of entire cancer genomes or "exomes" (protein coding region of the genome) at ever diminishing costs. Together with developments in computational biology, these advances are rapidly bringing forth new understanding of cancer genome alterations and the tumorigenic mechanisms that result. One of the first cancer genomes to be sequenced was that of a cell line (and its paired normal counterpart) derived from a patient with metastatic melanoma.[112] This effort uncovered more than 33,000 somatic base substitutions, of which 187 were nonsynonymous coding mutations. As expected, most base mutations were C→T transitions indicative of UV exposure.

This initial discovery provided a glimpse into one of the central challenges of melanoma research: to identify which somatic changes are meaningful. The high UV-associated base mutation rate in melanoma suggests that nearly 2% of all genes may harbor nonsynonymous coding mutations in a typical cutaneous melanoma, most of which are likely to be "passenger" events with little biologic consequence to melanoma genesis or progression. Thus, cataloging all significant genomic alterations that might represent "driver" events will require not only sequencing hundreds of tumor specimens but also the principled application of increasingly sophisticated analytical methods for data deconvolution. Initial insights toward this end emerged from a whole-exome sequencing study of melanoma, which utilized a computational algorithm designed to model the effects of evolutionary selection on the cancer genome.[11] This approach facilitated the discovery of several new melanoma genes that might otherwise have been obscured by the high UV-associated mutation rates pervasive in melanoma genomes. Concomitantly, large-scale US (Cancer Genome Atlas) and international efforts (International Cancer Genomic Consortium) have taken on the ambitious goal of comprehensively characterizing the genomes of diverse human tumors including melanomas.[113] Thus, it seems certain that the next decade will witness additional major breakthroughs in melanoma genome characterization that inform the biology and treatment of this malignancy.

While sequencing of large numbers of melanomas will help to identify which mutations are statistically significant, additional approaches are needed to distinguish the complete spectrum of functional "drivers" of melanoma genesis, progression, and maintenance. Such genetic events may confer transforming activity, dictate prognosis, or correlate with responsiveness (or resistance) to emerging targeted therapeutics. While the initial exome sequencing efforts in melanoma have been very informative, they have not included parallel characterization of other molecular characteristics, such as DNA methylation, mRNA and miRNA expression, protein expression and activation, and clinical characteristics and outcomes. The availability of all of these types of data will allow for integrated analysis approaches that will help to elucidate key molecular events and pathways. In addition, hypotheses about critical drivers of melanoma biology will benefit from functional testing. Increasingly, such testing is being performed, at least initially, through the use of high-throughput screening methods using RNAi libraries, collections of small molecules, and/or expression libraries of open reading frames of genes of interest. These approaches allow not only for evaluation of individual genes/targets, but also for more global approaches for data analysis to identify key networks and pathway. Such approaches can help to tailor investigations to focus on individual genes which may be most important functionally or most exploitable clinically.

One challenge in characterizing the molecular biology of melanoma is the recognition that it can be impacted by increasingly effective therapeutics. As noted earlier, multiple studies have been undertaken to characterize the molecular basis of resistance. To date, all studies that have been reported have identified the continued presence of the same mutation in the BRAF gene at the time of disease progression that was present before treatment was started.[114] At the same time, a variety of new genomic features have been identified in the progressing lesions that confirm certain longstanding assumptions while challenging others. As noted earlier, NRAS mutations arise alongside mutated BRAF in the progressing lesions of ~25% of patients.[52] This stands in marked contrast to the mutual exclusivity of these mutations in treatment-naïve tumors, and highlights how such studies must not be constrained by assumptions based on previous observations about the molecular biology of this disease. On the other hand, while some mutations in MEK1/2 cause marked resistance to both BRAF and MEK inhibitors, in other cases the presence of MEK1 mutations does not preclude clinical response to RAF inhibition.[55,115] Thus, not all MEK1 mutations are equivalent, similar to prior findings involving distinct BRAF mutations.[24] The characterization of progressing lesions has also confirmed the molecular heterogeneity of melanoma. Such heterogeneity has been demonstrated by the finding of independent resistance mechanisms within different progressing tumors of individual patients, and within different regions of individual tumors.[72,115,116]

In addition to the aforementioned advances in the field of targeted therapy, melanoma treatment is being revolutionized by improved understanding and targeting of the antitumor immune response. Ipilimumab, an antibody that blocks the inhibitory CTLA4 molecule on the surface of T-cells, was the first therapy to ever demonstrate a survival benefit in a randomized clinical trial in patients with metastatic melanoma, and it gained regulatory approval in 2011.[117] Agents that target other inhibitory molecules, including PD-1 and PD-L1, have recently demonstrated very promising clinical safety and activity in early phase clinical trials.[118,119] While the resistance studies described previously focused on the molecular biology of resistance to MAPK pathway inhibitors, there is also growing evidence that somatic mutations

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may be critical to the effectiveness of immunotherapy.[120] In addition to creating new antigens that may be recognized by the immune systems, oncogenic signaling pathways in tumors can also influence the antitumor immune response.[121,122]

In the future, integration of melanoma molecular biology with a growing knowledge of the tumor microenvironment and the immune system will likely be needed to propel additional discoveries. While this presently stands as a daunting task, the remarkable progress of the last decade provides a proof-of-concept that advancing our understanding of the molecular basis of this disease will have a tremendous positive impact on the quality of life and survival of patients with this highly aggressive disease.

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94 Cutaneous Melanoma

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INTRODUCTION

Melanoma arises from the malignant transformation of the melanocyte, the cell responsible for the production of the pigment melanin. Precursor melanocytes arise in the neural crest and, as the fetus develops, migrate to multiple areas in the body including the skin, meninges, mucous membranes, upper esophagus, and eyes. Melanomas can arise from any of these locations through the malignant transformation of the resident melanocytes. By far the most common location is the hair follicle–bearing skin arising from melanocytes at the dermal/epidermal junction. In the National Cancer Database, 91.2% of melanomas are cutaneous, 5.3% are ocular, 1.3% are mucosal, and 2.2% are of unknown primary site.[1] Each of these types has significant differences in the etiology and genetic makeup, in particular related to the degree of ultraviolet (UV) radiation exposure and their frequency of driver oncogenic mutations. The current understanding of melanoma biology comes from studies of genetic analyses of melanomas correlated with clinicopathologic presentations, which have elucidated two key features of this cancer: (1) cutaneous melanoma, as opposed to mucosal or uveal melanoma, is usually a carcinogen-induced cancer with a high mutational load, demonstrated by the molecular fingerprinting of UV light damage, and (2) the majority of melanomas are dependent on a particular oncogenic signaling pathway, the mitogen-activated protein kinase (MAPK) pathway, through usually mutually exclusive driver mutations in cKit, NRAS, BRAF, GNAQ or GNA11.[2–6] Cutaneous melanomas arising from the trunk and extremities, which are associated with intermittent UV radiation exposure, have high rates of BRAF (50%) or NRAS (20%) mutations.[2,7,8] Mucosal and acrolentigenous melanomas, with low rates of UV radiation exposure, have lower rates of BRAF mutations (5% to 20%) and higher rates of KIT mutations (5% to 10%).[2] The great majority of uveal melanomas have mutually exclusive mutations in the alpha subunits of G-protein–coupled receptors GNAQ and GNA11.[3,4]

The primary focus of this chapter is on cutaneous melanoma, but summary information is presented for the other forms of melanoma, as well as on the subtypes of cutaneous melanoma.

MOLECULAR BIOLOGY OF MELANOMA

Mutational Landscape in Melanoma

Studies of whole exome sequencing (sequencing of the approximately 1.6% of the genome that encodes for expressed proteins) and whole genome sequencing of melanomas compared to matched normal DNA of the same patients are leading to a greatly improved understanding of the genomic alterations in melanoma. The studies of the mutational load of cutaneous melanomas by next generation sequencing demonstrate that melanoma has significantly more sequence variations per megabase of DNA compared to most other cancers. For example, melanomas have 15 times more mutations per megabase of DNA than colorectal cancer and 4 times more than lung cancer.[9] As a high proportion of these mutations are cytosine to thymine (C>T) substitutions, typical of UV radiation–induced thymine dimmers, it is highly likely that the high rate of sequence variants in melanoma is due to the role of UV as the principal carcinogen in the disease.[10–12]

Two studies have reported on exome sequencing of relative large series of melanomas compared to normal matched DNA. After sequencing the exomes of 147 melanomas, it became evident that sun-exposed melanomas had markedly more UV-like C>T somatic mutations compared to sun-shielded acral, mucosal, and uveal melanomas. These studies confirmed the recurrent mutations in BRAF, NRAS, and cKit. Newly identified recurrently mutated cancer genes included PPP6C, encoding a serine/threonine phosphatase in 12% of sun-exposed melanomas, and were mutually exclusively in tumors with mutations in BRAF or NRAS. Furthermore, an activating mutation in RAC1(P29S) was noted in 9.2% of sun-exposed melanomas.[11] In a similar study, analysis of large-scale melanoma exomes from 121 paired samples[12] also confirmed the recurrent BRAF, NRAS, and cKIT mutations, and discovered six recurrently mutated melanoma genes (PPP6C, RAC1, SNX31, TACC1, STK19, and ARID2), two of which are the same as in the other study. Integration with chromosomal copy number data contextualized the landscape of driver mutations, providing oncogenic insights in BRAF- and NRAS-driven melanoma as well as those without known NRAS/BRAF mutations. In this study, the authors found a higher than expected number of genetic events dysregulating the RB and p53 pathways, which had previously thought to be mostly intact in melanoma.[12]

The first fully sequenced whole genome of any cancer was a melanoma cell line compared to a lymphoblastoid cell line generated from the same patient to provide the comparing source of normal DNA.[10] This study provided the first comprehensive catalogue of somatic mutations from an individual cancer. The dominant mutational signature reflects DNA damage due to UV light exposure. It also revealed an uneven distribution of mutations across the genome, with a lower prevalence in gene footprints, which indicated that DNA repair preferentially functioned in areas with transcribed regions. There are ongoing efforts to perform whole exome sequencing in large panels of melanomas. The largest effort is The Cancer Genome Atlas. It will report on whole exome sequencing in over 500 melanoma samples compared to normal exomes, with a subset of cases additionally undergoing whole genome sequencing, DNA methylation studies, RNA sequencing, microRNA sequencing, and reverse phase protein array analysis of phosphorylated proteins.

The studies of whole genome sequencing will be important to understand melanoma genetic alterations in nontranscribed genes since there can also be recurrent mutations in them. This is exemplified by the demonstration of two very common mutations in the promoter of telomerase reverse transcriptase (TERT) by two independent research groups. TERT is the gene coding for the catalytic subunit of telomerase. In one of the studies,[13] mutations in the TERT promoter were reported in 71% of melanomas examined. The mutations increased the transcriptional activity from the TERT promoter by two- to four-fold. This information may be of high relevance beyond melanoma, since examination of 150 cancer cell lines derived from diverse tumor types revealed the same mutations

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in 24 cases (16%). The other group reached the same conclusion by investigated a melanoma-prone family through linkage analysis and high-throughput sequencing.[14] They identified the same TERT promoter mutations as disease-segregating germline mutations. When they screened TERT promoter mutations in sporadic melanoma, they found them in 74% of human cell lines derived from metastatic melanomas and in 33% of primary melanomas.

Driver Mutations in Melanoma

Melanoma has become a notable example of a cancer histology dependent upon driver oncogenic mutations in the MAPK pathway, with additional genetic alterations in other pathways leading to uncontrolled cell growth and avoidance of apoptosis (Fig. 94.1). There is evidence of MAPK activation by defined point mutations in at least 70% of melanomas, resulting in constitutive signaling leading to oncogenic cell proliferation and escape from apoptosis.[15] The aberrations that lead to MAPK pathway activation in most cutaneous and mucosal melanomas consist in usually mutually exclusive activating mutations in the receptor tyrosine kinase KIT (2% to 3%), the G-protein neuroblastoma RAS viral oncogene homolog (NRAS) (15% to 20%), and the serine-threonine kinase BRAF (40% to 50%).[3–6] Uveal melanomas have a distinct set of driver mutations in GNAQ and GNA11, which are the alpha subunits of G-protein–coupled receptors.[3,4]

Mutations in KIT are found in exons 9, 11, 13, and 17, and there is no one predominant point mutation.[6] Because of this, molecular testing for KIT mutations must evaluate multiple regions of the gene by extended sequencing or multiplexed polymerase chain reaction (PCR) tests. Emerging evidence suggests that not all KIT mutations activate its function, resulting in some mutations being insensitive to KIT inhibitors used for patient treatment.[16] NRAS mutations cluster in the RAS hotspot mutation site Q61, usually Q61L and less frequently Q61R and Q61H. NRAS mutations are more frequent in older individuals and are equally common in melanomas arising from the skin with chronic or intermittent sun damage.[2,17] The vast majority of BRAF mutations in melanoma involve a substitution for valine at the 600th amino acid position to glutamine (V600E).[5,18] The frequency of the BRAF V600E mutation is inversely correlated with age since it is most frequent in melanomas that appear on the skin without chronic sun damage in young adults. BRAF V600K is the second most common variant, which increases in incidence with age. V600D or V600R mutations are much less frequent.[8] Overall, up to 90 different point mutations have been described in BRAF in different cancers, some of which are activating and others are inhibiting its enzymatic activity.[18]

Aberrations in the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin pathway, including the phosphatase and tensin homolog (PTEN), are also noted in a significant number of melanomas, but these do not seem to function as true drivers of the malignant phenotype. PTEN alterations include missense mutations, deletions, and insertions, as well as loss of heterozygosity and epigenetic silencing, making interrogation for mutations and genomic rearrangements in PTEN necessary.[19,20] The pathogenesis of melanoma, like most

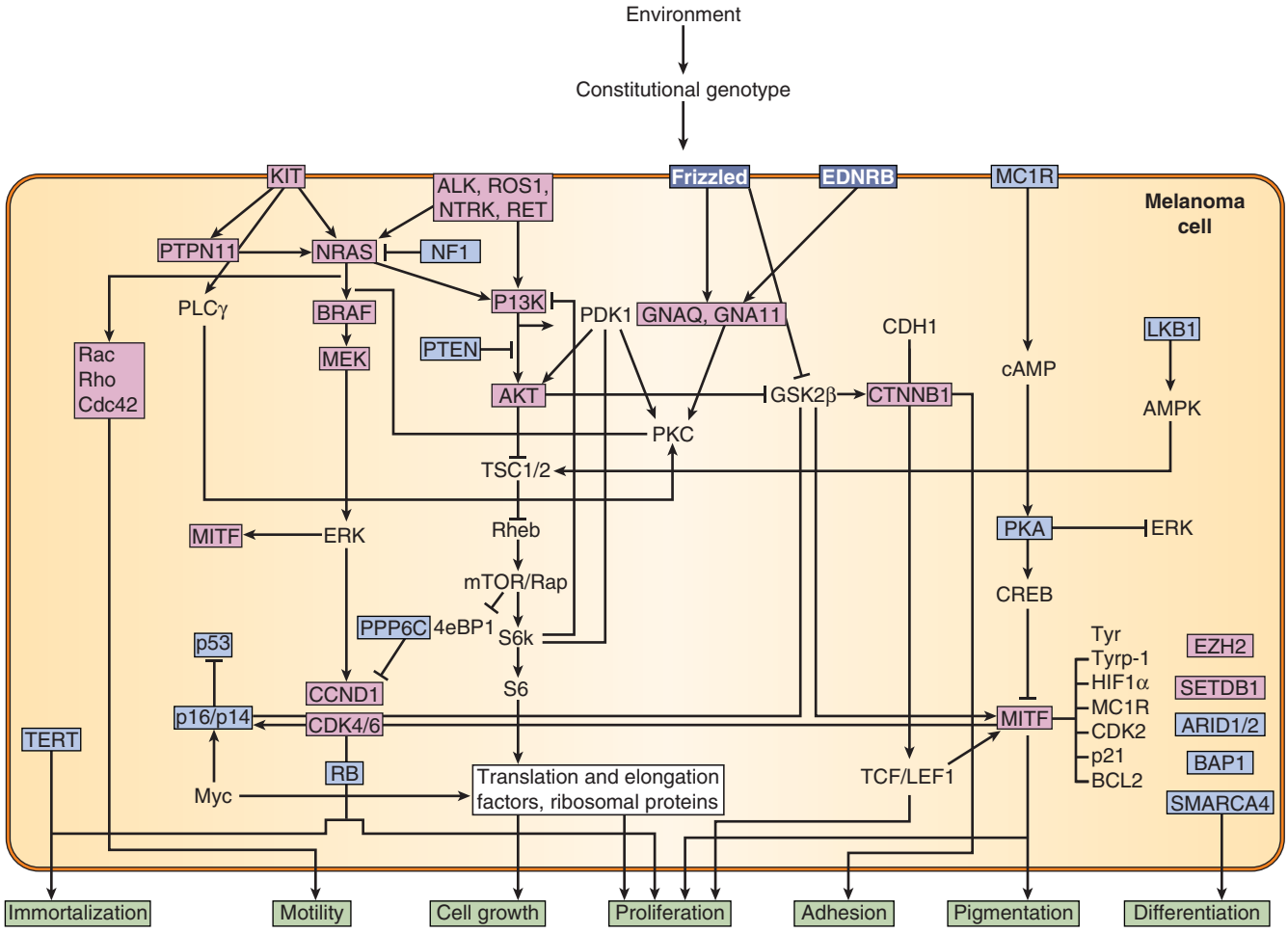

Figure 94.1 Signaling pathways disrupted by genetic alterations and their relationship to the hallmarks of melanoma. Proteins boxed in *red* are affected by gain-of-function mutations; those boxed in *blue* are affected by loss-of-function mutations. (Source: Bastian BC. The molecular pathology of melanoma: an integrated taxonomy of melanocytic neoplasia. *Ann Rev Pathol Mech Dis* 2014;9:239–271.)

other cancers, requires the presence of a driver oncogene and the dysregulation of cell cycle control and apoptosis to provide the full oncogenic signaling and ability to grow autonomously. These happen with the frequent mutations or genetic deletions of CDKN2A, cyclin D1, or the amplification cyclin-dependent kinase 4.[21]

Progression of Melanocytes to Cutaneous Melanoma

Genetic Events in Melanocyte to Melanoma Progression and Oncogene-Induced Senescence

BRAF and NRAS are founding mutations of cutaneous melanoma that are frequently present in benign nevi.[22] Despite of the presence BRAF and NRAS mutations, nevi have an exceedingly low proliferative activity and infrequently progress to melanoma. This is explained because of the phenomenon of oncogene-induced senescence preventing malignant progression to melanoma, where these mutations require functioning with additional genetic events that lead to dysregulation of cell cycle control to result in the development of a progressive melanoma.[15,23] The model for oncogene-induced senescence in melanoma is based on the identity of the main driver mutations (BRAF and RAS) in nevi, the initial phase of proliferative activity they spark, the formation of a benign nevus in association with the induction of senescence markers (cell cycle arrest, induction of the tumor suppressor $p16^{INK4a}$, endoplastic reticulum stress markers and increased SA-βGal activity, and possibly additional senescence biomarkers), and the subsequent cessation of expansion, which is typically maintained for decades.[24]

Cellular Changes in Melanocyte to Melanoma Progression

The transition from melanocyte to metastatic melanoma involves several histologic intermediates, including melanocytic atypia, atypical melanocytic hyperplasia, radial growth phase melanoma, vertical growth phase melanoma, and metastatic melanoma. Atypical melanocytes arising in a preexisting nevus or de novo are very common but rarely progress to melanoma. However, some patients develop confluent atypical melanocytic hyperplasia at the dermal/epidermal junction or nests of atypical melanocytes in the epidermis or at the dermal/epidermal junction. As this process progresses, it reaches a point at which a diagnosis of melanoma is warranted.

Early cutaneous melanomas usually proceed to grow radially, and this is called the *radial growth phase* (RGP) of melanoma, which may continue for years before progressing to the vertical growth phase (VGP) (Figs. 94.2 and 94.3). The RGP of a cutaneous melanoma may include either melanoma in situ or superficial

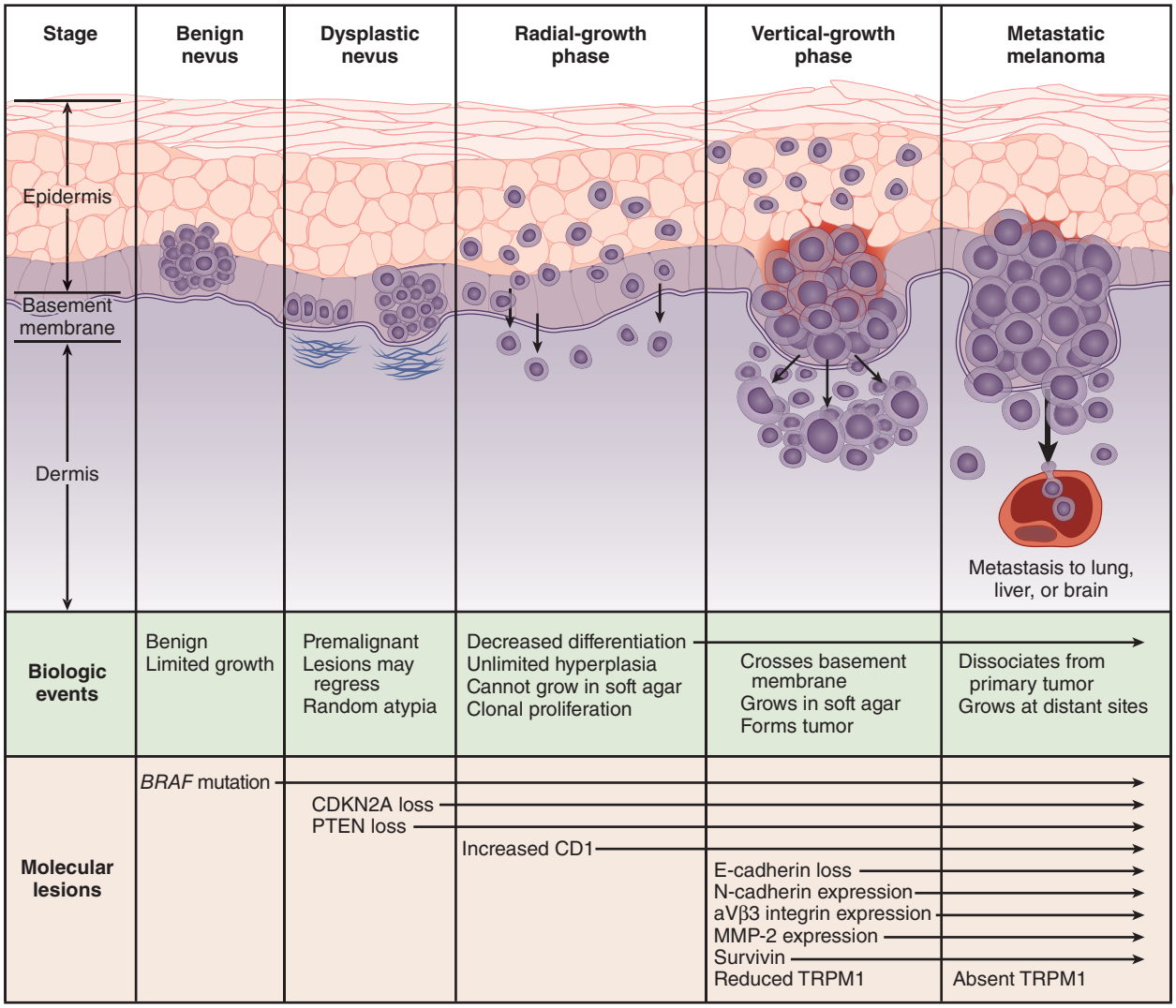

Figure 94.2 Biologic events and molecular changes in the progression of melanoma. (From Miller AJ, Mihm MC Jr. Melanoma. *N Engl J Med* 2006;355:51–65.)

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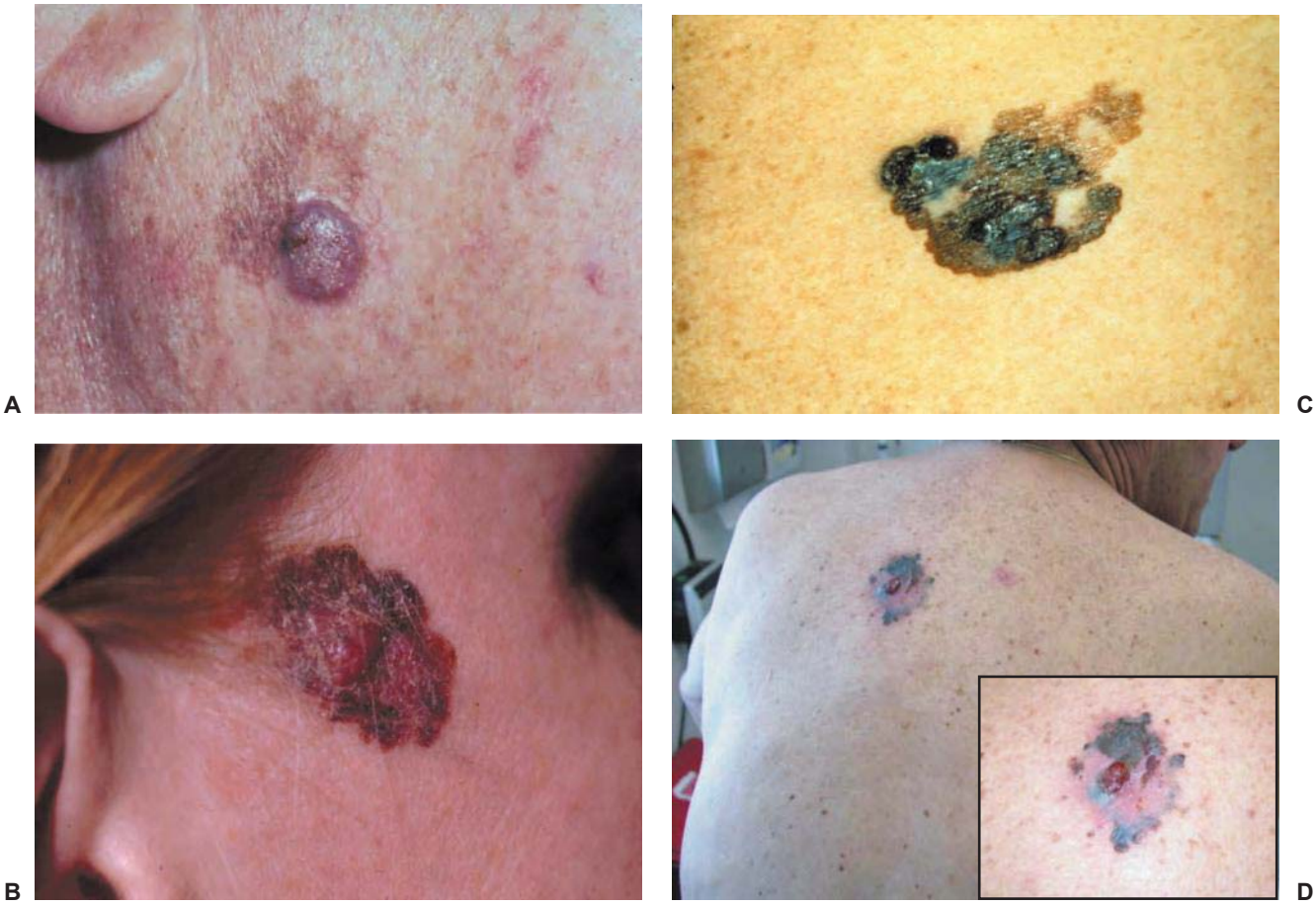

Figure 94.3 **(A)** A nodule of vertical growth phase melanoma arising from a radial growth phase pigmented macule on the right cheek. **(B)** Superficial spreading melanoma, 2.9 mm thick, arising on the temple of a young woman. There were microscopic satellites, and the patient died of disease within several years. **(C)** Superficial spreading melanoma with all the classic features of the ABCD mnemonic (*a*symmetry, *b*order irregularity, *c*olor variation, and *d*iameter >6 mm). **(D)** Large, ulcerated 2.5 mm superficial spreading melanoma with regression in elderly man.

invasion into the papillary dermis, or both. Melanomas in RGP present clinically as enlarging macules or very minimally raised papular lesions, which are typically (but not always) pigmented. These lesions are rarely symptomatic. If not recognized, these lesions typically progress to the VGP, manifest clinically by a nodular growth of the lesion, often described by the patient as a lesion that began to "raise up." This vertical growth usually arises as a nodule within the RGP component and encompassing only part of the RGP (see Fig. 94.3A,C). Thus, the VGP appears to represent further steps in the process of malignant transformation due to clonal changes in the cells of the RGP.

Some melanomas present as metastatic melanoma in lymph nodes, skin, subcutaneous tissue, or visceral sites without an apparent primary cutaneous site. In some cases, these have been associated with a history of a regressed primary melanocytic lesion. In other cases, such an explanation is less clear. In all of these cases, the prospect of early diagnosis of melanoma is compromised, and the risk of melanoma-associated mortality is increased.

EPIDEMIOLOGY

Malignant melanoma is the sixth most common US cancer diagnosis. The actual incidence of melanoma is increasing more rapidly than that of any other malignancy. It was estimated that 76,690 men and women (45,060 men and 31,630 women) will be diagnosed with and 9,480 men and women will die of melanoma of the skin in 2013.[25] This amounts to 4% of new cancer diagnoses and 1.5% of cancer deaths. In the early part of the 20th century, the lifetime risk of a white person developing melanoma was approximately 1 in 1,500. Currently, 1 in 49 men and women will be diagnosed with melanoma of the skin during their lifetime. Its incidence is second only to breast cancer for women from birth to age 39 years; similarly, it is the second most common cancer diagnosis for men through age 39 years, slightly less common than leukemia.[26] Overall 5-year survival rates for melanoma have increased from 82% in the late 1970s (1975 to 1977) to 91% in the more recent era (2002 to 2006).[26]

This is a disease that disproportionately affects whites over African Americans, Asians, or Hispanics. In the United States, whites account for 98.2% of cutaneous melanomas reported in the National Cancer Database, with African Americans accounting for 0.7% and Hispanics accounting for 1.1%.[1] This is best explained by a combined effect of UV sunlight exposure and fair skin. It is most striking that the highest per capita incidence of melanoma worldwide is in Australia, and that this high incidence afflicts primarily the Australians of Western European descent who have fair skin, and not the darker-skinned aboriginal population. It is also notable that these fair-skinned European descendants who moved to Australia have much higher incidences of melanoma than the Western European populations that remain in the higher latitudes of Europe. In migrant populations, individuals who move during childhood to areas with greater sun exposure develop melanoma at rates higher than those of their country of origin and similar to those of their adopted country.[27]

In nonwhite populations, there is a much higher proportion of melanomas in acral (subungual, palmar, plantar) and mucosal locations. However, the incidences of those types of melanoma are similar across races. Their higher relative proportion in Asians and African Americans can be best explained by the disproportionate

increase in nonacral cutaneous melanomas in fair-skinned whites rather than by an absolute increase in risk of acral and mucosal melanomas in nonwhite populations.

Ocular and nonacral cutaneous melanomas are 50- to 200-fold more likely in white populations than in nonwhite populations, but melanomas in acral and mucosal sites are within twofold of each other across racial groups. Similarly, the increased incidence of melanoma over the last few decades can be explained primarily by increased incidence in white populations, not in nonwhite populations.[28] These observations support the hypothesis that most cutaneous melanomas in white populations are etiologically related to sun exposure but that there may be a baseline risk of melanoma in other locations that is unrelated to sun damage. There are significant molecular differences between acral melanomas and melanomas arising on the skin associated with chronic sun damage, with *B-RAF* and *N-RAS* mutations in approximately 80% of melanomas on chronically sun-damaged skin, whereas those mutations were uncommon in melanomas from acral or mucosal sites or from skin without chronic sun damage.[2]

CHANGES IN INCIDENCE

Data from the Surveillance, Epidemiology, and End Results program reveal an increase in age-adjusted melanoma incidence rates from 8.2 per 100,000 in the 1970s (1974 to 1978) to 18.7 per 100,000 in more recent years (1999 to 2003).[29] From 1990 to 2003, during which there was a 16% decrease in male cancer deaths overall for all cancers, there was a 2% increase in mortality rate from melanoma. From 1991 to 2003, during which there was an 8% decrease in cancer deaths overall for women, there was only a 4% decrease in mortality rate associated with melanoma.[26]

In Australia, and to a lesser extent in the United States, there has been a substantial increase in awareness about melanoma and the value of screening by total-body skin examinations. There also has been a greater proportion of patients diagnosed at earlier and noninvasive stages of disease. Thus, part of the increase in incidence may be explained by increased early diagnosis of lesions with low metastatic potential. However, there has also been a significant increase in mortality from melanoma over the last few decades.[29]

GENDER AND AGE DISTRIBUTION

In the United States and Australia, the gender ratio of melanoma at diagnosis is 2 male to 1 female, but it depends on the age group. Analysis of incidence data for invasive melanoma diagnosed from 1992 to 2006 from 12 cancer registries that participate in the Surveillance, Epidemiology, and End Results program of the National Cancer Institute revealed that, by age, the men-to-women rate ratio ranged from 1.3 (95% confidence interval [CI], 1.2 to 1.3) for ages 40 to 64 years for incidence to 2.6 (2.5 to 2.7) for older than 65 years for both incidence and mortality. However, between the age of 15 and 39 years old, melanoma is more common in females (rate ratio = 0.6).[25] The median age of melanoma patients has increased from 51 years in the 1970s (1974 to 1978) to 57 years in a more recent time period (1999 to 2003). Nonetheless, the median age for diagnosis of melanoma is approximately 10 years lower than the current median age of diagnosis for the more common solid tumors, such as colon, lung, or prostate cancer. The large majority (approximately 80%) of patients with melanoma are diagnosed in the productive years from age 25 to 65 as shown for a representative population from the state of Virginia (Fig. 94.4). Melanoma is common in patients in their 20s and older, but it also is observed in teenagers, and occasionally even in infants and neonates. For women aged 25 to 35 years, melanoma is the leading cause of cancer-related death.

MELANOMA IN CHILDREN, INFANTS, AND NEONATES

Diagnosis and management of melanoma in children, infants, and neonates is complicated by several factors: (1) excisional biopsy of skin lesions often is not feasible under local anesthesia in young children, and (2) pigmented skin lesions with substantial cellular atypia but with structural symmetry may be Spitz nevi, which typically have benign behavior. Thus, some young patients with changing pigmented skin lesions are observed longer than would be advisable because biopsy is more problematic than in most adults. In addition, young patients may undergo incomplete shave biopsy to avoid a full-thickness excision, and information is lost about the architecture of the lesion, leaving a diagnostic dilemma between melanoma and Spitz nevus. Even in the best of circumstances, some melanocytic tumors are difficult to diagnose with certainty. This has led to a formal definition of melanocytic tumors of uncertain malignant potential.[30]

Melanoma deaths in children and young adults have a large effect on total years of life lost because of melanoma. Current recommendations for management of melanoma in children and infants are the same as for adults, and outcomes are generally believed to be comparable.[31]

ANATOMIC DISTRIBUTION

Cutaneous melanoma can occur at any skin site in the body. The most common sites in males are on the back and in the head and neck regions. In women, the most common sites are in the lower

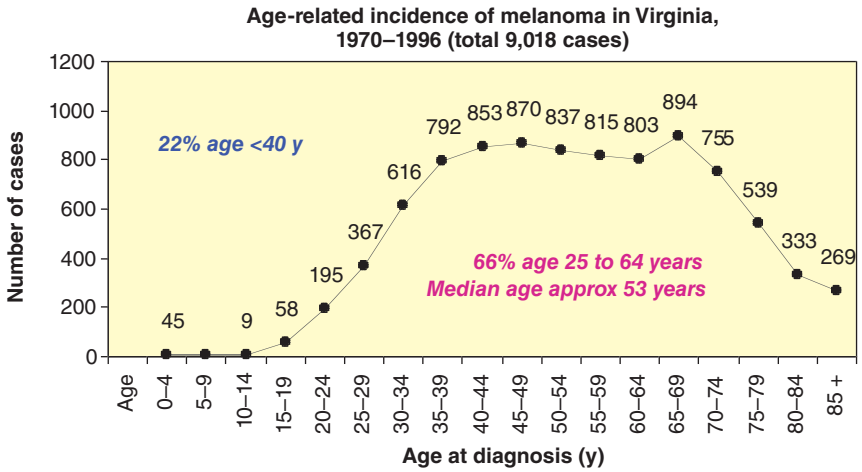

Figure 94.4 Age-related incidence of melanoma in Virginia, 1970 to 1996 (total of 9,018 cases).

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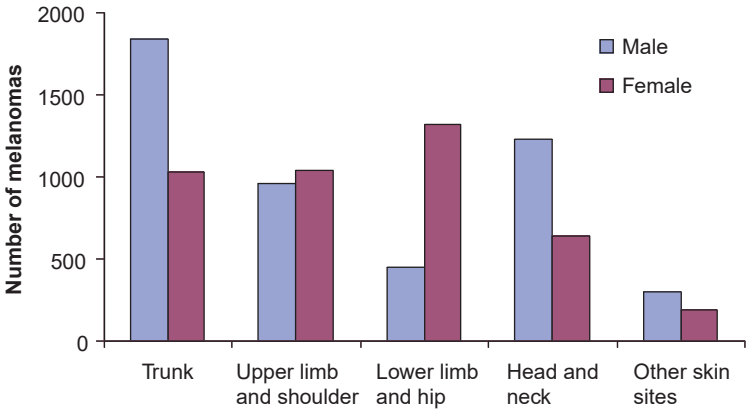

Trunk M:F = 1.8; lower limb M:F = 1/3; headand neck M:F = 1.9

Figure 94.5 Incidence of melanoma in Virginia, 1970 to 1996, by gender.

extremities, commonly below the knee (Fig. 94.5). Lentigo maligna melanoma (LMM) most commonly arises on sun-damaged surfaces of the head and neck in older patients. Acral lentiginous melanoma (ALM) is most common on subungual and other acral locations.

ETIOLOGY AND RISK FACTORS

Ultraviolet Light Exposure

The demographic features of cutaneous melanoma have implicated UV light exposure as a major etiologic factor in the development of melanoma. Multiple studies continue to support an etiologic association between UV irradiation and melanoma.[32] UVC radiation is generally absorbed by the ozone layer. UVB radiation (290 to 320 nm) is associated with sunburn and induction of tanning by melanin pigment production. There are substantial data to support its etiologic role in melanoma.[32] There is also some evidence implicating UVA radiation (320 to 400 nm), although UVA is more associated with chronic sun damage changes.[33] However, the relative role of each type of UV irradiation in melanoma etiology is debated. Animal data suggests that sun exposure early in life increases the risk of melanoma. Human skin grafted on mice will develop nevi and melanomas in the presence of UVB irradiation, further supporting the role of UVB irradiation in melanoma.[34] Similar to the animal modeling, sunburns early in life have been implicated in melanoma incidence.[35] However, chronic sun exposure in individuals who tan well may even be protected against melanoma. The role of sunlight intensity and frequency is debated, but both chronic and intermittent exposure may be relevant.[32] Current data suggest that UV radiation causes melanoma by a combination of DNA damage, inflammation, and immune suppression.[36]

Tanning bed use has been implicated in the etiology of melanoma, in particular tanning bed use in adolescence or early adulthood.[37] Tanning bed use has been formally classified as a carcinogen, and increased awareness of the harmful effects of UV exposure promise to control the increase in melanoma incidence.

Physical Traits

Several physical traits have been linked to increased incidence of cutaneous melanoma. These include blond or red hair, green or blue eyes, presence of multiple (>100) melanocytic nevi, and five or more atypical nevi. A prior diagnosis of melanoma is associated with an eight-fold increased risk of developing a secondary melanoma.

Familial Predisposition

It has been estimated that 5% of melanomas occur in high-risk families with an autosomal dominant inheritance with incomplete penetrance.[38] The most frequent and highest penetrance melanoma susceptibility gene is a germline mutation in CDKN2A, a tumor suppressor gene that encodes for two different proteins, p16INK4A and p14 ARF.[39] These proteins control cell cycle progression and apoptosis, and have roles in correcting DNA damage and cellular senescence. CDKN2A mutations have been reported in approximately 25% of melanoma-prone families, but this frequency varies highly on the selection criteria used and the region of the world where it is studied. The rare autosomal dominant inherited familial atypical multiple mole melanoma-pancreatic cancer syndrome is associated with CDKN2A mutations, and less frequently to BRCA2 mutations.[40] Another germline mutation linked to familial melanoma is cyclin-dependent kinase 4, which is linked to the function and p16INK4A and controls the retinoblastoma pathway. A germline mutation in microphthalmia-associated transcription factor (*MITF* E318K) represents a medium-penetrance susceptibility gene predisposing to familial melanoma, as well as to sporadic melanoma and renal cell carcinoma.[41,42] The E318K mutation in MITF disrupts sumoylation and enhances transcription of MITF-responsive genes. Other common risk factors include dysplastic nevus syndrome, xeroderma pigmentosum, and a family history of melanoma even without the known genetic traits. The association of melanoma with Li-Fraumeni syndrome, with germline mutations in p53, is currently unclear.[43]

Pregnancy and Estrogen Use

Older literature suggested anecdotally that the incidence of melanoma was higher in pregnant females and that they had a particularly bad outcome. However, multiple systematic and larger studies have shown no evidence of any negative (or positive) impact of prior, concurrent, or subsequent pregnancy on clinical outcome.[44,45] Similarly, there is no clear prognostic relevance for birth control pills or estrogen replacement therapy.[46] The prior sense of an apparent association of pregnancy and melanoma may be due to melanoma being the second most frequent cancer in females of childbearing age. The general recommendation for treatment of women with melanoma diagnosed during pregnancy is to manage them in the same fashion as patients who are not pregnant. Depending on the time during pregnancy at which a melanoma is diagnosed, there can be circumstances in which radiologic imaging may be limited because of concern for the fetus, and major surgery may be delayed until the fetus is at an age when it can

survive independently. However, the excision of a primary melanoma certainly can be done in almost any circumstance, under local anesthesia.

The other related question often asked by patients is whether it is advisable to become pregnant and to bear a child after treatment for melanoma. As just stated, there is no evidence that a subsequent pregnancy adversely impacts outcome. However, the more interesting and challenging question is the more personal or social issue of the potential for premature parental death due to melanoma. Thus, it is helpful for patients to understand their risk of future recurrence and melanoma-related mortality because that translates into the risk that the child will grow up losing a parent. Measures of the risk of future disease progression can be defined based on the initial prognosis and the subsequent elapsed time without recurrence, and such information may help to guide patients with this challenging question.[47]

PREVENTION AND SCREENING

Melanomas diagnosed and treated during the RGP have an excellent prognosis. Thus, prevention and early diagnosis can have a great impact on decreasing melanoma morbidity and mortality. The apparent leveling off of melanoma-related mortality rates in Australia and the United States likely is the result of better screening and prevention.

Sun Protection

UV exposure and sunburns, in particular, appear to be etiologic in most melanomas. Thus, protection from UV light, especially in fair-skinned individuals, is believed to have substantial benefit in preventing melanoma.

A clinical trial has provided evidence that regular sunscreen use helps prevent melanoma.[48] This was a randomized trial from March 1992 to August 1996 of 1,621 randomly selected adult residents of a Queensland township in Australia with an initial primary end point testing the prevention of squamous cell and basal cell carcinomas, which the study did demonstrate.[49] Prevention of the development of melanoma was a prespecified secondary end point. Participants were randomly assigned to either a planned sunscreen intervention group or a control group using sunscreen at their discretion. The intervention group received broad-spectrum, sun protection factor (SPF) 16 sunscreen every morning, and was instructed to reapply the sunscreen after a long sun exposure, heavy sweating, or after bathing. After a 10-year follow-up, regular sunscreen use decreased by half the rate of developing new melanomas. This conclusion was based on 11 participants in the intervention group and 22 in the control group being newly diagnosed with either invasive or in situ melanoma ($p = 0.051$). The incidence of invasive melanoma decreased by 73% in the intervention group compared with the control group (3 versus 11 patients, respectively; $p = 0.045$). Therefore, this study provides evidence that use of sunscreen can decrease the incidence of melanoma development.

There are limitations inherent in sunscreen use as the primary means to protecting from UV light damage. One is that certain body sites are not easily covered with sunscreen, such as the scalp. More important, even "waterproof" sunscreens wash off or become less effective with time. Most people also forget to reapply sunscreens frequently enough and may still get burns. There are also sociologic issues, which may differ for different populations and are arguable. However, it is worth considering the provocative findings of a study performed on young adults from Western Europe, who were randomized to receive either SPF10 or SPF30 sunscreen. In a blinded fashion, they were asked to report sun exposure times and sunburns. The number of sunburns was the same in both groups, and sun exposure was greater in the SPF30 group, suggesting that some populations may stay in the sun until they get a burn, and that sunscreen simply helps them to stay in the sun longer.[50] The sun-seeking behavior has been related to an evolutionary need that favored UV exposure to make vitamin D in the skin in populations that migrated to areas of the world with lower sun exposure. In mouse models, the exposure to UV light was linked to increased production of beta-endorphins and recurrent seeking of UV exposure.[51]

It is safe to say that the best protection from the sun is a building, the next best is protective clothing, and the third best is sunscreen. Patients should be advised to use all three. Avoiding midday sun from about 11 a.m. to 3 p.m. by staying indoors is advised, as well as wearing clothing with a thick enough weave that it blocks sunlight, or a formal SPF rating, when possible. Hats are particularly helpful for the face and scalp, which often are highly exposed to sunlight and not so readily covered fully with sunscreen. Otherwise, sunscreen can provide protection to sun-exposed areas when outside.

Screening for Early Diagnosis

Self-Examination

For many patients, they, their spouses, or other family members may be able to screen effectively for new suspicious skin lesions, and this should be encouraged. It is more common for women to detect melanomas than for men to do so, either for themselves or for their partners. In any case, there is value in educating patients about how to detect melanomas if they are at high risk. As many as half of melanomas are identified by the patient or family,[52] and patient self-examination has been associated with diagnosis of thinner melanomas.[53] Teaching aids for patients on how to perform skin self-examination are available from the American Cancer Society and the American Academy of Dermatology. Patients with melanoma or at high risk should be seen regularly by a dermatologist. It is reasonable to suggest that patients perform skin self-examinations more often than their dermatology visits, although there are no proven guidelines. Doing a self-examination once a month may be the easiest for the patient to remember.

The role of skin cancer screening to decrease incidence and mortality from cutaneous melanoma has been prospectively studied in the Schleswig-Holstein project.[54] This was an observational study comparing trends in melanoma mortality in a population-based skin cancer screening project conducted in the northern German region of Schleswig-Holstein, compared to neighboring regions in Germany and Denmark where no such screening was conducted. From July 1, 2003, to June 30, 2004, 360,288 individuals aged 20 years were screened by whole-body examination. They reported that mortality in Schleswig-Holstein melanoma declined by 48% when analyzed using log-linear regression to assess mortality trends. No such change in melanoma mortality rates was noted in the studied adjacent regions. This study provides strong evidence that skin cancer screening programs may reduce melanoma mortality.[54]

Management of the Patient with Numerous Atypical Moles

Some patients have numerous atypical moles. This presentation is commonly described as atypical mole syndrome, dysplastic nevus syndrome, or B-K mole syndrome.[55] These patients have a heightened risk of melanoma, and this is commonly a familial feature. When associated with a family history of melanoma, patients with dysplastic nevus syndrome have a risk of melanoma that may approach 100%. These patients deserve particular attention to melanoma prevention through sun protection and to early diagnosis through aggressive screening. However, the optimal approach for screening is not defined. At a minimum, routine skin examinations by a dermatologist are usually recommended, as often as every 3 months. Visual inspection of the atypical nevi

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may be augmented by routine digital photography to facilitate detection of subtle changes in radial growth or other changes over time. Although these approaches commonly permit identification of melanomas when they are in situ or thin, it is not known whether they improve survival. In addition, concern remains that visual inspection alone, even for very experienced dermatologists, is inadequate to diagnose all melanomas when they are still curable. Thus, substantial effort is in progress to develop more sensitive and specific diagnostic tools than visual inspection alone. One that is employed routinely in many practices is dermoscopy, also known as epiluminescent microscopy. This involves use of a handheld microscope at the bedside to examine skin lesions in an oil immersion setting. This appears to improve diagnostic accuracy in experienced hands, and increasing experience has made its use more feasible in general practice, especially with considerations for standardization.[56,57] When coupled with the use of a digital camera, the images can be stored and compared over time as well. Computer-assisted digital analysis of these images is also being studied but remains investigational.

Evaluation and management of patients with dysplastic nevus syndrome is complicated by the fact that very few dysplastic nevi will develop into melanoma. Estimates range from a risk of 1 per 1,000 nevi examined in a pigmented lesion clinic being melanoma to 1 per 10,000 nevi becoming melanoma per year.[56,57] Recommendations for management of dysplastic nevi include those from the Melanoma Working Group in the Netherlands and by a National Institutes of Health Consensus Conference.[58]

It is tempting to consider excision of all dysplastic nevi. Although that remains an option, there is no proof that this will decrease risk. Melanomas may arise de novo in 30% to 70% of cases, and so it is not clear that removal of all suspicious nevi will lead to a meaningful improvement in survival. However, it is certainly appropriate to biopsy any nevus that is suspicious, especially one that is changing.

Testing for Genomic Changes in Melanoma

Understanding the genetic makeup of melanoma has become the cornerstone of advances in the management of advanced disease, and it is likely to have an increasing role in the management of earlier stage melanoma. Genetic analyses can be focused on driver oncogenic events or can provide a broader understanding of the genomic aberrations in the cancer. Their study is becoming a standard of care practice in melanoma.

Commercially available tests identifying the BRAF V600E/K mutation have been approved by the U.S. Food and Drug Administration (FDA) and other regulatory bodies as companion diagnostics for the use of novel BRAF and MEK inhibitors. These assays are frequently based on specific PCR probes labeled with fluorescent tags that bind to wild-type and V600E or V600K mutant BRAF sequences. These assays are performed in sections of formalin-fixed paraffin embedded tissue blocks routinely used for pathologic analyses. As with all techniques used to detect somatic mutations, they are limited by the amount of mutant sequence in the initial sample as well as DNA integrity in the sample, and their ability to detect non-V600E BRAF mutations since the primers used are usually restricted to this particular BRAF mutation. Multiplexed single nucleotide extension assays (i.e., Sequenom [San Diego, CA] or SNaPshot [Vanderbilt-Ingram Cancer Center, Nashville, TN]) evaluate a list of specific base mutations of interest, but do not identify mutations outside the interrogated bases. These techniques are designed for simultaneous interrogation of different point mutations. They are particularly suited for the targeted interrogation of known oncogenes that contain mutation hotspots, such as NRAS, BRAF, and GNAQ/GNA11, being relevant for melanoma.[59]

Traditional Sanger sequencing has been the gold standard for the detection of point mutations, but it has been shown to have lower sensitivity than the PCR-based assays, thereby leading to a higher frequency of false negative results. Pyrosequencing provides information from the sequencing 300 and 500 nucleotides at a time, resulting useful for the analysis of mutations clustered in a small gene region. It is a highly sensitive technique, being able to detect mutant DNA when only 5% of the total sample is from the cancer tissue.

Copy number analyses have been very useful in the discovery and description of genes and pathways involved in melanoma pathogenesis. Initially, probe sequences were derived from bacterial artificial chromosomes using array comparative genomic hybridization. More recently, single nucleotide polymorphism–based arrays have been introduced. But currently the approach of choice for the analysis of the DNA alterations in melanoma at the genome level is massively parallel sequencing techniques. These techniques enable the sequencing of exomes and entire genomes of tumor samples (compared to normal DNA from the same patient), with the simultaneous sequencing of a large number of genes and determination of mutations, genetic alterations, and copy number changes. The price and complexity of this type of analysis has rapidly improved, making it feasible to use beyond research studies. Limited panels performing next generation sequencing in what have been called "actionable" genes have been implemented for clinical use. These provide information based on sequencing data of 200 or so genes for which the available literature suggests that they could provide information which may be interpretable to decide on treatment options, in particular in terms of clinical trial participation with new targeted agents.[60]

A clinically applicable approach to genetic testing of melanomas is first performing a targeted testing for the mutation status of BRAF, NRAS, and KIT in cutaneous and mucosal melanoma samples before pursuing alternative mutation interrogation with higher throughput approaches. Next-generation sequencing may be applicable in situations where known mutations are not identified, and the identification of additional genetic mutations is needed.

DIAGNOSIS OF PRIMARY MELANOMA

Characteristics of Primary Melanoma

The classic appearance of primary cutaneous melanoma is summarized by the mnemonic ABCD for *a*symmetry, *b*order irregularity, *c*olor variation, and *d*iameter >6 mm (see Fig. 94.3). Because melanomas arise from melanocytes, which contain the melanin-synthetic pathway, melanomas classically are distinguished by their pigmentation. Melanomas may have shades of brown, black, blue, red, and white. However, there is a wide range in the appearance of melanomas. Some melanomas are pitch black. Others are shades of brown. Some have no visible pigment and appear skin-colored. Still others have a red color only. When melanomas have all of the classic ABCD features, they are typically easy to diagnose. However, those melanomas that lack some of these features can be difficult to diagnose. In addition, in patients with large numbers of atypical nevi, which may also have ABCD features, this mnemonic is often inadequate to aid in early diagnosis. The other important findings that may aid in early diagnosis are a change in a lesion over time or new development of a skin lesion. These warrant evaluation, and in high-risk patients there should be a low threshold for biopsy. In addition, some dermatologists recommend considering the "ugly duckling" sign: A lesion that stands out as different from the patient's other nevi should be evaluated and possibly biopsied.[61] This can be particularly helpful in a patient with a large number of clinically atypical nevi. Both of these approaches may help to identify amelanotic (nonpigmented) melanomas, which often do not meet the ABCD criteria. Some melanomas are not diagnosed until they become symptomatic, and whereas awareness of the symptoms of bleeding, itching, pain, and ulceration are worth noting, these usually connote deep vertical growth and are hallmarks of a late diagnosis, not an early one.

Biopsy

Biopsy of a suspicious skin lesion is necessary for an accurate diagnosis and for optimal staging. The correct way to perform such a biopsy is to make a full-thickness biopsy of the entire lesion, with a narrow (1 to 2 mm) margin of grossly normal skin. The depth of excision should include the full thickness of dermis and thus should extend into the subcutaneous tissue, but it does not need to include all of the subcutaneous tissue except in very thin patients or patients with very thick polypoid lesions that may go deep into the subcutis. This allows assessment of the architecture of the lesion, which is critical for differentiation of melanoma from Spitz nevus, and it permits an accurate measure of tumor thickness, which is critical for prognosis and affects the surgical treatment recommendations. Of importance, desmoplastic melanoma often arises from LMM and is difficult to diagnose both clinically and histologically. Shave biopsies of these lesions can often lead to failure to appreciate the desmoplastic melanoma in the dermis and may substantially delay diagnosis.

For some large lesions (e.g., >2 cm diameter) in cosmetically sensitive locations (e.g., face or genitalia), there may be a rationale for an incisional biopsy, but that also should be performed as a full-thickness skin biopsy. Ideally, it should include the most suspicious area of the lesion and also should include, if possible, a portion of the edge of the lesion where it transitions to normal skin to enable assessment of the junctional change. The incisional biopsy may be an elliptical incision or it may be a full-thickness 4- to 6-mm punch biopsy. Punch biopsies are problematic if too small, if they do not include full-thickness skin, if they are crushed during removal, if they are oriented inaccurately in the paraffin block, or if they are too small to include both the edge of the lesion and the most suspicious or most raised part of the lesion.

Orientation of the incision used for an excisional biopsy should be considered in the context of the prospect for the future need for a wider re-excision. On extremities, the incision and scar should be oriented longitudinally rather than transversely, although some exceptions may be considered near joints to avoid crossing a joint. When in doubt about the optimal orientation, it is very reasonable to perform the excisional biopsy as a simple circular excision, leaving the wound open for secondary or delayed primary closure.

Biopsy of subungual lesions is more challenging. The pigmentary changes seen in patients with subungual melanoma usually extend along the length of the nail, but the lesions usually arise at the proximal end of the nail bed. Access to that location often requires removal of all or a large part of the nail. One or more punch biopsies of the base of the nail bed often constitute the most realistic method for obtaining a biopsy of such lesions, and it may need to be repeated to be diagnostic. A punch biopsy tool can remove a circle of the nail, providing access to the nail bed for punch biopsy of the suspicious area.

Melanoma Subtypes: Histologic Growth Patterns

Classically, four main histologic growth patterns are described for melanomas, but two others are also worth mentioning.

Superficial Spreading Melanoma

The most common type is superficial spreading melanoma, which accounts for about 70% of primary cutaneous melanomas (see Fig. 94.3C). It is typical for the trunk and extremities, except on acral sites. It is associated with pagetoid growth of atypical melanocytes in the epidermis. Superficial spreading melanoma is commonly associated with sun exposure.

Nodular Melanoma

Nodular melanomas lack an RGP, may be nonpigmented, and commonly are diagnosed when relatively thick. Thus, these carry the worst prognosis of the various subtypes of melanoma. They account for about 20% of cutaneous melanomas. By definition, nodular melanomas are in VGP when recognized.

Acral Lentiginous Melanoma

ALMs account for <5% of melanomas.[62] They are typically found on acral sites (subungual, palmar, plantar) and on mucosal surfaces (anorectal, nasopharyngeal, female genital tract). ALM occurs across all races and ethnicities. Its etiology is likely independent of UV light exposure. Because other cutaneous melanomas are uncommon in African, Asian, and Hispanic populations, ALMs on acral sites are proportionately more common in these populations than in fair-skinned whites. ALM is typically associated with a prolonged RGP before vertical growth; however, its locations make it harder to diagnose than other forms of melanoma. Subungual lesions can be detected by linear pigment streaks arising from the base of the nail, but these are not always evident. They can be confused with subungual hematomas, which can lead to diagnostic delay. When there is a question of whether a pigmented subungual lesion may be melanoma or a hematoma, the location of the pigment can be marked and then followed over a short interval (e.g., 3 weeks), during which time a hematoma should move toward the end of the nail, but a melanoma should not.

Subungual melanomas can also present with breakage of the nail or a nonpigmented thickening or drainage, and these are often confused with chronic fungal infections. Any concerning pigmented subungual lesion should be biopsied, but it is sometimes challenging and requires splitting or removing part of the nail. A punch biopsy near the nail bed matrix is often appropriate. In addition, when there is spontaneous chronic inflammation or breakage of the nail, biopsy for melanoma should be considered, even in the absence of pigmentation.

Lentigo Maligna Melanoma

LMMs typically occur in older individuals, in chronically sun-damaged skin, and commonly on the face. They tend to have shades of brown or black, whereas the red and blue colors seen in other melanomas are not typical of LMM. They may also develop areas of regression manifested by depigmentation of part of the lesion. Overall, LMMs account for about 10% to 20% of melanomas in the National Cancer Database experience,[1] 47% of melanomas of the head and neck, and only 2% of melanomas of other regions.[62] LMMs usually have an extensive RGP that extends for many years before developing invasion. When melanoma is just in situ, this RGP portion is called *lentigo maligna* or Hutchinson freckle, as opposed to LMM. These are not to be confused with the benign pigmented macule, lentigo. Lentigo malignas evolve a VGP to become invasive LMMs at a rate estimated to be between 5% and 33%.[63] LMMs are commonly diagnosed as thin lesions. However, more substantial vertical growth can occur, as seen in Figure 94.3A.

Lentiginous Melanoma

Early RGP melanomas sometimes are difficult to classify into the typical patterns of lentigo maligna, superficial spreading melanoma, or ALM. A report defined a distinct entity of lentiginous melanoma. Its features include diameter ≥1 cm, elongated and irregular rete ridges, confluent melanocytic nests and single cells over a broad area of the dermal/epidermal junction, focal pagetoid spread, cytologic atypia, and possible focal dermal fibrosis.[64] Over time, this may represent a growing proportion of melanomas that have traditionally been grouped as superficial spreading melanoma, lentigo maligna, ALM, or unclassified melanomas.

Desmoplastic Melanoma

Desmoplastic melanoma is an uncommon form of melanoma, histologically manifest by dermal melanocytes in a dense stromal

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response. These lesions are usually nonpigmented and usually have lost the melanin production pathway. They usually stain negative for MART-1/MelanA, gp100, and tyrosinase, but they do stain for S100. The lack of pigmentation and the dense stromal response often interfere with clinical and histologic diagnosis. It occurs most commonly in the head and neck, but it may occur in other body sites.[65] Desmoplastic melanoma may appear de novo as a nonpigmented skin papule or as a dermal/VGP component arising from a preexisting lentigo maligna or other pigmented junctional lesion. Desmoplastic melanomas may have neurotropic features and have been associated with a high rate of local recurrence.[66] However, recent reports suggest that if adequate margins are taken, the risk of local recurrence is low.

The overall mortality risk for desmoplastic melanomas is comparable to that of other invasive melanomas of similar depth of invasion.[67] Multiple studies support the contention that desmoplastic melanomas have a significantly lower risk of nodal metastases than other melanomas,[68–72] with only 1.4% sentinel node positivity among 155 patients with pure desmoplastic melanoma, compared with 18.5% in those with mixed desmoplastic melanoma.[67,68,71,72] There has been a debate about whether to abandon histologic staging of regional nodes in patients with desmoplastic melanoma.[69] It may be appropriate to consider a higher threshold for performing sentinel node biopsy (SNBx) in patients with pure desmoplastic melanoma, but there is no consensus on this question.

Prognostic Factors for Primary Melanomas

The best predictor of metastatic risk is the depth of invasion, measured with an ocular micrometer, from the granular layer of the skin to the base of the primary lesion. This was originally described by Breslow[73] and remains an important factor in staging and prognostic stratification. However, many other histologic and clinical features have relevance for estimating the risk of future metastasis and mortality. These include age, angiolymphatic invasion, mitotic rate, gender, and body site.

Depth of Invasion

Breslow thickness is the depth of invasion measured from the granular layer of the epidermis to the base of the lesion. Melanoma cells involving adnexal structures are considered junctional and are not included in the Breslow depth. The current melanoma staging system of the American Joint Committee on Cancer (AJCC) identifies tumor (T) stage based on Breslow thickness such that T1 lesions are <1 mm thick, T2 lesions are 1 to 2 mm thick, T3 lesions are 2 to 4 mm thick, and T4 lesions are >4 mm thick.[74]

Clark et al.[75] defined depth based on the layer of skin to which the melanoma has invaded. Clark level I melanomas are melanomas in situ, limited to the epidermis or dermal/epidermal junction. Clark level II melanomas invade into the superficial (papillary) dermis, and these are usually RGP lesions. Clark level III melanomas fill the papillary dermis. Clark level IV melanomas invade into the deep (reticular) dermis and have significant metastatic risk. Clark level V melanomas are uncommon and contain invasion into the subcutaneous fat.

It has become apparent that Clark level does not add much additional prognostic value to Breslow thickness and has been removed from the 2010 version of the AJCC staging system.[74] Breslow thickness has an effect on survival, local, regional, and systemic recurrence rates, and that association is continuous, without any apparent breakpoints. Although the staging system requires categorization of thickness ranges, the continuous nature of the risk association should be kept in mind. Thickness is considered in defining the margins of excision for primary melanomas.[76,77]

Ulceration

Ulceration of the primary lesion has been identified as an important negative prognostic feature[76] and is incorporated in the current staging system such that T1a, T2a, T3a, and T4a melanomas are nonulcerated, and T1b, T2b, T3b, and T4b melanomas are ulcerated. In an analysis of prognostic features in a large multicenter database, the prognosis of an ulcerated lesion was comparable to that of a nonulcerated lesion one T level higher. Thus, the overall stage assignment groups ulcerated lesions with nonulcerated lesions one T level higher (e.g., T2b and T3a are both stage IIA). The staging system is summarized in Tables 94.1 and 94.2 and is described in detail elsewhere.[74]

Patient Gender and Skin Location of Primary Melanoma

The incidence of melanoma is higher for men than women overall, but in adolescents and young adults it is more common in women.[25] Furthermore, for essentially all patient subgroups, the prognosis is better for women than men. Thus, among patients with stage III and IV melanoma, men outnumber women approximately 1.5:1. Women are more likely to have melanomas on the extremities, whereas men are more likely to have melanomas on the trunk and head and neck. The clinical outcome for patients with melanomas on extremities is better than that for patients with truncal or head-and-neck melanomas; thus, the prognostic impact of gender is difficult to distinguish from the impact of tumor location. There may still be, however, a prognostic benefit for female gender independent of tumor location.[76,78] In addition, location of tumors has prognostic relevance in that head-and-neck melanomas have poorer prognosis than trunk or extremity melanomas, and melanomas on acral sites have poorer prognosis than other extremity melanomas.[78,79] A particular location associated with poor prognosis is the mucosal melanoma. Anorectal, female genital, and head-and-neck melanomas of mucosal origin have a mortality risk of 68% to 89% over 5 years.[1,79,80]

Patient Age

The impact of age on prognosis is confusing. There is a greater risk of lymph node metastasis in young patients at the time of SNBx,[81] especially for patients younger than age 35 years, but the melanoma-associated mortality risk increases with age for all thickness ranges.[1,76] This paradox has not been explained. It suggests a possible age-specific curative potential for patients with micrometastatic nodal disease. Alternatively, it is worth considering that the attribution of mortality to melanoma progression is not always straightforward. Older patients have other competing causes for death that could lead to earlier mortality in the presence of metastatic disease. Nonetheless, age does appear to have independent prognostic significance for patients with melanoma.

Growth Pattern

Overall, nodular melanomas have the worst prognosis, associated with their diagnosis at a thicker stage. Lesser risk is associated with ALM, superficial spreading melanoma, and LMM, in that order, all associated with decreasing average Breslow thickness. Generally, the histologic growth pattern of melanoma has little prognostic relevance when Breslow thickness is taken into account. The VGP component appears to be the component of melanoma that determines metastatic risk, and these VGP components are similar, independent of the growth phase in the RGP component. LMMs are a possible exception, in that they appear to have a better prognosis than other histologic types, independent of thickness. Desmoplastic melanoma, superficial spreading melanoma, LMM, and ALM have comparable prognosis, for distant metastases and survival, when stratified by thickness.[68,81]

Mitotic Rate

It is reasonable to expect that the growth rate of melanomas is linked to the rate of tumor cell division. Accordingly, mitotic rate in the dermal component has been identified as a negative

TABLE 94.1

Melanoma Tumor, Node, Metastasis Classification

T Classification	Thickness (mm)	Ulceration Status	Mitotic Rate
TX	Unknown	—	—
T0	No evidence of primary tumor	—	—
Tis	Melanoma in situ	—	—
T1a	≤1.0	No	<1/mm^2
T1b	≤1.0	Yes	≥1/mm^2
T2a	1.01–2.0	No	Any
T2b	1.01–2.0	Yes	Any
T3a	2.01–4.0	No	Any
T3b	2.01–4.0	Yes	Any
T4a	>4.0	No	Any
T4b	>4.0	Yes	Any
N Classification	**No. Nodes with Metastasis**	**Presentation**	**In Transit or Satellite Metastasis(es)**
NX	Not assessable	—	—
N0	0	—	No
N1a	1	Clinically undetectable[a]	No
N1b	1	Clinically detectable[b]	No
N2a	2–3	Clinically undetectable[a]	No
N2b	2–3	Clinically detectable[b]	No
N2c	0	—	Yes
N3	≥4 or matted		—
N3	≥1	Any	Yes
M Classification	**Metastatic Site**	**Serum LDH Level**	
M0	None detected	—	
M1a	Distant skin, subcutaneous or node	Normal	
M1b	Lung	Normal	
M1c	All other visceral	Normal	
M1c	Any	Elevated	

LDH, lactate dehydrogenase.
[a] Clinically undetectable nodes are those diagnosed only with sentinel node biopsy or elective lymphadenectomy, and lacking gross extracapsular extension. They are referred to also as micrometastases, but this definition differs from the pathologist's definition of a micrometastasis as one that is <2 mm in diameter.
[b] Clinically detectable nodes are also referred to as macrometastases, but this is a different definition than the pathologist's definition based on a diameter >2 mm. Patients with gross extracapsular extension are also considered to have macrometasetasis.
Modified from Edge S, Byrd DR, Compton CC, et al., (eds.). *AJCC Cancer Staging Manual*. 7th ed. New York: Springer; 2010.

prognostic feature, especially with six or more mitoses per square millimeter.[81,82] Similarly, dermal expression of Ki67, a molecular marker of proliferation, is associated with greater risk of metastasis.[83] For thin melanomas, the presence of any mitotic figures has been associated with metastatic risk, whereas the absence of dermal mitoses is associated with an excellent prognosis.[84] The current staging system incorporates mitotic rate of ≥1/mm^2 in differentiating low-risk thin melanomas (T1a) from higher-risk thin melanomas (T1b), and data used to define the current staging system identify increasing risk with increasing mitotic rate for all thicknesses.[74] Increased mitotic rate is associated with a poorer prognosis across all thickness ranges, but is not yet incorporated formally in the staging system beyond the current cutoff of 1/mm^2 for thin lesions.[74,85,86]

Other Prognostic Factors

There is also evidence, and biologic rationale, that angiolymphatic invasion has negative prognostic significance,[81] and that microscopic satellites are associated with poorer prognosis. Satellitosis is incorporated in the current staging system[74] but will be considered separately because it defines the patient as stage III and thus goes beyond assessment of risk factors of the primary lesion alone.

Unresolved Issues in Melanoma Staging

The AJCC staging system is evidence-based and accounts for several important clinical and histopathologic findings. However,

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TABLE 94.2

Pathologic Stage Grouping for Cutaneous Melanoma

Clinical Staging[a]				Pathologic Staging[b]				Five-Year Survival (%)	Ten-Year Survival (%)
0	Tis	N0	M0	0	Tis	N0	M0	>99	>99
IA	T1a	N0	M0	IA	T1a	N0	M0	95	88
IB	T1b	N0	M0	IB	T1b	N0	M0	91	83
	T2a	N0	M0		T2a	N0	M0	89	79
IIA	T2b	N0	M0	IIA	T2b	N0	M0	77	64
	T3a	N0	M0		T3a	N0	M0	79	64
IIB	T3b	N0	M0	IIB	T3b	N0	M0	63	51
	T4a	N0	M0		T4a	N0	M0	67	54
IIC	T4b	N0	M0	IIC	T4b	N0	M0	45	32
III	Any T	N1–3	M0	IIIA	T1a–T4a	N1a	M0	82	63
					T1a–T4a	N2a	M0	73	57
				IIIB	T1b–T4b	N1a	M0	57	38
					T1b–T4b	N2a	M0	54	36
					T1a–T4a	N1b	M0	52	48
					T1a–T4a	N2b	M0	47	39
					T1a–T4a	N2c	M0	N/A	N/A
				IIIC	T1b–T4b	N1b	M0	49	24
					T1b–T4b	N2b	M0	38	15
					T1b–T4b	N2c	M0	N/A	N/A
					Any T	N3	M0	27	18
IV	Any T	Any N	Any M	IV	Any T	Any N	M1a	19	16
					Any T	Any N	M1b	7	3
					Any T	*Any N*	*M1c*	*10*	*6*

N/A, not available.
[a] Clinical staging includes microstaging of the primary melanoma and clinical-radiologic evaluation for metastases. By convention, it should be done after complete excision of the primary melanoma with clinical assessment for regional and distant metastases.
[b] Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial or complete lymphadenectomy. Pathologic stage 0 or stage IA tumors are the exception; they do not require pathological evaluation of the lymph nodes.
Modified from Edge S, Byrd DR, Compton CC, et al., (eds.). *AJCC Cancer Staging Manual*. 7th ed. New York: Springer; 2010.

several clinical settings are not fully addressed by the AJCC staging system. These include the following.

Positive Deep Margin on Biopsy

When a primary melanoma is diagnosed by shave biopsy, and the tumor extends to the deep margin, it is presumed that the melanoma was deeper than the original measured biopsy depth. Sometimes, on wide local excision there is residual melanoma with a greater depth than on the original biopsy. In that setting, it is appropriate to define the T stage based on the latter depth of invasion. However, in many cases, the wide excision does not reveal any more melanoma, or may reveal tumor that is more superficial. It is generally assumed that in those cases, any residual melanoma at the deep margin may have been destroyed by inflammatory changes after the biopsy. One approach for defining T stage in that setting is to call it TX. The other is to use the T stage of the original depth, even though that is incomplete. The latter has the advantage of distinguishing thin melanomas (e.g., a clinically thin melanoma with thickness <1 mm) from a thick melanoma (e.g., a 5-mm melanoma on shave biopsy, with positive deep margin). Thus, use of TX results in substantial loss of information for patients and their clinicians.

Local Recurrence After Original Incomplete Excision

Some patients present with melanoma after excisional biopsy or destruction (e.g., cryotherapy) of a pigmented skin lesion that was believed to be benign (clinically or histologically) on initial review. When such a lesion recurs and is found to contain melanoma, re-review of the original biopsy is appropriate, if available. Staging of such recurrent melanomas, when the original lesion was not known to be melanoma, is not well addressed.

Skin or Subcutaneous Lesion Without Junctional Involvement and Without Known Primary Melanoma

This is addressed later in this chapter. Cutaneous or subcutaneous nodules that occur in the absence of junctional melanocytic change, and in the absence of any other known primary, are among the most interesting presentations of melanoma. They may be in-transit metastases from primary melanomas that spontaneously regressed (stage IIIB), primary melanomas that arose from dermal nevi or that persisted in the dermis after arising from a partially regressed primary melanoma (stage IIB), or a distant metastasis

from an unknown primary melanoma (stage IV, M1a). A review of experience with these lesions at the University of Michigan suggests that they behave more like primary tumors arising in the dermis or subcutaneous tissue.[76] In the current staging system, these are considered stage III.

GENERAL CONSIDERATIONS IN CLINICAL MANAGEMENT OF A NEWLY DIAGNOSED CUTANEOUS MELANOMA (STAGE I–II)

Most melanomas present as clinically localized lesions without clinical or radiologic evidence of metastatic disease. Nonetheless, some of these patients have occult metastases, and the definitive surgical management includes both therapeutic resection and pathologic staging evaluation for regional metastases. The vast majority of primary melanomas are diagnosed on histologic assessment of skin biopsy performed by a dermatologist or a primary care practitioner. The patient then presents to a surgeon or other physician for definitive treatment.

Clinical Evaluation and Radiologic Studies for Patients with Clinical Stage I–II Melanoma

In patients with clinically localized melanoma, there is a wide range of clinical practice in the appropriate radiologic staging studies to be performed. Certainly all patients with such disease should have a complete history and physical examination, with attention to symptoms that may represent metastatic melanoma, including headaches, bone pain, weight loss, gastrointestinal symptoms, and any new physical complaints. Physical examination should carefully assess the site of the primary melanoma for clinical evidence of persistent disease and should evaluate the skin of the entire region (e.g., whole extremity or quadrant of torso, or side of the face) for dermal or subcutaneous nodules that could represent satellite or in-transit metastases. Biopsy should be done for any suspicious lesions and with a very low threshold for biopsy. In addition, physical examination should include thorough evaluation of both the major regional nodal basins (e.g., epitrochlear and axillary for a forearm melanoma) and also any atypical lymph node locations, such as the triangular intermuscular space on the back for upper back primaries.

There is a great deal of uncertainty and debate about appropriate initial staging studies. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) from 2013 recommend no staging radiographs or blood work for melanoma in situ, and recommend imaging for low-risk thin melanomas (stage IA) "only to evaluate specific signs or symptoms." For clinical stage I–II, no other imaging is recommended. For stage III melanoma, consideration of imaging is recommended, to include chest radiograph (CXR), computed tomography (CT) scans, or positron emission tomography (PET)/CT scans, with consideration of magnetic resonance imaging (MRI) of the brain, and other imaging is suggested only as clinically indicated. More complete staging studies are suggested for stage III melanoma.[87]

CXR for asymptomatic patients with a new diagnosis of clinically localized melanoma yielded suspicious findings in 15% of patients, of whom only 0.1% had a true unsuspected lung metastasis.[88] In a similar study, the yield of true positive CXR was 0% of 248 patients.[89] In patients with stage IIB melanoma, initial staging CT scans identified occult metastasis that changed management in 0.7% of patients.[90] Even in patients with positive SNBx, staging PET scan identified no melanoma metastases in 30 patients, even though there were lymph node metastases in 16% of cases.[91] In patients with clinical T1b–T3b melanomas, true positive rates for all imaging studies was 0.3%, and false-positive rates were 50% to 100% for CXR, 88% for CT and PET/CT scans.[92] Thus, there is a large body of data that argues that CXR, CT, and PET/CT are all of little or no value in initial staging of melanoma stage 0–IIIA.

PET with fluorodeoxyglucose (FDG) has a role in staging patients with advanced melanoma,[93] but its role in earlier-stage disease is less clear both because it is expensive and because it is associated with substantial radiation exposure. In one study, patients with clinically localized melanomas >1 mm thick, with local recurrence, or solitary in-transit metastases, FDG-PET scanning was performed prior to sentinel node biopsy. Sensitivity for detection of sentinel nodes was only 21%, although specificity was high (97%). In addition, 21% of patients had PET evidence of metastases, but none was confirmed by conventional imaging at that time, and the sensitivity for predicting sites of future disease recurrence was only 11%. Overall sensitivity for detecting occult stage IV disease was only 4%, and this is not recommended for initial staging.[94] These findings are similar to other experiences with PET imaging for intermediate-thickness melanomas.[95,96]

Also, some clinicians send blood for a complete blood count, for serum chemistries, including liver function tests, and for a lactate dehydrogenase (LDH) level, especially as they may be useful prior to surgery under general anesthesia. These also are of low clinical yield in terms of the melanoma but may detect unappreciated concurrent illness that may affect therapeutic decisions, including preoperative assessment. Specifically, if there is microcytic anemia, it should be worked up, with the differential diagnosis to include gastrointestinal metastasis of melanoma. Elevated LDH should prompt a more extensive staging workup, and elevated liver function tests should prompt a hepatobiliary ultrasound or CT scan unless there is another known explanation.

Wide Local Excision for Clinical Stage I–II Melanoma: General Considerations

Wide excision of the primary melanoma is performed to provide local control. Multiple randomized, prospective clinical trials support current recommendations for the extent of the margins of resection. The wide excision also provides an opportunity to evaluate the tissue adjacent to the primary lesion for microscopic satellites, which, if present, have clinical and prognostic significance.

There has been considerable debate about the appropriate margins of excision for primary melanomas, and it is helpful to understand the evolution of thought and data about this topic. In the early 1900s, melanoma was a rare disease, and when it was diagnosed, it was often locally advanced. Surgical resection was often associated with recurrence disease, and there were no guidelines for appropriate and successful surgical management of the primary lesion. In 1907, Handley reported a study that involved histologic examination of tissue sections taken at varied distances from the primary melanoma in a human tissue specimen that he obtained from a patient with a large primary melanoma. In that study, he found microscopic evidence of melanoma cells as far as 5 cm from the primary tumor. He recommended wide re-excision of melanomas with a measured margin of 5 cm from the primary lesion. This recommendation became standard management for melanoma for many decades, with patients typically undergoing radical resections requiring skin grafts ≥10 cm in diameter.

As melanoma became a more frequent diagnosis, there was greater awareness of it, and lesions were often diagnosed at an earlier (thinner) stage. In addition, these large re-excisions usually contained no detectable melanoma cells separate from the primary lesion. These observations, and concern for the morbidity of large resections and skin grafts, led to a questioning of the need for 5-cm margins of resection. It is ironic that the origin of this aggressive resection practice was based on data from a single patient in a single study; however, limiting the margins of excision has required multiple large, randomized, prospective trials. These trials are summarized in Table 94.3 and are detailed in the follow sections.

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TABLE 94.3

Prospective Randomized Clinical Trials of Melanoma Excision Margins

Clinical Trial	*N*	Thickness Ranges (mm)	Margins–Study Groups (cm)	Local Recurrence	Disease-Free Survival	Overall Survival
World Health Organization Melanoma Program Trial No. 10	612	0–2	1 vs. 3–5	None for 0–1 mm with 1 cm margins; for 1–2 mm, more local recurrences with 1 cm margins (NS)	—	No difference
French ■ Cooperative ■ Surgical trial	337	0–2	2 vs. 5		No difference; 10-y DFS 85% and 83%, respectively	No difference; 10-y survival 87% and 86%, respectively
Swedish ■ Cooperative ■ Surgical Trial	989	0.8–2	2 vs. 5	<1% overall	No difference; relative hazard rate for 2 cm margin 1.02 (0.8, 1.3)	No difference; relative hazard rate for 2 cm margin 0.96 (0.75, 1.24)
Intergroup ■ Melanoma trial	740	1–4	2 vs. 4	0.4% (first, recurrence) 0.9% (first recurrence), 2.1% ever for 2 cm margins; 2.6% ever for 4 cm margins	—	10-y disease-specific survival 70% for 2 cm margins; 77% for 4 cm margins ($p = 0.074$, NS)
British Cooperative ■ Group trial	900	≥2	1 vs. 3	(Locoregional = local + in-transit + nodal) Increase with 1 cm margin (hazard ratio 1.26, $p = 0.05$)	—	Similar; trend to better survival with 3 cm margins, $p > 0.1$ (NS)

DFS, disease-free survival; NS, not significant.

CLINICAL TRIALS TO DEFINE MARGINS OF EXCISION FOR PRIMARY CUTANEOUS MELANOMAS

The World Health Organization (WHO) Melanoma Program Trial No. 10 randomized 612 melanoma patients with melanomas ≤2 mm in thickness to excision margins of 1 cm versus 3 to 5 cm.[97,98] Patients were stratified into two subgroups: Breslow depth <1 mm versus 1 to 2 mm. There were no differences in survival rates or in rates of distant recurrences with 1-cm margins versus 3- to 5-cm margins with follow-up beyond 15 years.[99] There were more local recurrences for the group with 1-cm margin (eight versus three patients), but this was not a significant difference. There were no local recurrences for melanomas <1 mm thick treated with 1-cm margins. The lack of local recurrences with thin melanomas (<1 mm) after 1-cm margins of excision support this as a standard excision margin for T1 melanomas. The numerically slightly higher (but statistically insignificant) local recurrence risk with thinner margins for T2 melanomas has left questions about the appropriate margin for thicker lesions.

French and Swedish Cooperative Surgical Trials

The French Cooperative Group randomized 337 patients with melanomas up to 2 mm in thickness to 2- or 5-cm margins.[100] Ten-year disease-free survival rates were 85% and 83%, respectively, and ten-year overall survival (OS) rates were 87% and 86%, respectively.[100] The Swedish Melanoma Study Group randomized 989 patients with primary melanoma 0.8 to 2 mm thick on the trunk or extremities to 2- or 5-cm margins. Local recurrences were observed in only eight patients overall (<1%). In a multivariate Cox analysis, estimated hazard rates for OS and recurrence-free survival for those with 2-cm margin were 0.96 (95% CI, 0.75 to 1.24) and 1.02 (95% CI, 0.8 to 1.3), respectively, compared with the 5-cm margins.[101] Both of these studies support 2-cm margins as adequate for melanomas up to 2 mm thick and find no added benefit to 5-cm margins.

Intergroup Melanoma Trial

The Intergroup Melanoma Surgical Trial addressed the question of surgical margins in 740 patients with intermediate-thickness melanomas (1.0 to 4.0 mm thick) randomized to either 2- or 4-cm margins.[102] Patients were stratified by tumor thickness (1 to 2 mm, 2 to 3 mm, and 3 to 4 mm), anatomic site (trunk, head and neck, and extremity), and ulceration (present or absent). Patients with melanomas on the head and neck or distal extremity were not randomized for margin of excision because 4-cm margins are not readily performed in such locations. Thus, 468 patients (group A) were actually randomized for margin of excision. All patients were also randomly assigned to undergo either an elective lymph node dissection (ELND) or observation after wide local excision, and this component of that study is discussed separately.[102]

Among the 468 patients in group A (randomly assigned to excision with 2- versus 4-cm margins), only 3 (0.6%) experienced a local recurrence as the first site of failure, and 11 (2.3%) had local recurrence overall.[102] Among the 272 patients in group B (nonrandomly assigned to excision with a 2-cm margin), a higher rate of local recurrence was observed, with 3.7% having a local recurrence as a first recurrence and 6.2% overall experiencing a local recurrence during the course of their disease.[102] Among these 468 patients in group A, the incidences of local recurrence as first relapse were 0.4% versus 0.9% for 2- and 4-cm margins, respectively, and the incidences of local recurrence at any time were 2.1% versus 2.6%, respectively. In addition, the time to local recurrence and the median survival after local recurrence were unaffected by the extent of the margin. Ten-year disease-specific survival rates for the two groups were 70% and 77% for 2- and 4-cm margins, respectively ($p = 0.074$, not significant). Thus, this study supports a 2-cm margin as adequate

for melanomas 1 to 4 cm thick, and this was associated with rates of local recurrence (as first recurrence) well <1%. Multivariate analysis of data from this study further supported the lack of benefit of wider margin of excision for local control and identified ulceration of the tumor and head-and-neck location only as significant negative prognostic features.

British Cooperative Group Trial

The British randomized trial compared 1- versus 3-cm margins of excision in patients who had cutaneous melanomas ≥2 mm thick (T3, T4).[103] Nine hundred patients with T3 and T4 melanomas were accrued, of whom 25% had T4 melanomas. It is the only randomized trial evaluating margins of excision that included patients with T4 melanomas. Patients with melanoma on head and neck, hands, or feet were excluded. No patients had any surgical procedure to stage the regional nodal basins (sentinel node biopsy or ELND) or systemic adjuvant therapy. The trial was stratified according to tumor thickness (2 to 4 mm versus >4 mm). There were few local recurrences; local recurrences and in-transit metastases were not statistically more frequent in the 1-cm margin group. Locoregional recurrences were defined broadly to include local, in-transit, or regional nodal recurrences. Using that definition, a 1-cm margin of excision was associated with a significantly increased risk of locoregional recurrence (hazard ratio [HR], 1.26; $p = 0.05$). Overall survival was comparable for the two groups ($p = 0.6$); there was a nonsignificant trend toward higher death rate in the group with 1-cm margins (128 versus 105 deaths; HR 1.24, $p = 0.1$). This study has been controversial, and its relevance to current practice is questioned because of the lack of surgical staging of the regional nodes, but it does challenge the safety of 1-cm margins for melanomas >2 mm thick.[103] These results support excision >1 cm for thicker melanomas. The data from the Melanoma Intergroup study support 2-cm margins for melanomas 2 to 4 mm thick. No data have formally compared 2-cm margins with 3-cm margins for T4 melanomas.

SURGICAL STAGING OF REGIONAL NODES

Thin and RGP melanomas are commonly cured by excision alone; however, thicker melanomas may have metastatic potential. Initial management includes an assessment for metastases and consideration of treatment options that may be beneficial in providing regional control and systemic control. Melanoma may metastasize by lymphatic or hematogenous routes. Usually, lymphatic dissemination presents earlier than hematogenous dissemination. Thus, emphasis is placed on staging the regional nodes in patients with melanoma. The finding of lymphatic metastases is associated with a higher risk of systemic disease. Another potential benefit of staging the regional nodes is to select patients for curative resection. There are substantial data on this issue that bear on the current recommendations for surgical staging of nodes, and these are summarized here.

Lymphatic anatomy is variable and is poorly understood in comparison to venous and arterial anatomy. Classic work by Sappey defined aspects of lymphatic drainage patterns from skin and defined the skin regions that typically have lymphatic drainage to major nodal basins. More recently, lymphoscintigraphy has permitted mapping the actual lymphatic drainage patterns from the skin at the site of the primary melanoma. This sometimes identifies lymphatic drainage that differs from Sappey's predictions.

In the past, the standard recommendation was to perform ELNDs for melanomas of intermediate thickness (1 to 4 mm). Despite some retrospective data supporting this approach,[104] subsequent retrospective and prospective studies have failed to show a significant survival advantage to routine ELND.[105–107] In the early 1990s, a new procedure was developed and popularized for surgical staging of node-negative primary melanomas, which is called *intraoperative lymphatic mapping and sentinel lymph node biopsy*. This approach has become routine practice for melanoma management.

The concept and method for SNBx was originally developed by Cabanas[108] for management of penile carcinomas, but it was not pursued extensively. The initial experience with lymphatic mapping and SNBx for melanoma was the work of Morton et al.[109] at the University of California Los Angeles and the John Wayne Cancer Institute. They injected a vital blue dye (isosulfan blue) intradermally and found that this stained the draining lymphatics and stained, in turn, the first node(s) into which these lymphatics empty. This was validated in human clinical experience and it was rapidly adopted as an effective way to identify the first lymph node(s) to which the melanoma drains.[110] The sentinel node(s) serve as sentinels for the remainder of the node basin. Lymphatic mapping permits identification of the specific nodes that drain the relevant area of skin, and so these nodes (typically one or two nodes) can be excised for detailed histopathologic assessment while sparing the remaining nodes in that basin, which are critical for drainage of other skin areas, thus minimizing morbidity, in particular lymphedema.

Lymphoscintigraphy has been coupled with the blue dye injection to support identification of the sentinel node(s), using handheld probes for detection of γ radiation emitted by technetium-99 (^{99}Tc), the radionuclide commonly used in lymphoscintigraphy. Most surgical oncologists performing SNBx use a combination of radionuclide injection several hours preoperatively (in the nuclear medicine suite, up to 1 mCi of ^{99}Tc) and intraoperative intradermal injection of isosulfan blue dye (up to 1 ml) a few minutes prior to the incision. The injection of radiocolloid is shown in Figure 94.6. The sentinel node(s) should be both blue and radioactive ("hot"). However, sometimes the blue dye may fail to enter the node in the short interval before the dissection. Alternatively, if the dissection takes longer than anticipated, the blue dye may transit through the node by the time the node is identified. In addition, technical issues may result in the blue dye and radiocolloid being injected in slightly different areas, such that they identify different nodes. The gamma probe is used to guide the dissection to the sentinel node(s), as suggested in Figure 94.7.

Lymphatic mapping and SNBx using both blue dye and radiocolloid increases sentinel lymph node identification rates to 99% compared with 87% with blue dye ($p < 0.0001$).[111] However, radiocolloid alone has not been formally compared with radiocolloid plus blue dye. There is substantial multicenter and single-center experience with use of radiocolloid alone, which is associated with successful identification of the sentinel node(s) in >99% of patients and with a mean of approximately two sentinel nodes per

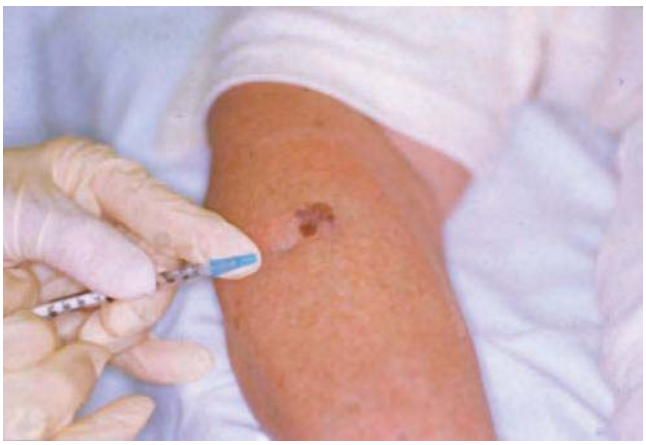

Figure 94.6 Injection of technetium-99 sulfur colloid intradermally near primary melanoma.

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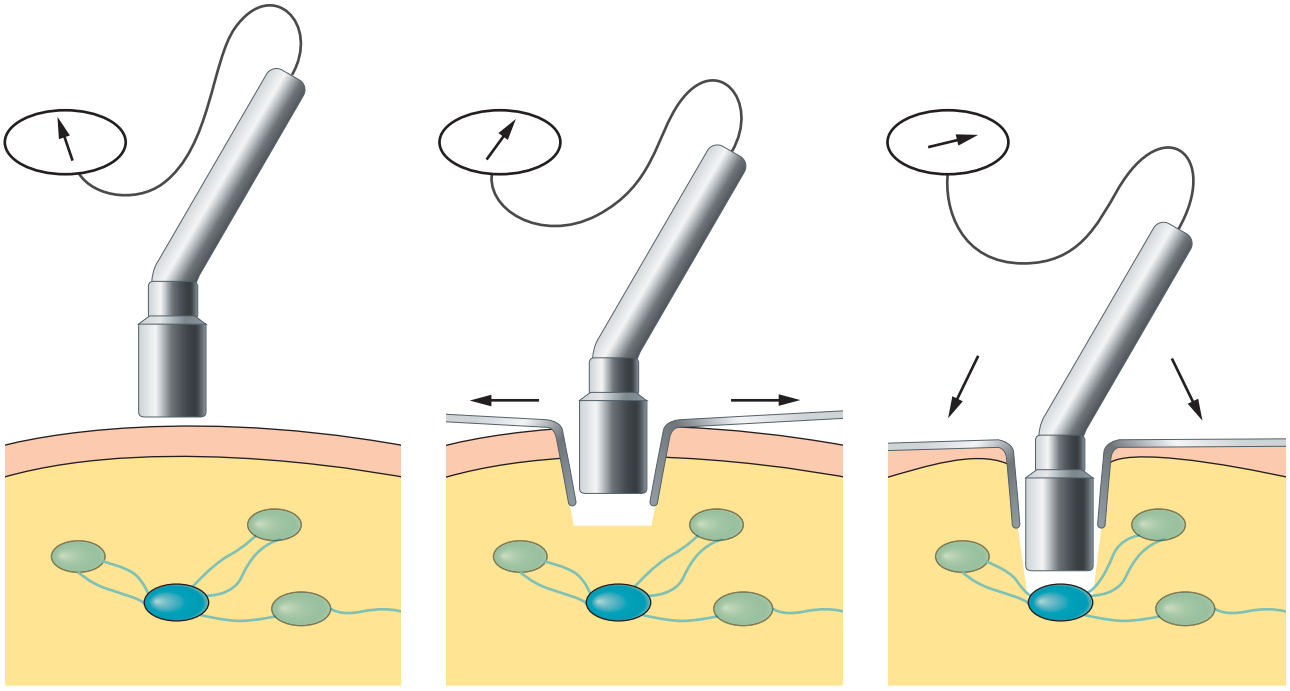


Figure 94.7 Schematic of a way to identify and remove the sentinel node using a handheld gamma camera.

patient.[112] The effectiveness of blue dye alone is limited because some patients have drainage to lymph node basins that may not be predicted (e.g., drainage from the right upper back to the left axilla) or drainage to atypical nodal basins (e.g., the triangular intermuscular space on the back, epitrochlear or popliteal nodes, or subcutaneous "in-transit" nodes that are outside a traditional nodal basin).[113,114] Examples of unusual lymph node locations mapped by lymphoscintigraphy are shown in Figure 94.8. Thus, in the large majority of clinical settings, it is most appropriate to perform radiocolloid lymphoscintigraphy in lymphatic mapping for SNBx of melanoma.

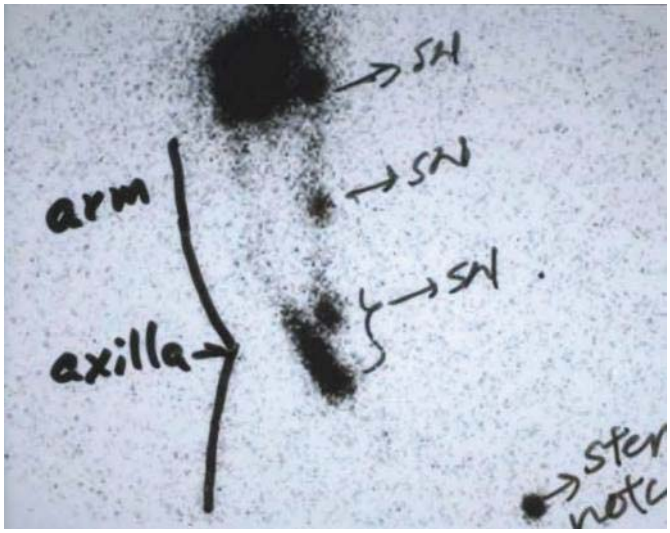
In experienced hands, lymphatic mapping should identify a sentinel node in 98% to 100% of cases, and it should be feasible to perform the SNBx with minimal morbidity, on an outpatient basis, and in many cases under local anesthesia with sedation. The early reports of SNBx stress a long learning curve, but as the technology of gamma probes has improved, the technique is less operator dependent. In addition, lymphatic mapping has now been performed long enough that surgical residents trained since the mid-1990s typically have had experience with it for melanoma and for breast cancer. The standard evaluation of a sentinel node includes evaluation of multiple sections of the node, often combined with immunohistochemical staining for melanoma markers (e.g., S100, HMB45, tyrosinase, and/or MART-1/MelanA).

Typical results of SNBx reveal that the rate of positive nodes increases with increasing tumor thickness, as would be expected, from <5% for the thin melanomas that undergo SNBx (e.g., T1b lesions) to approximately 40% for thick melanomas. Current experience with SNBx in most series supports the prognostic value of SNBx in thick melanomas (>4 mm)[115] as well as in thinner lesions. When ELND was performed, it was typically recommended only for melanomas 1.5 to 4 mm thick. However, in the Duke experience, the relative risk of distant versus regional metastases is not dramatically higher for thick melanomas, and this supports a clinical approach that includes the potential for curative resection of regional metastases in these cases.[105] In addition, the low morbidity of SNBx supports a threshold for SNBx in thinner melanomas than the 1.5-mm criterion that was used for performance of ELND.

The overall rate of positive SNBx in most series (typically for melanomas >1 mm) is in the range of 15% to 25%. The percentage of patients with false-negative SNBx in experienced hands and with use of radiocolloid and the handheld gamma probe, with or without blue dye, is typically in the range of 1.9% to 4%.[111] The most rigorous definition of false-negative rate is false negative/(false negative + true positive), and 3% false negative in the setting of 20% true positive represents 13% false-negative rate. False-negative rates have been estimated by seeking nodes containing metastases in the remaining nodal basin after a negative SNBx. In other settings, it is done by defining patients who return with clinically evident nodal metastases after a prior negative SNBx in the same node basin. These may or may not be equivalent. Nonetheless, there is a small percentage of patients who have negative SNBx who later return with nodal metastases in the same nodal basin. Although the procedure is very accurate and does identify the large majority of nodal metastases, it is prudent to follow patients for nodal recurrence even after a negative SNBx.

Lymphatic mapping and SNBx has been applied generally for all cutaneous sites and may also be useful for melanomas of mucous membranes.[116] A challenging area for SNBx is the head and neck. In particular, melanomas of the scalp and of the face may drain to parotid nodes or periparotid nodes, for which SNBx is more complex, more technically challenging, and associated with greater potential morbidity. In addition, false-negative SNBx are more common than in trunk and extremity melanomas, occurring in approximately 10% of patients, for a true false-negative rate that may approach 30%. However, in many cases, it can be performed reliably and still has a place in management.

More recent technology that offers promise for improving sentinel node localization are the development of mobile gamma cameras that can replace the single gamma detector of the gamma probe with an array of hundreds of detectors that permit real-time imaging that rivals that of the fixed gamma camera. This approach has the potential to improve identification of nodes in atypical loca-



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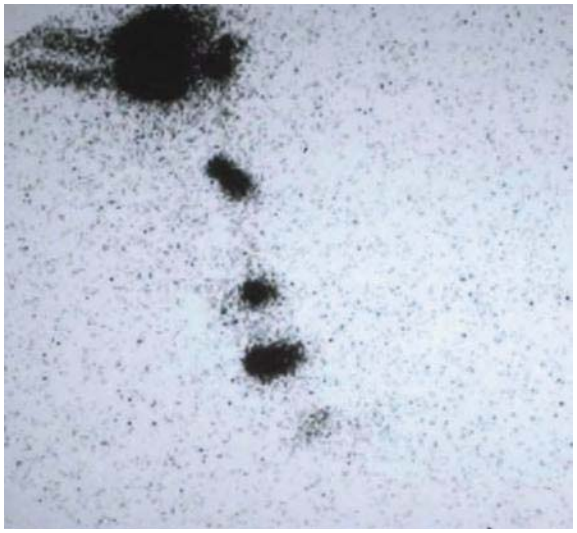

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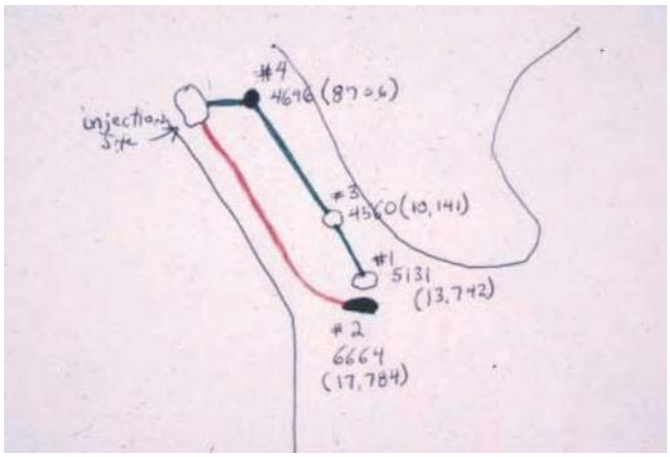

C

Figure 94.8 Lymphatic mapping from near the elbow along two separate lymphatic channels toward the axilla. An early image **(A)** shows the lymphatic channels clearly. A later image **(B)** shows the sentinel nodes clearly, but the channels are much less evident. One of the sentinel nodes is in subcutaneous tissue near the elbow and is almost missed due to proximity to the injection site (no. 4) **(C)**. The two nodes near the axilla were actually just distal to the true axillary space. One of them was a true sentinel node (no. 2) and contained tumor (designated by the *solid black node*), whereas the other node near it was the third node in that lymphatic channel, so that the first node in that channel was truly sentinel (no. 4) and also contained tumor. The other two nodes downstream from no. 4 were both negative for tumor.

tions and for ensuring adequate clearance of the sentinel nodes.[117] Also promising is single photon-emission computed tomographic/CT imaging, which can provide very discrete localization of sentinel nodes, which may be helpful in selected challenging locations. Despite the high accuracy of SNBx for nodal staging, the false-negative rate may be as high as 10% to 20%,[118] and these new technologies offer a possibility to reduce that false-negative rate.

In performing SNBx, melanoma metastases are sometimes clinically evident in the operating room as small pigmented spots just under the capsule of the node. When these are present, the hottest part of the node is usually precisely at that location (unpublished clinical observations). This may be particularly relevant for some large nodes, where the pathologist can be guided to the portion most at risk of metastasis for detailed histologic assessment (Fig. 94.9). Morton et al.[119] have formalized a technique that may identify the part of the node that is most likely to contain metastases, based on injecting carbon black dye and isosulfan blue dye. This has not yet become standard, but this or other refinements may further increase the accuracy of staging by this procedure.

The Multicenter Sentinel Lymphadenectomy Trial 1 was initially reported in 2006, and updated in 2014, as a randomized, prospective trial of 1,269 patients with melanomas 1 to 4 mm thick who were randomized to SNBx or observation in addition to wide local excision of the primary lesion.[110,120,121] The finding was that there was no difference in 5-year disease-specific survival (87.1% versus 86.6%).[120] Patients who developed clinically positive nodes during follow-up after initial observation of the node basins had worse survival than those with positive nodes found at the time of SNBx; however, this posthoc analysis carries inherent weaknesses.[120] In this trial, patients were randomized 3:2 to wide excision plus SNBx, or wide excision only, respectively. In the group receiving SNBx, 225 of 814 (27.6%) patients underwent early completion lymph node dissection (CLND), compared to 132 of 533 (24.7%) in the control group having delayed CLND. Lymphedema was significantly greater (20.4% versus 12.4%, $p = 0.04$) for those in the observation group who underwent delayed CLND, and hospitalization was longer for delayed

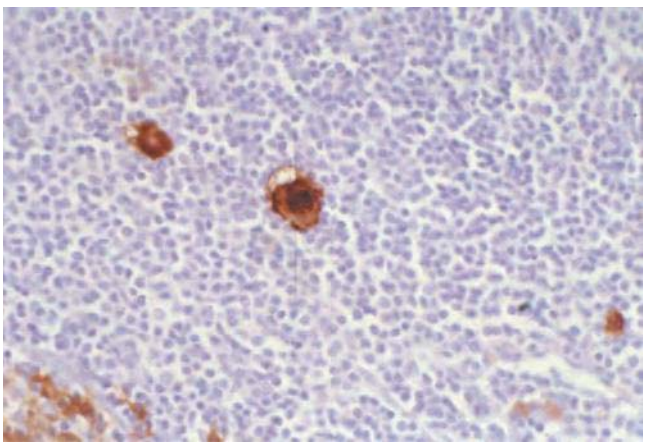

Figure 94.9 Immunohistochemical detection of isolated melanoma cells in a sentinel node when stained for the melanoma marker S100.

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CLND.[122] The follow-up results in 2014 supported the conclusion that biopsy-based staging of intermediate-thickness or thick primary melanomas provides important prognostic information and identifies patients with nodal metastases who may benefit from immediate CLND. In this series, SNBx-based management prolonged disease-free survival for all patients and prolonged distant disease-free survival and melanoma-specific survival for patients with nodal metastases from intermediate-thickness melanomas.[121]

One important consideration should be kept in mind, which is often overlooked in considering the potential value of SNBx and subsequent CLND. That consideration is the value to patients of regional control of their tumor, even in the absence of survival benefit. A study evaluating patients' perception of their own utilities for health states suggested that the development of recurrent disease markedly decreases patient perception of their health state, even if it does not impact survival.[123] This study thus suggests that regional tumor control may have value to patients, even in the absence of a survival benefit.

The rationale for performing SNBx for melanoma includes the following: (1) A negative SNBx is a good prognostic indicator that may provide comfort to low-risk patients. (2) A positive SNBx for patients with T1–T3a clinically N0 melanomas (clinical stage I–IIA) renders them candidates for adjuvant high-dose interferon (HDI) therapy, which offers some clinical benefit. (3) Patients with T4 melanomas or with microscopic satellites (N2c, stage IIIB) are further upstaged by the finding of a positive sentinel node, which helps these patients in risk assessment and may make them candidates for selected clinical trials. (4) Many clinical trials require surgical staging of regional nodes, and, thus, sentinel node mapping makes patients candidates for trials that may prove to be of benefit. (5) Identification of melanoma in a sentinel node permits selection of patients for CLND to increase the chance of regional tumor control. (6) Excision of the sentinel node may be curative if there is no tumor beyond the node, even if CLND is not feasible. This hypothesis is being explored explicitly in the Multicenter Selective Lymphadenectomy Trial 2 (MSLT2).

Selection of Patients for Sentinel Node Biopsy

SNBx is generally recommended for patients with melanomas at least 1 mm thick. For thinner melanomas, there is debate about the appropriate criteria for performing SNBx.[81] A common practice is to offer SNBx for thin melanomas with adverse prognostic features, including ulceration. The 2010 staging system also identifies a mitotic rate of ≥1 as an adverse prognostic feature, and this is associated with higher risk of sentinel node metastasis. Earlier data also support the relevance of mitotic rate as a prognostic factor in primary melanomas[82] or Clark level IV.[78] There is debate about performing SNBx for thin melanomas that are in VGP, that have dermal mitoses, or that occur in young patients.[81,84] Also, there is rationale for offering SNBx for melanomas <1 mm thick that have a positive deep margin on biopsy and thus are not fully evaluable for depth.

Pure desmoplastic melanomas have a similar overall metastatic and mortality risk as other melanomas, but their risk of regional nodal metastases appears to be lower than that of other melanomas.[67,124] Thus, it may be reasonable to limit SNBx for pure desmoplastic melanomas. However, there is limited experience with managing regional nodes in desmoplastic melanoma, and some desmoplastic melanomas can metastasize to regional nodes.[65]

Sentinel Node Biopsy Subsequent to a Prior Wide Local Excision

SNBx should be performed at the same procedure as wide local excision. However, there are some circumstances in which wide local excision may be performed without SNBx, and there is then a question of whether SNBx can be performed reliably after a prior wide local excision. Such circumstances include a thin melanoma on original biopsy, found to be deeper on re-excision or on second-opinion pathology review. A multicenter experience with 76 patients having SNBx performed after a prior wide local excision revealed a 99% success rate in SNBx, a mean yield of two sentinel nodes per patient, with a 15% overall sentinel node–positive rate, a 4% rate of melanoma recurrence in a negative mapped basin, and only a 1% rate of isolated first recurrence in a node. These and other data support performing SNBx after prior wide local excision, although performing it concurrently with the original wide local excision is preferred.[125]

MANAGEMENT

Clinically Localized Melanoma

Melanoma In Situ (Clinical TISN0M0, Stage 0)

For melanomas confined to the epidermis and epidermal/dermal junction that are diagnosed as melanoma in situ, this is a lesion that is curable in the vast majority of cases by wide excision alone. On initial evaluation, the regional nodes should be examined, as should the skin and subcutaneous tissue between the primary site and these regional node basins. Melanoma in situ by definition is not invasive or metastatic; however, metastatic melanoma to regional nodes has been observed occasionally from melanoma in situ with histologic evidence of regression.[126] Thus, it is prudent to examine the nodes clinically. However, in the absence of clinical evidence of metastasis, there is no need to perform radiologic staging studies. Definitive management involves re-excision with a margin of 5 mm. The standard recommendation is to perform a full-thickness re-excision including underlying subcutaneous tissue, although there are no formal data that a full-thickness skin excision is less adequate for melanoma in situ. However, variation in thickness within the original biopsy specimen may lead to occult invasion that is not observed on the evaluated sections. Thus, it is prudent to perform a full-thickness excision of skin and subcutaneous tissue to the underlying deep fascia. A 5-mm margin is the standard recommendation, but melanoma in situ can extend beyond its visible extent. Thus, if cosmetically acceptable, it is reasonable to obtain a margin of as much as 1 cm, especially if the original biopsy was incomplete. If the margins are positive or close, re-excision to a widely clear margin is recommended. SNBx is not indicated. No adjuvant therapy is needed if the margins are widely clear.

Clinical Follow-Up After Surgical Treatment

Melanomas in situ are curable in the vast majority of cases with surgery alone. However, they rarely may be associated with metastasis, probably attributable either to an invasive component that was not detected because of sampling error, or to an associated regressed invasive component.[126]

Thus, in accord with the NCCN Guidelines®, it is appropriate to follow these patients for local recurrence, in-transit metastasis, or regional node metastasis on an annual basis. The risk of recurrence is not high enough to require specialty follow-up, but a focused physical examination of the patient by the primary care physician is appropriate. More important, patients with melanoma in situ are at increased risk of subsequent primary melanomas, and so close dermatologic follow-up with full-body skin examinations is recommended.

Thin Primary Melanoma (Clinical T1A)

The classic definition of a thin melanoma was based on the original report of Breslow[73] of the association between depth of invasion (Breslow thickness) and subsequent risk of metastasis and death. In that report, patients with melanomas <0.76 mm thick had no

TABLE 94.4

Brief Review of Literature on Intratumoral Therapies

Therapy	Regimen	Response Rate Injection Lesions	Response Rate Distant Lesions	Citations
Interferon alpha	10 million IU 3×/wk rec IFNa2b	45% (31% CR, 14% PR)	CR 6%; PR 12% (RR 18%)	Von Wussow, 1988
Rose Bengal (PV-10)	10% PV-10 w/v in saline, 0.5 ml/cc lesion volume	46%	27%	Thompson 2008
Interleukin-2	0.3–6 MIU/lesion, based on size 3 ×/wk	79% (79% CR)	0%	Weide 2010
Electrochemical with bleomycin	Bleomycin (intralesional or IV) plus electrical pulse	96%	New lesions arose soon	Campana 2009, 2012
Imiquimod	Topical daily or BID	Up to 50%	Low	Berman 2002
BCG	Intralesional injection	90%	17%	Morton 1974
Talimogene laherparepvec (T-Vec; HSV-1 encoding GM-CSF)	Intralesional injection	26%	26%	Senzer 2009; Kaufman 2010; Korn 2009; Andtbacka 2013
BCG + imiquimod	BCG then, when inflamed, add imiquimod ($n = 9$)	67% (56% CR, 11% PR)	—	Kidner 2012
Diphencyprone	Prepared in acetone; administered at increasing doses to cutaneous lesions	100% (57% CR, 43% PR), based on seven patients	Not reported	Damian 2009
2,4-Dinitrochlorobenzene	Intralesional	60%	Not reported	Goodnight 1979

CR, complete response; PR, partial response; RR, response rate; IV, intravenously; BID, twice a day; BCG, Bacillus Calmette–Guérin; HSV-1, herpes simplex virus 1; GM-CSF, granulocyte macrophage–colony-stimulating factor.

subsequent metastasis. Thus, the definition of a thin melanoma had been a melanoma <0.76 mm thick. However, subsequent studies have shown a continuous risk association with increasing thickness, without an absolute "cutoff" at 0.76 mm,[73] and melanomas <0.76 mm in thickness do have approximately a 5% risk of subsequent metastasis.[127] Additional studies have defined additional histopathologic features that affect the prognosis of thin melanomas. The current AJCC staging system addresses several prognostic features of thin melanomas such that T1a melanomas are those <1 mm thick, with less than one mitosis per square millimeter, and without ulceration. In the absence of any clinical evidence of metastasis, these are clinical stage IA melanomas and have a 5-year survival rate of 94%.[74,78]

In most centers, the surgical management of patients with T1a melanomas includes wide excision with a 1-cm margin (including skin and all underlying subcutaneous tissue, to the deep muscle fascia). The margin should be measured from the visible edge of the pigmented lesion or from the biopsy scar, whichever is larger. Excisions of this size can almost always be closed primarily, with exceptions being on the face, palms, and feet, where skin grafts or rotation flaps may be needed.

Surgical Methods in Wide Local Excision (Applies for All Primary Melanoma Thicknesses)

For melanomas of the trunk and proximal extremities, wide local excisions should involve measuring the appropriate margin (usually 1 to 2 cm) around the entire scar from the biopsy, or from the visible edge of residual melanoma, and extending the incision to make an ellipse that is approximately three times as long as it is wide. Ideally, the direction of the scar should be longitudinal on the extremities, occasionally with some modification at joints, and should be along skin lines on the trunk and neck. On the upper back, it is usually best for the scar to run transversely, to minimize tension on it. When the initial biopsy scar is not in the direction that is desired for the final excision, an effective approach is first to mark out the oval shape that is required for the appropriate margins, then rather than extending that to an ellipse that is in the same direction, the ends of that oval can be extended in the desired direction, resulting in a sigmoid-shaped oval, which has two advantages: The closure results in a scar that is more in the desired direction, and the sigmoid shape allows the tension to be distributed in two directions. The excision should include all skin and subcutaneous tissue to the deep fascia, but not including the fascia. When a major cutaneous nerve runs along the deep fascia to innervate distal cutaneous structures, it is appropriate to preserve that nerve. Wide excisions can almost always be performed under local anesthesia, with or without intravenous sedation, in the patient who is thus motivated.

Clinical Follow-Up for Thin Melanomas (Stage IA)

There are no definitive data showing a survival advantage for close follow-up after surgical management of primary or metastatic melanoma; however, there is an expectation from patients for follow-up, and there are treatable recurrences and metastases that can be identified best by physician follow-up. The National Comprehensive Cancer Network® (NCCN®) has issued useful guidelines for treatment and follow-up of melanoma.[128] The risk of metastasis for thin melanomas is in the 5% to 10% range, and less for RGP lesions. In the uncommon case of recurrent thin melanomas, the recurrences usually occur late, often beyond 5 years from diagnosis; the annual risk of recurrence is fairly constant over a long time,[47] so annual follow-up for many years is recommended rather than frequent follow-up in the first few years. Follow-up suggestions are listed in Table 94.7.

Clinical T2A, T2B Melanomas

Melanomas 1 to 2 mm thick, with or without ulceration, should be managed with an initial history and physical examination to elucidate signs or symptoms that could suggest metastatic disease. In the absence of such findings, there is very low yield of additional staging studies, and they are not recommended. In those patients without evidence of metastasis, definitive management includes

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wide excision with a 1- to 2-cm margin and SNBx. There are definitive data from the Melanoma Intergroup trial that a 2-cm margin is adequate for these patients,[107] and even a 1-cm margin was associated with the same survival as a 3- to 5-cm margin in long follow-up of the WHO Trial 10 (see Table 94.3).[99] However, there has been a slight increase in local recurrence in patients with 1- to 2-mm lesions who had 1-cm margins (versus 3- to 5-cm margins). This is not statistically significant in the patients studied, but it may signal a slight increase in local recurrence risk. When it is feasible to take a 2-cm margin without a skin graft (trunk and proximal extremities in most cases), this is recommended to minimize the chance of local recurrence. However, when the lesion is located on the face or distal extremities, where such a margin may be difficult to achieve without a skin graft, a 1- to 1.5-cm margin is acceptable. If a skin graft will be necessary even to close a 1-cm margin (rare), it is recommended that a 2-cm margin be taken because the morbidity and cost of the skin graft will already be needed. In addition, for lesions that are barely above 1 mm in depth (e.g., 1.03 mm), it certainly is reasonable to use a 1-cm margin.

SNBx is routinely recommended for patients with melanomas 1 to 2 mm thick.[129] If the SNBx is positive, then subsequent management should follow recommendations given later for stage IIIA melanoma (T2a with positive SNBx involving one to three nodes) or stage IIIB melanoma (T2b with positive SNBx involving one to three nodes). However, if the SNBx is negative, then the patient is considered to have been pathologically staged as T2aN0M0 (stage IB) or T2bN0M0 (stage IIA), and no additional surgical management is required and no adjuvant systemic therapy is indicated, other than clinical trials.

Clinical T3A Melanomas (Clinical Stage IIA)

Melanomas 2 to 4 mm thick, without ulceration, represent T3a lesions, and in the absence of metastases, these are clinical stage IIA lesions. They should be managed clinically with a history and physical examination as detailed previously and may be considered for a staging studies and serum LDH level. Definitive management includes wide excision with a 2-cm margin and SNBx for histologic staging of the regional nodes. If the SNBx is negative, then no additional surgical or systemic therapy is indicated other than possible clinical trials. If the SNBx is positive, then management for stage IIIA melanoma should be followed.

Clinical T3B Melanomas (Clinical Stage IIB)

Melanomas 2 to 4 mm thick with ulceration represent T3b lesions and thus are clinical stage IIB melanomas. These are high-risk localized melanomas. Initial management should include a careful history and physical examination. Staging studies are generally of low yield, but in selected high risk cases may be considered, and if there are symptoms suspicious for metastatic disease, there is value in performing indicated imaging studies.[130] Given the higher risk of synchronous metastases that may be detected at diagnosis, systemic staging with CT scans of the chest, abdomen, and pelvis (or PET/CT scan) plus MRI scan of the brain may be indicated if there are symptoms or signs suggestive of systemic metastasis.

In the absence of clinical evidence of metastasis, definitive management is wide excision with a 2-cm margin and an SNBx. If the nodes are negative, the summary stage is IIB (T3bN0M0). For these patients, no additional surgical therapy is needed. However, HDI and pegylated-interferon therapies have been approved for use as postsurgical adjuvant therapy for patients with resected stage IIB-III melanoma. It is worth noting that the randomized clinical trials of adjuvant interferon were performed before the recent revision of the AJCC staging system, when ulceration was not incorporated in the staging system. Thus, the patients with stage IIB in whom interferon was tested did not include the current patients with stage T3bN0. Nonetheless, it is available for such patients, whose risk is comparable to that of patients with nonulcerated thick melanomas (T4aN0).

Thick Melanomas (T4A, T4B, Greater than 4 mm Thick)

Thick melanomas have been commonly associated with a risk of metastasis and mortality in the range of 50% over 5 to 10 years. Ulceration increases this risk: T4a melanomas are clinical stage IIB, and T4b melanomas are clinical stage IIC. Initial workup should include a history and physical examination, and serum LDH plus more aggressive radiologic imaging as indicated by signs and symptoms. For these high-risk patients, consideration should be given to more complete staging with CT scans of the chest, abdomen, and pelvis plus MRI of the head. Definitive management includes wide excision with at least a 2-cm margin plus SNBx. There are no definitive prospective, randomized data regarding margins for melanomas thicker than 4 mm, but margins of at least 2 cm are recommended. The general experience is that 2-cm margins provide adequate local control for these lesions, suggesting that the strong data supporting the adequacy of 2-cm margins in 1- to 4-mm melanomas may be extrapolated to thicker lesions.[131]

As SNBx has been employed routinely since the early 1990s, most studies show that sentinel node status has independent prognostic value for patients with thick melanomas.[131] Because these patients have a high risk of sentinel node positivity (approximately 35% to 40%), there is a high chance of regional nodal recurrence, and SNBx, followed by CLND, offers the prospect of increasing the chance of regional control. In patients with negative sentinel nodes, adjuvant interferon should be considered because it is approved by the FDA for these patients. This should be discussed in detail with patients.

SPECIAL CONSIDERATIONS IN MANAGEMENT OF PRIMARY MELANOMAS

Primary Melanomas of the Head and Neck

For melanomas on the head and neck, there are important anatomic constraints, and there are times when the optimal margins are not feasible (e.g., a 2-cm margin for a lesion 1 cm below the eye), but to the extent possible, the optimal margins should be obtained and closed with an advancement flap, skin graft, or limited rotation flap. In the unusual circumstance of a large-diameter lentigo maligna on the face that is not amenable to surgical resection because of cosmetic results or comorbid patient conditions, it may be treated with superficial or Grenz X-rays with local control rates reported above 90%.[132] Anecdotal reports of off-label topical treatment with imiquimod ointment have also resulted in effective local control of superficial melanomas.[133,134] This is being used increasingly, with good results in reported experience, but recurrence may occur.[135] Initial experience suggests that imiquimod is not effective at eradicating dysplastic nevi.[136] Desmoplastic melanomas commonly occur in the head and neck region and may have reported local recurrence rates up to 40% to 60% after resection.[137] Other series vary substantially in local recurrence rates of desmoplastic melanomas. One reports local recurrences as first recurrences in 14% of patients, which exceeds that of other histologic types,[69] and another reports no difference in local recurrence rates compared to other melanomas, although the presence of neurotropism was associated with higher risks of local recurrence.[70] An explanation for the high local recurrence rates in some series of desmoplastic melanoma may include inadequate margins of excision because of anatomic constraints in the head and neck. In addition, because desmoplastic melanomas are usually amelanotic, the surgical margins may be underestimated, and the his-

tologic appearance of desmoplastic melanoma can interfere with accurate detection of microscopically positive margins, especially in fibrotic skin. Thus, in patients with desmoplastic melanoma, every effort should be made to obtain adequate margins.[137] If that is not possible, postoperative adjuvant radiation should be considered with 2- to 3-cm margins around the resected lesion because this may reduce subsequent local recurrences.

Neurotropic melanomas of the head and neck have a propensity to recur at the skull base by tracking along cranial nerves, and postoperative adjuvant radiation including the resection bed and the cranial nerve pathway should be considered for this variant.

Primary Melanomas of the Mucous Membranes

Mucosal melanomas of the head and neck, anorectal region, and female genital tract are usually diagnosed when they are thick. They are associated with higher risks of distant metastases and death compared to cutaneous melanoma. They are also associated with higher risks of local recurrence and regional nodal metastases. Staging of these lesions is not addressed completely in the AJCC staging system for cutaneous melanomas, but there are general similarities that can be applied to mucosal melanomas. The depth of invasion is difficult to measure because they are often biopsied in a fragmented way, but they usually are deep lesions, with depths often of ≥1 cm. They should be resected with wide margins if possible. Resection of melanomas of the nasopharynx, oropharynx, and sinuses is limited by the bony structures of the skull and the base of the brain. Vulvovaginal melanomas may be widely resected in many cases but may also be constrained by efforts to preserve urinary and sexual function. They may also be associated with extensive radial growth in addition to the invasive lesion, which can lead to multifocal local recurrences. Anorectal melanoma may usually be resected widely by an abdominoperineal resection, but this morbid operation is not associated with higher survival rates than local excision only.[138] Adjuvant local radiation therapy may be of value when widely clear margins are not feasible.[139] However, no randomized, prospective trials of radiation have been performed in this setting. SNBx has been performed for vulvovaginal melanomas, but its impact on ultimate clinical outcome is not known.[140] It may also be performed for anorectal melanomas,[141] but pelvic and systemic metastases are more concerning for ultimate outcome than the risk of groin metastases. SNBx is not generally feasible for mucous membrane melanomas of the head and neck because of technical considerations.

Mucosal melanomas have not specifically been tested for their response to interferon therapy, but they are considered eligible for interferon, which is reasonable to consider after resection of thick mucosal melanomas with or without lymph node involvement. These patients may also be eligible for clinical trials in the adjuvant setting.

Primary Melanomas of the Fingers and Toes

For melanomas of the plantar aspect of the foot, especially on the anterior weight-bearing surface or on the heel, skin grafts are inadequate for bearing the weight of walking. Thus, it is often effective to rotate the skin of the instep of the foot to cover defects in those areas, with skin grafting of the instep area if needed.

For subungual melanomas of any finger or toe, the appropriate management is amputation at the interphalangeal joint of the toe or just proximal to the distal interphalangeal joint of the finger. Even for subungual melanomas in situ, such an amputation is indicated. These lesions often are found to contain invasion on the final specimen that is not evident on original biopsy, and it is not feasible to resect the entire nail bed with any margin without taking the bone of the distal phalanx because the two are intimately associated. It is important for amputations of the fingers, especially the thumb, to attach the severed deep flexor tendon to the remaining proximal phalangeal bone, to retain adequate flexor strength after surgery. This can be done by passing a braided multifilament suture through the phalangeal bone and the ligament via holes drilled in the bone in two places. The skin incision for these amputations can be designed by measuring 1 to 2 cm (depending on thickness) from the nail bed and including at least that amount of skin with the amputation. This almost always leaves some skin on the plantar or palmar surface (except when the subungual melanoma has extended well out onto the plantar/palmar surface) that can be used to close the surgical defect and provides a sturdy skin surface.

For melanomas of the proximal toe or finger, the considerations are similar to those for distal and subungual digital melanomas. For melanoma of the toe, amputation of the toe is usually the best choice because the functional morbidity of losing a toe is small. The exception is the great toe, but even amputation of that toe is feasible, although retention of the first metatarsal head is valuable for gait and balance. For small-diameter, thin melanomas proximally located on the fingers, and for toes when appropriate, it occasionally may be feasible to perform a wide excision and skin grafting (rarely primary closure) with preservation of the digit.

SNBx can be performed accurately from these lesions and should usually be performed for melanomas of the fingers or toes if they are at least T1b lesions.

THE ROLE OF RADIATION THERAPY IN THE MANAGEMENT OF PRIMARY MELANOMA LESIONS

The general management of primary melanoma lesions is surgical resection. However, there is a role for definitive or adjuvant radiation therapy in certain histologic variants including lentigo maligna, desmoplastic melanoma, or neurotropic melanoma, and for palliation of unresectable primary disease. Lentigo maligna commonly occurs as a large lesion in the head and neck region of elderly patients. If the patient is medically inoperable or if the proposed resection would result in a poor cosmetic outcome, he or she can be treated with superficial or Grenz X-rays with local control rates above 90%.[132] Desmoplastic melanomas also commonly occur in the head and neck region and have high local recurrence rates. Postoperative adjuvant radiation may be delivered with 2- to 3-cm margins around the resected lesion if margins are inadequate, or following resection of a locally recurrent lesion, and thus as this can substantially reduce subsequent local recurrences.[137] Neurotropic melanomas of the head and neck have a propensity to recur at the skull base by tracking along cranial nerves, and postoperative adjuvant radiation including the resection bed and the cranial nerve pathway should be considered for this variant. Large unresectable primary lesions should be considered for palliative radiation therapy or be enrolled in clinical trials. Of note, the concurrent use of interferon-α2b with radiation or its use 1 month following radiation has been reported to cause increased radiation toxicity and should be used cautiously.[142]

CLINICAL FOLLOW-UP FOR INTERMEDIATE-THICKNESS AND THICK MELANOMAS (STAGE IB–IIC)

Suggestions for follow-up are listed in Table 94.4. For intermediate-thickness melanomas, history and focused physical examination may be done as often as every 3 months and as infrequently as annually, with LDH, and complete blood count at least annually, and other scans done as indicated for symptoms. CT or PET/CT is not likely to have much yield if the other studies and clinical examination are all unremarkable. However, there are circumstances in which they may be useful. Especially for the high-risk

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primary (e.g., T4b) on the lower extremity, pelvic CT scan or PET/CT may be helpful in identifying iliac nodal recurrences that are difficult to detect on examination. In addition, for high-risk melanomas, brain MRI may be helpful in detecting small brain metastases when they are asymptomatic and amenable to treatment with gamma knife radiation therapy.

Most first recurrences will be in local skin, in-transit skin, or lymph nodes, which can be detected on physical examination and can be treated surgically with some chance of cure. The most common first sites of visceral metastasis are lung and liver. Other frequent sites of metastasis include the gastrointestinal tract, brain, bone, distant skin or nodes, and adrenal glands. Clinical follow-up should elicit any information on headaches, weight loss, change in appetite, bone pain, or other symptoms that could be associated with these metastatic sites. There should be a low threshold for performing radiologic studies to work up such symptoms. However, routine extensive scans have not been shown to improve clinical outcome. In a study of follow-up for patients with stage II-III melanoma, melanoma recurrences were detected based on symptoms in 68%, physical examination findings in 26%, and CXR in 6%.[143] In another study of patients with stage I-II melanoma followed with physical examination, blood tests, and CXR, recurrences were detected by physical examination (72%), patient symptoms (17%), and CXR (11%).[144] The diagnostic yield of laboratory tests is low, but elevations of LDH or other liver function tests may signal a liver metastasis or other new metastasis. New microcytic anemia can be a first sign of gastrointestinal blood loss due to a small bowel metastasis.

REGIONALLY METASTATIC MELANOMA (STAGE III): LYMPH NODE METASTASIS, SATELLITE LESIONS, AND IN-TRANSIT METASTASES

Melanoma has a high propensity to regional metastasis in any of several presentations, all presumably via intralymphatic dissemination. These are the most common first metastases. The presence of regional metastasis is a negative prognostic finding; however, there is some chance of long-term disease-free survival and cure for patients with regional metastases, and they should be managed with curative intent whenever feasible.

There is a wide range of outcomes for patients who develop regional (stage III) metastases. Prognostic features of the primary melanoma have been associated with clinical outcome even after the development of metastases.[145] However, in the assessment of prognosis of patients with stage III melanoma performed for the current AJCC staging system, only ulceration of the primary lesion had independent prognostic impact,[74,76] and this has been incorporated in the staging system.

Regional metastases are defined as follows:

- *Local recurrence* is best defined as recurrence of melanoma in the scar from the original excision or at the edge of the skin graft if that was used for closure.
- *Satellites metastases* may occur either simultaneously with the original diagnosis or arise subsequent to original excision. Typically, recurrences that are separate from the scar but within 2 to 5 cm of it are considered satellite metastases (Fig. 94.10).
- Regional recurrences beyond 5 cm of the scar but proximal to regional nodes are considered *in-transit metastases* (Fig. 94.11).
- *Regional node metastases* are typically in a draining nodal basin that is near the lesion.

Thus, for example, melanomas of the forearm usually drain to an axillary node. However, the most proximal regional node may be an epitrochlear node or simply a subcutaneous node in an atypical location. With the use of lymphoscintigraphy and SNBx routinely in melanoma, such atypical nodal locations are increasingly defined.[146] It is occasionally difficult to distinguish whether an in-transit metastasis is a regional skin metastasis or a true nodal metastasis.

Management of Local Recurrence

Local recurrence is common after a primary lesion is inadequately excised. This type of local recurrence thus represents a failure of initial surgical management and may not represent the same high risk of distant metastasis and mortality that is associated with local recurrence after what is otherwise considered adequate surgical resection. However, local recurrences after adequate wide excision are associated with a very poor prognosis. In the Intergroup Melanoma trial, local recurrences were associated with 9% to 11% overall 5-year survival rate, as compared with 86% for those without local recurrence.[102]

Despite the bad prognosis associated with local recurrences, some patients either may be cured or may have extended tumor control by surgical resection. It is best to re-resect the entire scar down to the level of fascia, and perhaps including fascia, because there may be more tumor in the scar than is clinically evident, and this type of resection can generally be performed with minimal morbidity. Excision with a 1- to 2-cm margin is reasonable if the recurrences are limited to the scar. In the setting of associated

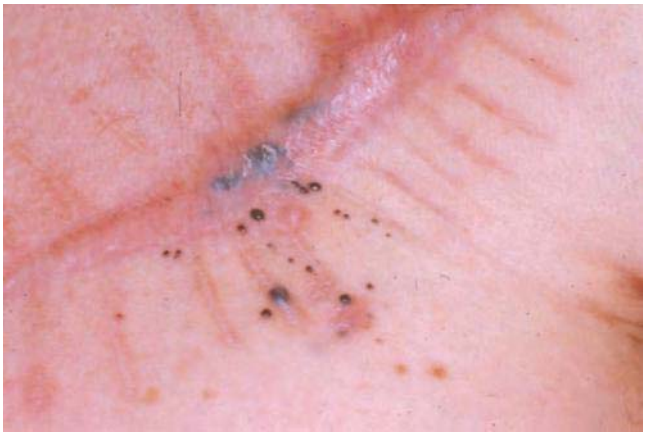

Figure 94.10 Local and satellite metastases after wide excision of melanoma on the chest.

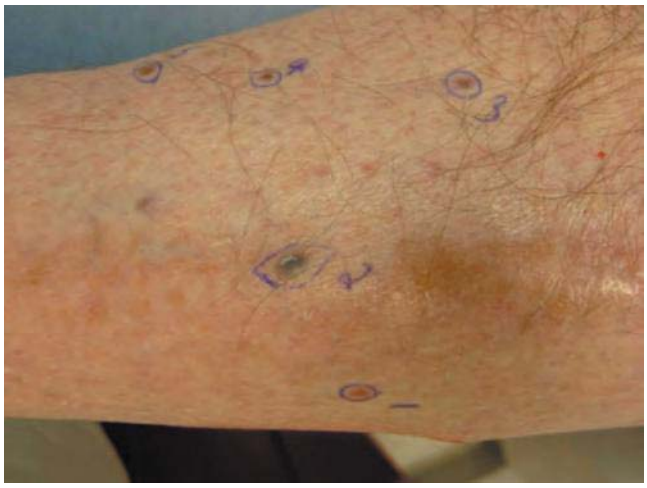

Figure 94.11 Close-up view of in-transit metastases involving the dermis, along the skin of the leg.

satellite metastases, more extensive resection may be appropriate with a skin graft. In patients with concurrent distant disease, a less aggressive approach to the local recurrence may be justified, and simple excision to a clear margin may be acceptable. In addition, it is appropriate to consider SNBx by mapping from the site of the local recurrence.[147] This is usually successful even if there has been a prior SNBx or a prior CLND. This may enable regional control in such high-risk patients in whom the sentinel nodes may be positive in up to 50% of cases.[147] Unresectable recurrent lesions should be considered for palliative radiation therapy.

Management of Satellite and In-Transit Metastases

The presence of in-transit or satellite metastases is a negative prognostic feature, with clinical outcomes similar to those observed for patients with palpable nodal metastases. Satellite and in-transit metastases have comparable biologic and prognostic significance.[148] When a patient presents with a solitary in-transit metastasis or a localized cluster of in-transit metastases, it is reasonable to perform excision of this along with SNBx. The margin of excision should be adequate to obtain free margins. This usually requires a 5- to 10-mm margin. A fairly frequent clinical scenario that is difficult to manage is the patient with multiple in-transit metastases. This most commonly occurs in the lower extremity from primary lesions below the knee, but it may occur in other locations. There is no ideal management for such patients because the natural history almost always involves systemic dissemination of disease, which may occur simultaneously, within a few months, or many years after the in-transit metastases. The large majority of such patients will continue to develop new in-transit metastases over time, and so true control of this process is uncommon. However, there is no reliable systemic therapy for this process; thus, surgery remains the best first option for regional control, when feasible. In some scenarios, surgical management of a symptomatic lesion may be valuable for palliation while addressing the appropriate management of other in-transit disease.

Because these patients typically continue to progress with more in-transit metastases and shorter intervals between metastases, other options for management are needed. Radiation therapy may be considered after surgical resection in this setting. Other regional options include intralesional therapy with interferon-α, interleukin (IL)-2, bacillus Calmette-Guérin (BCG), oncolytic replication competent virus injections and dyes like Rose Bengal[149] or application of diphencyprone,[150] or topical treatment of superficial metastases with imiquimod, all of which can induce responses in the treated lesions and occasionally in untreated lesions.

With the marked improvements in systemic treatments for advanced melanoma the indication for surgical excision of satellite lesions and in-transit melanoma decreases. The presence of these lesions is a hallmark of a melanoma that has ability to metastasize. Therefore, an early systemic intervention with the existing locoregional skin lesions being used as indicators for the effectiveness of the treatment has the potential to change the natural course of that melanoma as opposed to serving as a temporary local therapy. Systemic treatments that could be used in this setting are anti–cytotoxic T-lymphocyte antigen (CTLA4), anti–programmed death 1 PD-1 or anti-PD-L1 antibodies, and the neoadjuvant use of BRAF and other targeted inhibitors.[151]

Isolated Limb Perfusion and Infusion

An option for management of some patients with extensive regional recurrences in an extremity is hyperthermic isolated limb perfusion (ILP) with melphalan or isolated limb infusion. ILP can lead to complete responses in 60% to 90% of patients, with complete responses reported in 25% to 69% of patients.[152,153] Some patients will fail to respond and others may have short-duration responses, but a subset of patients may have durable complete responses and long-term survival.[153] Retreatment with ILP or isolated limb infusion is feasible with some benefit. There also is some morbidity associated with ILP, including a low risk of limb loss. ILP may also shrink an unresectable recurrence, rendering it resectable. Tumor necrosis factor-α has been explored as a regional therapy agent for use in combination with melphalan in ILP for melanoma, with some encouraging findings in initial assessments. However, a randomized, prospective clinical trial performed through the American College of Surgeons Oncology Group, Z0020, showed no improvement in response rates or clinical outcome with melphalan plus tumor necrosis factor-α compared with melphalan alone.[152]

ILP has also been studied in the adjuvant setting after surgical treatment of a primary melanoma on the extremity, but no benefit was seen with this therapy in the adjuvant setting.[154]

Intratumoral Therapies with Potential Systemic Effects

Intratumoral therapies have been studied to induce regression of injected lesions in patients who are not good candidates for aggressive surgery or in patients with many cutaneous in-transit metastases. Intralesional BCG has been used successfully and occasionally with regression of uninjected lesions as well as injected lesions,[155] A recent randomized phase 3 trial reported clinical responses after intralesional injection of melanoma metastases with an oncolytic herpes virus encoding granulocyte macrophage–colony-stimulating factor (GM-CSF), called talimogene laherparepvec, with improved clinical response rates compared to control patients receiving GM-CSF alone.[156] In that study, there were regressions of treated and untreated lesions, with a 26% response rate (11% complete response) versus 6% with GM-CSF control (1% complete response), with durable response rate of 16% versus 2% ($p < 0.0001$), with a trend to better survival at interim analysis.[156] Review of the literature reveals a range of outcomes with intratumoral injection therapies or topical treatments of cutaneous metastases (see Table 94.4). A general finding is that noninjected lesions regress in some patients, but only if the injected lesions regress.

Other approaches studied for direct treatment of individual metastases, but not included in Table 94.4, include focused radiation therapy, pulsed dye laser therapy, intralesional GM-CSF, electrochemotherapy with cisplatin, or any of several oncolytic viruses administered intralesionally.[157] The German S3 guidelines for melanoma management discuss intralesional medical therapy of local and regional recurrences for melanoma, based on a summary of the literature, and specify that "patients with satellite and in-transit metastases should be treated within the context of clinical studies if possible," and that the highest response rates to intralesional therapy have included intratumoral IL-2, intratumoral electrochemotherapy with bleomycin or cisplatin, or local therapy with diphencyprone. All of these warrant investigation alone or in combination with other active therapies.

Management of Regional Lymph Node Metastases

In patients with metastases to regional nodes, prognosis is related to tumor burden in the nodes and the number of nodes involved with tumor. In numerous studies, the number of metastatic nodes is the dominant prognostic factor in stage III melanoma.[76,158] The extent of lymph node involvement has been studied in various ways. For the current staging system, differentiation was made between clinically occult metastases (sentinel node positive, clinically negative) and clinically positive (palpable) metastatic nodes. This was a significant prognostic distinction.[74,76] Patients with nonulcerated primary melanomas and one to three positive sentinel nodes are stage IIIA, and 5-year survival probability is significantly

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better than 50%. However, a palpable node represents stage IIIB disease. Prognosis also is worse for patients with four or more tumor-involved nodes or with satellite or in-transit metastases in addition to nodal metastases (stage IIIC).

Management of Patients After a Positive Sentinel Node Biopsy (Stage IIIa if Nonulcerated Primary Lesion, One to Three Positive Nodes)

The rationale for performing SNBx, when first developed, was to avoid the morbidity of CLND in the 80% to 85% of patients with negative regional nodes, but simultaneously to stage patients accurately and to select those patients with regional nodal metastasis for CLND. However, experience with SNBx for melanoma has been that most patients with positive sentinel nodes have only one positive node, and only about 15% of patients have melanoma metastases identified in CLND specimens.[159] This finding has prompted consideration of abandoning CLND for some patients after positive SNBx. Review of data from the National Cancer Database showed that only about 50% of patients with positive sentinel nodes in the United States undergo CLND.[160] Thus, there is a wide range of practice without clear consensus. Several studies have identified features of the positive sentinel node that predict a low risk of a positive CLND, with the suggestion that CLND may not be necessary in such situations. Features such as the number of positive sentinel nodes, the tumor burden, and the location of tumor in the node plus features of the primary melanoma all are associated with greater risk of positive nonsentinel nodes.[161] However, most clinical experience with SNBx and evaluation of nonsentinel nodes is complicated by the fact that sentinel nodes are evaluated by a much more rigorous histopathologic approach than nonsentinel nodes, and thus the incidence of positive nonsentinel nodes may be greater than the reported 15%. One study used multiantigen reverse transcriptase-PCR to evaluate nonsentinel nodes from patients whose formal pathology report was negative for melanoma and found molecular evidence of melanoma metastases in 54% of these patients.[162] This PCR approach is typically more sensitive than standard histology and may detect positive nodes that have such a low tumor burden as to be clinically insignificant. However, the current limited data suggest that the true rate of positive nonsentinel nodes after a positive SNBx may be somewhere between 15% and 50%.

The standard recommendation has been to perform CLND of any lymph node basin with a positive sentinel node for melanoma.[163] However, some patients refuse to have CLND or are not eligible for it because of medical contraindications. A recent article summarized the combined experience from 16 institutions and reported on the clinical outcome of 134 patients who had positive SNBx but who did not undergo CLND.[164] Their outcomes were compared with a cohort of patients with positive sentinel nodes who did undergo CLND. At a median follow-up of 20 months, 15% of patients had developed recurrent melanoma in lymph nodes as a component of a first recurrence. This was not significantly different from the outcome in patients who underwent CLND.[164] Thus, there is now justification for reconsidering the best surgical management after positive SNBx. Morton and colleagues initiated the MSLT2, which is randomizing patients with positive SNBx to (1) CLND or (2) close observation with lymph node basin ultrasound.[164] This trial will take several years to accrue and additional years for mature data. Until then, there will likely be evolution of perspectives about CLND after SNBx. There is support both for CLND and for close observation after positive SNBx, and thus equipoise exists for the MSLT2 trial. In the midst of this debate, the 2012 joint guidelines from the Society of Surgical Oncology and the American Society of Clinical Oncology recommend CLND for all patients with positive sentinel lymph nodes.[129]

Management of Palpable Metastatic Melanoma in Regional Nodes: Therapeutic or Completion Lymphadenectomy

The other clinical settings for lymphadenectomy include various presentations with clinically evident regional nodes: after a negative SNBx, after observation of a nodal basin, or from an unknown primary melanoma. If lymph node recurrence appears after a prior complete dissection in the same basin, the surgical management may include a repeat node dissection, but if there is confidence that the original dissection was thorough, the repeat procedure may be more limited to the site of evident tumor recurrence.

Metastasis to a regional node represents stage III (A, B, or C) disease and is associated with a subsequent risk of distant metastasis in the range of 40% to 80%. Nonetheless, there is a significant chance of cure after complete lymphadenectomy for stage III melanoma[105,158] with overall 25-year survival rates of 35%.[165] Thus, lymphadenectomy for stage III melanoma is performed with curative intent. However, even if the patient develops distant disease in the future, there is benefit in achieving regional control, which is obtained in about 90% of patients.[105] When regional nodal disease is left in place, it can become extensive, with skin involvement, even ulceration, and with extension to involve major neurovascular structures. An example of extensive axillary recurrence with skin involvement is shown in Figure 94.12. Aggressive surgical management of less extensive disease can avoid these changes in most patients.

There are some specific considerations related to lymph node dissections in different node basins. These are summarized as follows.

Axillary Dissection

Axillary dissection should include all node-bearing tissue in levels I, II, and III. The long thoracic nerve and thoracodorsal neurovascular bundle should be identified and preserved unless involved with tumor. The superior border of dissection should be the axillary vein, anteriorly, which should be skeletonized. However, deep to the axillary vein and plexus, the axillary space extends superiorly and medially substantially above the level of the axillary vein, and that region should be cleared surgically, with careful attention to preservation of the long thoracic nerve, which runs along the chest wall to its origin from spinal nerves. The intercostobrachial nerve and lower intercostal nerves that run through the axillary space may usually be sacrificed. The pectoralis major and minor muscles are usually preserved along with the medial pectoral nerve and vessels, but in reoperative cases or cases with involvement of one or both of

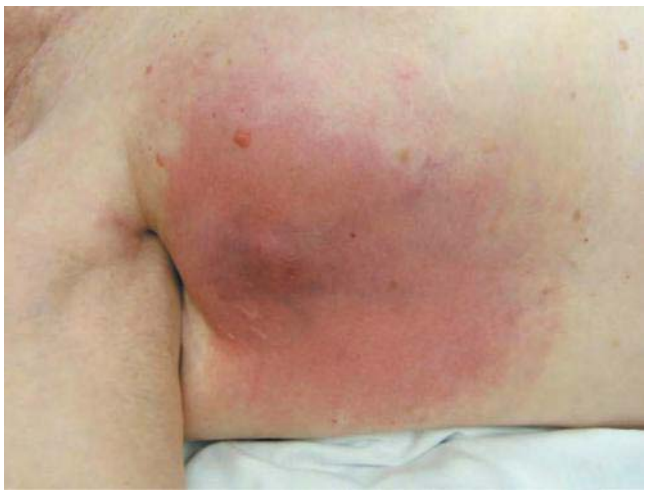

Figure 94.12 Extensive axillary adenopathy before resection.

these muscles, part of all of them may be sacrificed. It is rare for the long thoracic node to be involved with tumor, and it should be preserved because denervation of the serratus anterior muscle leads to "winged scapula" and can be associated with chronic pain related to destabilization of the shoulder. When there is bulky axillary disease, though, it is not uncommon for the thoracodorsal nerve to be involved, and patients usually tolerate sacrifice of that nerve when necessary. However, the possibility of resection of it should be discussed with patients preoperatively, especially when there is bulky adenopathy. In addition, if there is bulky axillary disease, the tumor often abuts or involves the axillary vein. The axillary vein usually consists of more than one vessel running in parallel; thus, sacrifice of the lowest limb of the axillary vein often is accomplished without evident morbidity. Even sacrifice of the entire axillary vein (one or several trunks) is usually tolerated well and can be considered in cases of advanced disease when necessary to enable complete resection of recurrent tumor. A troublesome finding is tumor involvement of the brachial plexus. Definitive therapy of that may require forequarter amputation, which is usually an unappealing option when the risk of systemic recurrence is also likely to be high, as it is in such cases. One alternative is to resect as much as possible while preserving the plexus, followed by adjuvant radiation therapy to the axilla. Another is to resect part of the brachial plexus, which has been reported, and in experienced hands can be done with reasonable outcomes.

In the vast majority of cases, even with some bulky disease, axillary dissection can be performed with minimal morbidity, with full expectation of full range of motion and function after recovery from the surgery over approximately 8 to 12 weeks. Lymphedema is an expected long-term complication, but it is usually a significant clinical issue in only about 10% of patients, and it can often be treated well with compression sleeve and/or manual lymphatic drainage therapy.

Inguinal and Iliac Dissection

For patients with metastatic melanoma to inguinal nodes, complete groin dissection is indicated. The nomenclature and clinical practice patterns vary for this procedure. As described by Spratt et al.,[166] the inguinal region can be defined as including the superficial inguinal region, which is superficial to the fascia that lies immediately superficial to the femoral vessels, and the deep inguinal region, which is deep to that fascia and includes the femoral vessels. The saphenous vein enters the femoral vein in the upper third of the inguinal region and passes through a foramen in the deep fascia. Cloquet node is the deep inguinal node that is classically considered to be the transitional node between the inguinal region and the iliac region. Although it is variable in its location, presence, and size, a superficial groin dissection should include removal of that node and identification of it for histologic evaluation. If that node contains metastatic melanoma, an iliac and obturator dissection is usually indicated. When patients have extensive nodal disease in the inguinal region, a complete inguinal dissection is appropriate, with skeletonization of the femoral artery and vein, often with a Sartorius flap to cover these vessels. However, for completion node dissection after a positive SNBx, a superficial inguinal dissection with excision of Cloquet node may be performed. Cloquet node is accessible through the foramen in which the saphenous bulb is found and is located lateral to the saphenous bulb.

Some surgeons describe the iliac region as the deep groin, but this terminology can lead to ambiguity. An iliac node dissection involves skeletonizing the external iliac vessels and is generally combined with removal of the iliac node-bearing tissue and obturator fat pad (obturator dissection). This dissection extends from the inguinal ligament to the takeoff of the internal iliac vessels and can be performed in continuity with the inguinal dissection or through a separate lower-quadrant abdominal wall incision and a retroperitoneal approach.

Patients with known inguinal metastases should undergo CT scan of the pelvis or PET/CT scan. Clinical evidence of iliac adenopathy or a positive Cloquet node at the time of groin dissection is an indication for iliac and obturator dissection. There is a range of clinical practice; some surgeons perform iliac dissections routinely for patients with extensive inguinal adenopathy.

The risk of lymphedema with inguinal or ilioinguinal dissection is greater than for axillary or cervical node dissections. Although most patients recover well, some degree of lymphedema probably occurs in most patients with this procedure. It may require a fitted compression stocking or massage therapy approaches.

Cervical Dissection

Metastatic melanoma to a cervical node is appropriately managed by complete neck dissection. A modified radical neck dissection should be performed, with preservation of the internal jugular vein, sternocleidomastoid muscle, and spinal accessory nerve. However, if these structures are invaded by tumor or involved with tumor, they can be resected. Sacrifice of the spinal accessory nerve can cause significant morbidity but is occasionally necessary. Melanomas of the face, ear, or anterior scalp often drain to parotid or periparotid nodes. In such cases, superficial parotidectomy is indicated as part of a modified radical neck dissection. In some situations, such as metastases to submental nodes near the midline or very low cervical nodes from a medial shoulder primary, there may be rationale for a neck dissection that is limited based on lymphatic anatomy.

MANAGEMENT OF REGIONAL METASTASES IN PATIENTS WITH VISCERAL OR OTHER DISTANT DISEASE

Regional metastases are common also in patients with advanced distant disease. Management of regional metastases can be important for clinical management even in the setting of distant disease, especially when painful or presenting with skin invasion and impending ulceration.

Melanoma Radiation Therapy

Data exist to support the use of adjuvant radiation to reduce primary and regional nodal recurrences in selected patient populations and for its use for palliation of unresectable primary and nodal recurrences or distant metastases. There is no current consensus regarding the optimal dose fractionation schedule for melanoma. Controversy surrounding the radiosensitivity of melanoma began in the early 1970s when cell survival curves for several human melanoma cell lines were published showing a broad shoulder indicative of high levels of potentially lethal damage repair. This fosters the hypothesis that melanomas were less likely to respond to conventionally fractionated radiation at 2 to 2.5 Gy per fraction and that higher dose per fraction schedules might result in superior clinical outcomes.[167] These studies caused many investigators to adopt high-dose (≥4 Gy per fraction) fractionation schedules for melanoma, and several investigators published improved clinical outcomes with these large fractional doses compared to conventional fractionation.[168] This led the Radiation Therapy Oncology Group (RTOG) to initiate RTOG 83-05, which was a prospective randomized trial comparing the effectiveness of high dose per fraction radiation and conventionally fractionated radiation in the treatment of melanoma. RTOG 83-05 randomized 126 patients with measurable lesions to 8.0 Gy × 4 fractions (32 Gy total) in 21 days delivered once weekly or 2.5 Gy × 20 fractions (50 Gy total) in 26 to 28 days delivered 5 days a week. The study was closed early when interim statistical analysis suggested that further accrual would not reveal a statistical difference between the arms. The 8.0 Gy × 4 fraction arm had a complete remission of 24% and

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partial remission of 36%, and the 20 × 2.5 Gy arm had a complete remission of 23% and partial remission of 34%.[168] This randomized trial demonstrated that melanoma is a radioresponsive tumor, and conventional and high dose per fraction schedules are equally effective clinically.

Despite the results of this study, many investigators still report that melanoma is a radioresistant histology and most current retrospective clinical reports regarding radiation for melanoma have used a high dose per fraction schedule. Although high dose per fraction treatments can result in increased risk of late radiation toxicity, there are little data to suggest that high dose per fraction schedules such as 30 Gy in five fractions over 2.5 weeks, which is currently commonly used in the adjuvant treatment of nodal basins following lymph node dissection,[169] results in increased late toxicity compared to conventionally fractionated regimens to 50 to 70 Gy. High-dose fractionation schedules are more convenient for the patient, less expensive, allow patients to proceed with systemic therapy sooner, and should be considered as a reasonable option unless critical structures are in the irradiated volume that would be treated above their radiation tolerance or the volume has previously been irradiated. They are particularly appropriate for patients with widespread disease and short life expectancies as they can provide rapid palliation, and late-radiation toxicity is not a concern for this patient population.

The Role of Radiation Therapy in the Management of Regional Nodal Disease

Patients with positive SNBx or palpable regional nodal metastases (stage III disease) are treated with therapeutic inguinal, axillary, or cervical lymph node dissections. Several large retrospective studies have identified lymph node extracapsular extension, large lymph nodes (≥3 cm in diameter), four or more involved lymph nodes, or recurrent disease after previous lymph node dissection as adverse risk factors that increase the risk for nodal basin recurrence following therapeutic nodal dissection to 30% to 50%.[76,170,171] Given the potential morbidity of recurrent unresectable nodal disease with pain, ulceration, bleeding, and lymphedema, these high-risk patients have been treated with high dose per fraction or conventionally fractionated postoperative adjuvant radiation delivered to nodal basins. Retrospective reports from several centers report 5-year locoregional control rates after radiation ranging from 80% to 93%.[169,171] There are no prospective randomized data comparing high dose per fraction schedules to conventional fractionation schedules to compare efficacy or toxicity between these treatment techniques; however, locoregional control rates appear to be equivalent with both schedules, resulting in 87% 5-year locoregional control rate in at least one report.[171]

Despite the improvement in locoregional control, postoperative adjuvant nodal irradiation has not shown a survival benefit. Most reports quote 5-year survival rates of 33% to 0%, which is similar to historic rates of patients not undergoing radiation or patients undergoing radiation following a re-excision of nodal failures, because of high rates of distant metastatic spread in this patient population.[169,171,172] Radiation complication rates in these retrospective studies vary depending on the site irradiated and include lymphedema, fibrosis, nerve plexopathies, and osteonecrosis, with lymphedema of the arm and leg being the major toxicity reported in most series.[169,171,172] Given the retrospective nature of these studies, it is not clear if some of these toxicities are solely attributable to radiation or are related to surgical morbidity or tumor recurrence.

In a multi-institutional report, the outcomes of 615 patients from MD Anderson and Roswell Park Cancer Centers with advanced regional nodal metastatic disease who underwent lymphadenectomy with and without adjuvant radiation were reported retrospectively.[173] On multivariate analysis, the number of positive lymph nodes, the number of lymph nodes removed, and the use of adjuvant radiation were associated with improved regional control. At a median follow-up of 60 months, regional failure occurred in 10% of patients with adjuvant radiation and 41% without adjuvant radiation. Distant metastatic disease developed in 55% of patients treated with adjuvant radiation and 74% of patients treated without adjuvant radiation. On multivariate analysis, disease-specific survival was reported to be significantly improved by the addition of adjuvant radiation.[173] In summary, retrospective data suggest that postoperative adjuvant radiation for patients with stage III disease results in improved locoregional control with reasonable complication rates but with no obvious survival benefit in most reports.

The Trans Tasman Radiation Oncology Group (Study 96.06) is the first prospective single-arm phase 2 study of adjuvant postoperative radiation therapy following lymphadenectomy in malignant melanoma with sufficient statistical power to answer its primary end points of regional in field relapse and late toxicity rates and secondary end points of adjacent relapse, distant relapse, OS, progression-free survival (PFS), and time to in-field recurrence. A total of 234 patients were treated with CLND followed by 48 Gy delivered in 20 fractions with 2.4 Gy per fraction over 4 weeks to the nodal basin using specified treatment guidelines. The reported overall pattern of first relapse showed a regional in-field recurrence rate of 6.8% and a distant relapse rate of 63%. The 5-year OS was 36% and the 5-year regional control rate was 91%. Grade 3 toxicity from axillary and inguinal lymphedema was 9% and 19%, respectively.[174] Burmeister et al.[175] reported results of the first multicenter prospective randomized trial (Trans Tasman Radiation Oncology Group 02.01/ANZMTG 01.02) comparing postoperative adjuvant radiation (48 Gy in 20 fractions) versus observation of patients with high-risk nodal metastatic melanoma (one or more parotid node, two or more cervical or axillary, or three or more groin-positive nodes, extracapsular spread, or ≥3 cm diameter cervical or axillary node or ≥4 cm groin node) following lymphadenectomy. A total of 250 patients were randomized and 217 were eligible for analysis; with a median follow-up of 27 months, the regional nodal failure rates were 19% for the radiation arm and 31% for the observation arm ($p = 0.041$) and median disease-free survival times and OS times were not significantly different ($p = 0.56$ and 0.12, respectively).[175] This study also found that the extent of extranodal extension (ENE) of melanoma independently predicted in-basin lymph node recurrences (14% for those without ENE, 17% with limited ENE, and 32% with extensive ENE, $p = 0.001$, multivariate).[175] Therefore, adjuvant radiation therapy to a nodal basin in patients with high risk of relapse after surgery improved local control at the expense of increased toxicities, but since the main risk in these patients is systemic relapse, then its use needs to be carefully evaluated in the setting of lack of improvement in PFS and OS.

ADJUVANT SYSTEMIC THERAPY (STAGES IIB, IIC, AND III)

Adjuvant Interferon Therapy

After over 50 years and in excess of 100 randomized clinical trials aimed at decreasing the relapse rate of melanomas at a high risk of relapse after surgery, only interferon-α (in two different formulations) has gained regulatory approval by either the FDA and/or the European Medicines Agency. Interferon-α–based adjuvant therapy has been administered in several variations: (1) HDI-α for 1 month followed by 1 year of intermediate dosing, (2) interferon-α at an intermediate dose administered for 1 or 2 years, (3) interferon-α at a low dose administered for 1 or 3 years, or (4) pegylated interferon administered for a target period of 5 years (Table 94.5). The multiple randomized clinical trials testing the benefits of interferon-α using these different regimens in the adjuvant setting have been subject of metanalysis.[176]

TABLE 94.5

Listing of Major Adjuvant Phase 3 Clinical Trials with Interon Alpha

Study Reference	No. of Patients Eligible for Analysis	TNM Stage	Therapy and IFN Subspecies	IFN Dose and Schedule—Treatment Arm	Median Follow-up at Time of Reporting (y)	DFS	OS	% Node positive
High Dose								
NCCTG 83-7052 Creagan[1]	262	II-III (T2-4N0M0/ TanyN+M0)	IFN-α2a vs. observation	IM 20 MU/m^2 3×/wk for 4 mo	6.1	NS	NS	61
ECOG E1684 Kirkwood[2]	287	II–III (T4N0M0/ TanyN+M0)	IFN-α2b—high dose vs. observation	IV 20 MU/m^2 5×/wk for 4 wk → then → SC 10 MU/m^2 3×/wk for 48 wk	6.9, 12.1	S	S (S at 6.9 y NS at 12.1 y)	89
ECOG E1690 Kirkwood[3]	642	II–III (T4N0M0/ TanyN+M0)	IFN-α2b—high dose vs. low dose vs. observation	High dose: IV 20 MU/m^2 5×/wk for 4 wk → then → SC 10 MU/m^2 3×/wk for 48 wk Low dose: SC 3 MU/m^2 2×/wk for 2 y	4.3, 6.6	S	NS	75
ECOG E1694 Kirkwood[4]	774	II–III (T4N0M0/ TanyN+M0)	IFN-α2b—high dose vs. GMK vaccine	IV 20 MU/m^2 5×/wk for 4 wk → then → SC 10 MU/m^2 2×/wk for 48 wk	1.3, 2.1	S	S	77
Italian Melanoma Intergroup Chiarion-Sileni[5]	330	III (TanyN1-3M0)	Intensified IFN-α2b every other month vs. IFN-α2b high dose for 1 y	Intensified high dose: IV 20 MU/m^2 5×/wk4 wk every other month for four cycles Standard high dose: IV 20 MU/m^2 5×/wk for 4 wk → then → SC 10 MU/m^2 3×/wkfor 48 wk	5.0	NS	NS	100
Intermediate Dose								
EORTC 18952 Eggermont[6]	1388	II–III (T4N0M0/ TanyN+M0)	IFN-α2b for 1 y vs. 2 y vs. observation	IV 10 MU 5×/wk for 4 wk → then → SC 10 MU 3×/wk for 1 y *OR* SC 5 MU 3×/wk for 2 y	4.65	NS	NS	74
EORTC 18991 Eggermont[7]	1256	III (TanyN+M0)	PEG IFN-α2b vs. observation	SC 6 μg/kg/wk for 8 wk → then → SC 3 μg/kg/wk for 5 y	3.8	S	NS	100
Low Dose								
AMCG Pehamberger[8]	311	II (T2-4N0M0)	IFN-α2a vs. observation	SC 3 MU 7×/wk for 3 wk → then → SC 3 MU 3×/wk for 1 y	3.4 (mean)	S	NS	0
FCGM Grob[9]	499	II (T2-4N0M0)	IFN-α2a vs. observation	SC 3 MU 3×/wk for 3 y	>3	0.74 (HR), S	0.70 (HR), S	0
WHO-16 Cascinelli[10]	444	III (TanyN+M0)	IFN-α2a vs. observation	SC 3 MU 3×/wk for 3 y	7.3	NS	NS	100

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Scottish Melanoma Cooperative Group Cameron[11]	96	II–III (T3-4N0M0/ TanyN+M0)	IFN-α2a vs. observation	SC 3 MU 3×/wk for 6 mo	6.5	NS	NS	Not available
EORTC 18871/ DKG-80 Kleeberg[12]	728	II–III (T3-4N0M0/ TanyN+M0)	IFN-α2b vs. IFN-γ vs. ISCADOR M (Weleda, Basel, Switzerland) vs. observation	IFN-α2b: SC 1 MU every other day for 12 mo	8.2	NS	NS	58
				IFN-γ: SC 0.2 mg every other day for 12 mo		NS	NS	
				ISCADOR M		NS	NS	
UKCCCR/AIM HIGH Hancock[13]	674	II–III (T3-4N0M0/ TanyN+M0)	IFN-α2a vs. observation	SC 3 MU 3×/wk for 2 y	3.1	NS	NS	70
DeCOG Hauschild[14]	840	III (T3anyN+M0)	IFN-α2a for 18 mo (A) vs. 3 y (B)	SC 3 MU 3×/wk for 18 mo vs. 3 y	4.3	NS	NS	18
DeCOG Garbe[15]	441	III (TanyN+M0)	IFNα2a (A) vs. IFNα2a + dacarbazine (B) vs. Observation (C)	SC 3 MU 3×/wk for 24 mo (A) vs.	3.9	S	S	100%
				SC 3 MU 3×/wk for 24 mo + DTIC 850 mg/m^2 every 4–8 wk for 24 mo (B) vs.		NS	NS	

TNM, tumor, node, metastasis; IFN, interferon; DFS, disease-free survival; OS, overall survival; NCCTG, North Central Cancer Treatment Group; IM, intramuscular; NS, not significant; ECOG, Eastern Cooperative Oncology Group; IV, intravenous; SC, subcutaneous; S, significant; AMCG, Austrian Melanoma Cooperative Group; FCGM, French Melanoma Cooperative Group; HR, hazard ratio; WHO, World Health Organization; EORTC, European Organisation for Research and Treatment of Cancer; UKCCCR, United Kingdom Co-ordinating Committee on Cancer Research; DeCOG, Dermatologic Cooperative Oncology Group.
Provided by Ahmad Tarhini (Adapted from Davar D, Tarhini AA, Kirkwood JM. Adjuvant immunotherapy of melanoma and development of new approaches using the neoadjuvant approach. *Clin Dermatol* 2013;31:237–250).

High Dose

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Intermediate

6. Eggermont AM, Suciu S, MacKie R, et al. Post-surgery adjuvant therapy with intermediate doses of interferon alfa 2b versus observation in patients with stage IIb/III melanoma (EORTC 18952): randomised controlled trial. *Lancet* 2005;366:1189–1196.
7. Bouwhuis MG, Suciu S, Testori A, et al. Phase III trial comparing adjuvant treatment with pegylated interferon Alfa-2b versus observation: prognostic significance of autoantibodies—EORTC 18991. *J Clin Oncol* 2010;28:2460–2466.

Low Dose

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14. Hauschild A, Weichenthal M, Rass K, et al. Efficacy of low-dose interferon {alpha}2a 18 versus 60 months of treatment in patients with primary melanoma of >= 1.5 mm tumor thickness: results of a randomized phase III DeCOG trial. *J Clin Oncol* 2010;28:841–846.
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TABLE 94.6

Summary of Ongoing Phase 2 Adjuvant Trials in High-Risk Melanoma

Study Reference	No. of Patients	TNM Stage	Therapy	Dose and Schedule—Treatment Arm	Primary Endpoint	ClinicalTrials.gov Identifier
EORTC 18071	950	III (T_{any}, N+ except in transit, M0)	Ipilimumab vs. placebo	IV, 10 mg/kg, 4× every 21 d, then starting from week 24 every 12 wk until week 156 or progression, 3 y	RFS	NCT00636168
US Intergroup E1609	1,500	III (IIIB, IIIC), IV (M1a, M1b)	Ipilimumab at 10 mg/kg (Arm A) or 3 mg/kg (Arm C) vs. high-dose interferon-alpha (Arm B)	IV, 10 mg/kg (A) or 3 mg/kg (C), 4× every 21 d, then starting from week 24 every 12 wk, 4× Vs. IV 20 MU/m^2 5×/wk for 4 wk, then SC 10 MU/m^2 3×/wk for 48 wk	RFS and OS	NCT01274338
COMBI-AD	852	III BRAF V600E/K mutation-positive	Dabrafenib + trametinib vs. placebo	Dabrafenib (150 mg twice daily) and trametinib (2 mg once daily) orally for 12 mo	RFS	NCT01682083
BRIM 8	725	IIC, III BRAF V600 mutation positive by Cobas (Roche, Basel, Switzerland)	Vemurafenib vs. placebo	Vemurafenib 960 mg orally twice daily for 52 wk	DFS	NCT01667419
DERMA	1349	IIIB or IIIC (tumor expression of MAGE-A3 gene)	GSK 2132231A (D1/3-MAGE-3-His fusion protein) vs. placebo	GSK 2132231A IM solution, 13 injections over 27 mo	DFS	NCT00796445

TNM, tumor, node, metastasis; EORTC, European Organisation for Research and Treatment of Cancer; IV, intravenous; RFS, relapse-free survival; OS, overall survival; SC, subcutaneous; DFS, disease-free survival; IM, intramuscular.
Provided by Ahmad Tarhini (Adapted from Davar D, Tarhini AA, Kirkwood JM. Adjuvant immunotherapy of melanoma and development of new approaches using the neoadjuvant approach. *Clin Dermatol* 2013;31:237–250).

Data from a total of 10,499 participants enrolled in 18 randomized clinical trials were reviewed, allowing the evaluation of the therapeutic efficacy of interferon in terms of disease-free survival (17 trials) and OS (15 trials). Adjuvant interferon was associated with significantly improved disease-free survival (HR = 0.83; 95% CI, 0.78 to 0.87, $p < 0.00001$) and OS (HR = 0.91; 95% CI, 0.85 to 0.97; $p = 0.003$). Subgroup analyses failed to detect a significant impact of different regimens of interferon-α administration or patient subgroups with different benefit to this mode of therapy. The use of adjuvant interferon-based therapy has become the standard of care therapy in most melanoma centers and in the community, and it is the basis of most comparator arms in ongoing adjuvant therapy clinical trials (Table 94.6).

High-Dose Interferon-α2B

Adjuvant HDI-α2b was approved by the FDA in 1996 for the treatment of resected stage IIB and stage III melanoma based on the results of the Eastern Cooperative Oncology Group (ECOG) trial E1684.[177] Interferon-α was administered by intravenous infusion, 20 million U/m^2, for 5 consecutive days every 7 days for 4 weeks during the "induction" phase. For the subsequent 48 weeks, 10 million U/m^2 were administered by subcutaneous injection on alternate days for a total of three doses every 7 days in the "maintenance" phase. The control arm was observation, which was the standard at the time that the trial was conducted. A total of 287 patients were enrolled, 80% of whom had stage III melanoma; 20% had stage IIB melanoma. Pathologic staging was performed with regional lymph node dissection because SNBx had not yet been introduced. Overall survival was the primary end point, and the trial was designed to detect a 33% improvement. A protocol-specified analysis with median follow-up of 6.9 years revealed a statistically significant 33% improvement by HR in OS compared to the observation arm after adjusting for other prognostic factors in a multivariate model ($p = 0.012$). Relapse-free survival was also significantly improved (39% improvement by HR compared to observation after adjusting for other prognostic factors; $p = 0.001$). Approximately three-fourths of the interferon-treated patients experiencing grade 3 or 4 toxicities by National Cancer Institute Common Toxicity Criteria. The most common were fatigue, asthenia, fever, depression, and elevated liver transaminases. A subsequent quality-of-life analysis of this trial population suggested that the toxicity associated with this regimen was largely compensated for by the psychological benefit derived from prevention of disease relapse.[178]

Since the reporting of E1684, several other studies of HDI have been conducted. Overall, nearly 2,000 patients with stage IIB and III melanoma have participated in four multicenter, randomized trials, conducted by the US Intergroup, investigating adjuvant HDI-α2b therapy. Data from these E1684, E1690, E1694, and E2696 clinical trials was updated to April 2001.[179] Analysis of treatment effects versus observation was based on data from 713 patients randomized to HDI or observation in trials E1684 and E1690. Overall, this updated analysis confirmed the original conclusions with the extended median follow-up intervals of 2.1 to 12.6 years. Relapse-free survival, but not OS, was significantly prolonged (two-sided log-rank $p = 0.006$) for patients treated with HDI versus observation. Among all patients, ulceration, recurrent disease at entry, enrollment in E1684, and age >49 years significantly negatively impacted relapse-free survival.

The North Central Cancer Treatment Group trial tested a regimen of 20 MU/m^2 dose of interferon-α2a administered intramuscularly three times per week for 12 weeks for stage II and III disease. This clinical trial demonstrated improvements in median disease-free survival and OS that were nonsignificant, with higher-risk patients appearing to benefit disproportionately.[180]

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Several clinical trials have tested a variety of regimens using lower doses of interferon, which have been conducted with the goal of decreasing toxicities while maintain efficacy. EORTC 18871 used a very-low-dose regimen (1 MU subcutaneously every other day), while the low-dose regimen of 3 MU subcutaneously thrice weekly was tested in WHO Melanoma Program Trial 16[181] (stage III), Scottish Melanoma Cooperative Group trial[182] (stage IIB/III), UK Co-ordinating Committee on Cancer Research AIM-High trial[183] (stage IIB/III), E169020 (T4, N1), and the 2010 German Dermatologic Cooperative Oncology Group (DeCOG) study[184] (T3Nx). None of these clinical trials showed a benefit in terms of OS. The 2008 German DeCOG study demonstrated a survival benefit for low-dose interferon but was powered primarily to assess the benefit of combination low-dose irradiation with dacarbazine and not designed to evaluate the low-dose regimen per se.[185] A number of trials have tested intermediate-dose interferon. EORTC 18952 demonstrated a 7.2% increase in distant metastases-free survival in patients with stage IIB/III, which was not statistically significant and no OS benefit was observed.[186]

Shortening the duration of therapy with HDI has also been a focus of clinical testing. The Italian Melanoma Intergroup enrolled 336 patients with stage III disease to receive either standard HDI or an intensified regimen (20 MU/m^2 intravenously 5 days/week for 4 weeks every other month for four cycles). At the 5-year mark, there were no statistically significant differences in either relapse-free survival or OS while toxicities did not increase.[187]

It had been postulated that the first month of intravenous HDI-α2b may be the main component providing benefit in HDI regimens. To test this concept, the ECOG E1697 randomized patients to 4 weeks of intravenous HDI with no maintenance therapy compared to observation. However, the interim analysis of E1697 after 1,150 of an originally planned 1,420 patients led to closing the study for futility.[188]

Pegylated Interferon-α

Pegylated interferon-α2b was approved by the FDA in 2011 for the adjuvant treatment of melanoma with microscopic or gross nodal involvement (stage III) within 84 days of definitive surgical resection including complete lymphadenectomy based on the results of EORTC 18991.[189] This was a randomized clinical trial comparing pegylated interferon-α2b with observation. Pegylation results in substantially slower clearance of interferon after administration. This allows for more stable drug exposure than can be achieved with the shorter-lived conventional interferon-α administered on alternating days by subcutaneous injection. Pegylated interferon can be administered less frequently and at a lower dose per injection but maintaining drug exposure over the course of several days. This results in a lower peak concentration after each dose while increasing the interval during which interferon is at biologically active concentrations in blood. Short-term[189] and long-term follow-up data has been provided on the EORTC 18991 clinical trial.[190] In this study, 1,256 patients with resected stage III melanoma were randomly assigned to observation (n = 629) or pegylated interferon 6 mcg/kg per week for 8 weeks by subcutaneous injection, followed by maintenance at 3 mcg/kg weekly (n = 627) for an intended duration of 5 years. Patients were prospectively stratified according to microscopic (N1) versus macroscopic (N2) nodal involvement, number of positive nodes, ulceration and tumor thickness, sex, and center. The primary end point was recurrence-free survival, and OS was a secondary end point. At 7.6 years of median follow-up, 384 recurrences or deaths had occurred with pegylated interferon versus 406 in the observation group (HR = 0.87; 95% CI, 0.76 to 1.00; p = 0.055); 7-year recurrence-free survival rate was 39% versus 35%. There was no difference in OS (p = 0.57). In stage III-N1 ulcerated melanoma, recurrence-free survival (HR = 0.72; 99% CI, 0.46 to 1.13; p = 0.06), and OS (HR = 0.59; 99% CI, 0.35 to 0.97; p = 0.006) were prolonged with pegylated interferon. Despite the anticipation that pegylated interferon would be better tolerated than HDI, pegylated interferon was discontinued for toxicity in 37% of patients.

Cytotoxic Chemotherapy and Biochemotherapy in Adjuvant Therapy of Melanoma

There has been a long list of prospective randomized clinical trials testing single-agent or combination-agent chemotherapy in the adjuvant setting, which have been nearly universally negative in terms of providing a clear clinical advantage over the control arm. For example, dacarbazine was not effective in the postoperative setting, whether administered alone or combined with BCG.[99] The only clearly positive clinical trial has been US intergroup study led by SWOG S0008, a phase 3 clinical trial of a biochemotherapy combination compared to standard HDI-α in patients with high-risk melanoma.[191] The investigators sought to determine whether a short course of biochemotherapy would be more effective than HDI as adjuvant treatment in patients with high-risk melanoma. S0008 enrolled patients with stage IIIA-N2a through stage IIIC-N3, who were randomized to either HDI following the E1684 schedule, or biochemotherapy consisting of dacarbazine 800 mg/m^2 on day 1, cisplatin 20 mg/m^2 on days 1 to 4; vinblastine 1.2 mg/m^2 on days 1 to 4; IL-2 9 MU/m^2 per day continuous intravenous administration on days 1 to 4; interferon 5 MU/m^2 per day subcutaneously on days 1 to 4, 8, 10, and 12; and granulocyte–colony-stimulating fator 5 ug/kg per day subcutaneously on days 7 to 16. Biochemotherapy cycles were given every 21 days for three cycles (9 weeks total). Patients were stratified for number of involved nodes (one to three versus four or more), micro- versus macrometastasis, and ulceration of the primary. Coprimary end points were relapse-free survival and OS. Between 2000 and 2007, 432 patients were enrolled. Grade 3-4 adverse events occurred in 64% of patients receiving HDI and 76% of patients randomized to biochemotherapy. At a median follow-up of 7.2 years, biochemotherapy improved relapse-free survival (p = 0.015; HR = 0.75; 95% CI, 0.58 to 0.97), with median relapse-free survival for biochemotherapy of 4.0 years (95% CI, 1.9 years—not reached) versus 1.9 years for HDI (95% CI, 1.2 to 2.8 years), and a 5-year relapse-free survival of 48% versus 39%. However, the OS was not different between the two arms (p = 0.55; HR = 0.98; 95% CI, 0.74 to 1.31), with median OS of 9.9 years (95% CI, 4.62 years—not reached) for biochemotherapy versus 6.7 years (95% CI, 4.5 years—not reached) for HDI, and 5-year OS of 56% for both arms. It was concluded that biochemotherapy could be considered as a shorter alternative to HDI for patients with high-risk melanoma.

Experimental Adjuvant Immunotherapy

There has been a long list of large adjuvant clinical trials with a variety of immunotherapy approaches for melanoma with negative results. The studies used whole tumor cell vaccines like Melacine (Corixa Corporation, Seattle, WA) or Cancervax (Marina del Ray, CA), MAGE-A3 (GlaxoSmithKline, Brentford, UK), ganglioside-based vaccines like GMK (Progenics Pharmaceuticals, Inc., Tarrytown, NY), and smaller studies with melanosomal antigen peptide vaccines. Furthermore, uncontrolled studies in patients with completely surgically resected stage IV (stage IV with no evidence of disease) led to the testing of adjuvant GM-CSF and a multipeptide vaccine in a placebo-controlled, randomized, prospective trial (ECOG E4697). That trial accrued approximately 800 patients but showed no significant beneficial impact of GM-CSF on clinical outcomes.[192] Recent data highlight the critical nature of vaccine adjuvants. Murine and human data suggest that use of incomplete Freund adjuvant with short peptide vaccines may interfere with protective antitumor T-cell responses by recruiting T cells selectively back to the vaccine site rather than to the tumor.[193] On the other hand, vaccines may be

made much more effective at inducing T-cell responses in patients by incorporating a toll-like receptor 9 agonist CpG.[194] Thus, new vaccine approaches will explore the value of more optimal adjuvants. Also, the availability now of clinically effective immune therapies for advanced melanoma promises to open the door to new combination immune therapies in the adjuvant setting that will be explored in clinical trials over the next few years (see Table 94.6).

Cytotoxic T-Lymphocyte Antigen 4 and PD-1 Blockade in Adjuvant Therapy

Ipilimumab is a fully human immunoglobulin G1 monoclonal antibody that blocks CTLA4, which has demonstrated improvement in OS in the treatment of metastatic melanoma in two randomized clinical trials.[195,196] This has led to clinical trials investigating the potential for ipilimumab in the adjuvant setting (see Table 94.6). EORTC 18071 is comparing ipilimumab at 10 mg/kg with maintenance therapy against placebo. Accrual is complete and results are pending. E1609 is an accruing clinical trial randomizing patients to ipilimumab at 10 mg/kg or 3 mg/kg for four doses, compared to a control arm receiving standard high-dose adjuvant interferon therapy. Enrollment on the 10 mg/kg arm was put on hold in 2013. Results are not anticipated until 2015. Early evaluation of clinical trials testing anti-PD-1 and anti-PD-L1 antibodies in patients with metastatic melanoma suggest that this class of immune modulating antibodies is likely to provide a high rate of durable tumor responses. S1404 is a planned clinical trial testing the anti-PD-1 IgG4 monoclonal antibody MK-3475 (lambrolizumab) compared to HDI. Results of this trial will take 3 to 4 years.

Neoadjuvant Therapy for Resectable Stage III or IV Melanoma

Neoadjuvant therapy for resectable stage III or stage IV melanoma remains an investigational approach. A study of neoadjuvant interferon for patients with palpable regional lymph node metastases was associated with an objective tumor response rate of 55%.[197] Further studies of this or other neoadjuvant therapies provide opportunities to investigate tumor biology in the tumor microenvironment and may lead to better understanding of the mechanism of antitumor effects of novel therapies.

Clinical Follow-Up for Patients with Regionally Metastatic Melanomas (Stage III)

There is no agreed follow-up plan for surgically resected melanoma. Most studies conducted to date were retrospective analyses of patients diagnosed and relapsing in the era when there were very limited truly active treatment options. Therefore, diagnosing patients at an earlier time point had very little chance of improving outcomes other than resulting in a lead time bias in the assessment of survival. This situation has led to a series of guidelines for follow-up of patients. Such guidelines have been proposed based on an analysis of the site and timing of first relapse in patients with surgically excised stage III melanoma at a single institution.[198] This was based on a review of the clinical records at Memorial Sloan-Kettering Cancer Center between 1992 and 2004 of patients who ultimately relapsed after surgical resection of stage III melanoma. In this group of patients, the overall 5-year relapse-free survival for patients with stage IIIA, IIIB, and IIIIC disease was 63%, 32%, and 11%, respectively. Site of first relapse was local/in-transit (28%), regional nodal (21%), or systemic (51%). First relapses were detected by the patient or family in 47% of cases, by the physician in 21%, and by screening radiologic tests in 32%. Based on these observations, the authors proposed that routine physical examinations beyond 3 years for stage IIIA, 2 years for stage IIIB, and 1 year for stage IIIC; radiologic imaging beyond 3 years for stages IIIA and IIIB; and 2 years for stage IIIC would be expected to detect few first systemic relapses.

National/International Guidelines for Follow-Up

Recommendations for patient follow-up are largely based on the time-dependent risk of recurrence, the likely sites of metastasis, and historical experience with whether recurrences are commonly identified by the patient, the physician, or by imaging or laboratory studies. However, there is also evidence that follow-up is comforting to patients and decreases psychological stress associated with the diagnosis.[199] No studies have shown a clear benefit in terms of survival with closer follow-up, but this could change as there are now effective therapies for advanced melanoma, so that diagnosis of patients when they are well enough to tolerate those therapies may be of clinical value. Thus, routine clinical follow-up continues to be a part of management, but studies of follow-up have mostly been retrospective, and there is a need for more rigorous studies of the global benefit, risk, and cost of follow-up visits, imaging, and serum markers. Two comprehensive guidelines on melanoma management provide guidance on follow-up based on systematic review of the literature, and expert opinion. The NCCN® has been reported in their 2013-14 guidelines,[128] summarized in Table 94.7. Also, evidence-based guidelines were published in 2013 from Germany, from the German Dermatological Society and the DeCOG.[200] These German S3 guidelines are also summarized in the Table 94.6. These two guidelines are very similar, but differ in the extent of cross-sectional imaging recommended, and the use of ultrasound for evaluation of the node basins. The German guidelines also recommend serum S100B levels in follow-up, whereas these are not recommended by the NCCN. Other minor differences are also evident. The NCCN Guidelines also recommend self-exam by patients on a regular basis as well as annual body skin exams for life, for all patients with a history of stage 0-IV melanoma in addition to the recommendations in the table.

Follow-up visits should include history and physical exam, which should focus on skin exam of the primary site, regional nodes, and in-transit sites. Self-examination by the patient is also recommended, which depends on education of the patient and/or family about what findings may signal recurrence. Chest X-rays were not recommended, but there was lower consensus (77%) for this than for most other German S3 recommendations.

MANAGEMENT OF DISTANT METASTASES OF MELANOMA (STAGE IV)

Any patient with distant metastases is considered stage IV. Distant metastases may include skin or soft tissue metastases distant from a known primary site or visceral, bone, or brain metastases. The prognosis is better for skin and subcutaneous tissue metastases, which are considered M1a, than for lung metastases (M1b) or other distant metastases (M1c). In addition, an elevated serum LDH in the setting of distant metastases is associated with a poor prognosis and also is considered M1c disease.[74]

Timing of Distant Metastases

It is uncommon for patients with melanoma to present initially with stage IV disease. Most patients who develop distant metastases do so after an interval from their original management for clinically localized disease or after management for regionally metastatic disease. Often, metastases become evident within 2 to 3 years of diagnosis, but delayed metastasis is also common, and for melanoma, regional and distant metastases have occurred after

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TABLE 94.7

Recommendations for Melanoma Follow-Up Adapted from the National Comprehensive Cancer Network V2.2014 and German S3 Guidelines

		Year 1	Year 2	Year 3	Year 4	Year 5	Years 6–10
IA	NCCN	H&P(q6-12)	H&P(q6-12)	H&P(q6-12)	H&P(q6-12)	H&P(q6-12)	[a]
	G-S3	H&P(q6)	H&P(q6)	H&P(q6)	H&P(q12)	H&P(q12)	H&P(q12)
IB-IIA	NCCN	H&P(q6-12)	H&P(q6-12)	H&P(q6-12)	H&P(q6-12)	H&P(q6-12)	[a]
	G-S3	H&P(q3) U/S(q6) S100B(q3)	H&P(q3) U/S(q6)[b] S100B(q3)	H&P(q3) U/S(q6) S100B(q3)	H&P(q6)	H&P(q6)	H&P(q6-12)
IIB	NCCN[d]	H&P(q3-6) Image(q4-12) MRI(q12)	H&P(q3-6) Image(q4-12) MRI(q12)	H&P(q3-12) Image(q4-12) MRI(q12)	H&P(q3-12) Image(q4-12) MRI(q12)	H&P(q3-12) Image(q4-12) MRI(q12)	[a]
	G-S3	H&P(q3) U/S(q6) S100B(q3)	H&P(q3) U/S(q6)[b] S100B(q3)	H&P(q3) U/S(q6) S100B(q3)	H&P(q6)	H&P(q6)	H&P(q6-12)
IIC-IV	NCCN[c,d]	H&P(q3-6) Image(q4-12) MRI(q12)	H&P(q3-6) Image(q4-12) MRI(q12)	H&P(q3-12) Image(q4-12) MRI(q12)	H&P(q3-12) Image(q4-12) MRI(q12)	H&P(q3-12) Image(q4-12) MRI(q12)	[a]
	G-S3	H&P(q3) U/S(q3) S100B(q3) CTs(q6)	H&P(q3) U/S(q3) S100B(q3) CTs(q6)	H&P(q3) U/S(q3) S100B(q3) CTs(q6)	H&P(q3) U/S(q6) S100B(q6)	H&P(q3) U/S(q6a) S100B(q6)	H&P(q6)

NCCN, National Comprehensive Cancer Network; H&P, history and physical exam; U/S, ultrasound exam of draining node basins; S100B = serum S100B level; MRI, magnetic resonance imaging.
[a] In addition, NCCN Guidelines recommend annual physical exams and history on an annual basis, as indicated, after 5 years. The German S3 guidelines extend to 10 years.
[b] Unless no SNBx, then like IIC.
[c] Image = consider chest X-ray, computed tomography, and positron emission tomography/computed tomography.
[d] For imaging in this stage, the NCCN Guidelines specify that imaging should be considered but is not mandatory.

disease-free intervals measured in decades.[201] In general, the interval to detection of distant metastases is shorter for patients who initially present with high-stage disease (e.g., stage IIB–III) and is longest for patients who present with clinically localized thin melanomas (e.g., stage IA).

Patterns of Metastases

Approximately 60% to 80% of first metastases are at local or regional sites including regional nodes. The most common first sites of visceral metastasis are lung and liver (about 10% each), and metastases to distant skin sites are also common. After an initial metastasis, subsequent metastases are more commonly visceral or distant and increasingly become multiple. Common visceral sites of metastasis are lung, liver, brain, gastrointestinal tract (especially small bowel), bone, and adrenal gland.

Prognostic Factors in Distant Metastatic Melanoma (Stage IV)

The new active systemic therapies for advanced melanoma are changing the prognosis of patients. However, no long-term follow-up is available for the most recent trials. Without treatment, or with mostly ineffective therapies, patients with stage IV melanoma were reported to have a median survival of 12 months, with 6 to 9 months for those who presented with visceral metastatic disease (M1c), as long as 15 months for those who presented with skin and lymph node metastases only (M1a), and patients with lung metastases as their only site of visceral organ involvement (M1b) had an intermediate prognosis.[76] Negative prognostic factors in stage IV melanoma also include a large number of metastatic sites, elevated LDH level, and poor performance status.[202]

Clinical Evaluation of Patients with Distant Metastasis (Stage IV)

When a patient is found to have a distant metastasis, the initial steps are to perform full staging studies. This typically should include MRI scan of the brain and either total-body PET/CT scan or CT scans of the chest, abdomen, and pelvis. Other scans or imaging studies (bone scan, soft tissue MRI, ultrasound, or plain films) may be indicated to evaluate known areas of metastasis (e.g., soft tissue masses in extremities) or to evaluate symptoms (e.g., plain films or bone scans for bony symptoms). Melanoma is usually highly avid for FDG uptake due to the strong Warburg effect (aerobic glycolysis even in the presence of sufficient glucose), and therefore metastatic lesions >5 mm are efficiently imaged by PET scans. An exception is uveal melanoma, which has variable FDG uptake even when metastatic. PET/CT scans are helpful in distinguishing tumor from scar in areas of prior surgery, although surgical sites may remain FDG-avid for up to 3 months after surgery. PET is substantially more sensitive for detection of small bowel metastases and lymph node metastases that are borderline in size.[203] PET/CT scan may also be helpful in assessing patients for resectability when there is limited disease on initial assessment.

Histologic or Cytologic Diagnosis

Patients being followed for a history of melanoma may develop new evidence of metastatic disease. In such patients, a new and growing mass in the chest or abdomen is likely to be metastatic melanoma, but tissue confirmation of metastatic melanoma is usually recommended. New masses can represent new primary lung cancers, lymphoma, sarcoid, inflammatory masses, or other changes, and the management and prognosis of these lesions usually differs dramatically from the management and prognosis of stage IV melanoma.

If the lesion is in an accessible area of the lung and is about 1 cm in diameter or greater, a CT-guided transthoracic needle biopsy is usually feasible and appropriate for making the diagnosis. If there is a solitary lung mass, and especially if the mass is <1 cm in diameter, then thoracoscopic resection with preoperative localization can be performed with great success and with low morbidity.[204] In the event that the lesion is malignant, then the biopsy may also have some therapeutic value.

Fine needle aspiration biopsy of soft tissue masses or lymph nodes can be rapid and accurate diagnostic approaches either at the bedside or with radiologic localization. Similarly, biopsies of many other tissue lesions can be accomplished by minimally invasive techniques. A fine needle aspirate will be diagnostic in most cases, but a core needle biopsy, when feasible, can improve diagnostic accuracy further. Immunohistochemical stains for S100, HMB45, tyrosinase, and MART-1/MelanA can all be helpful in confirming a diagnosis of melanoma.

Testing for BRAF and Other Genetic Analyses

A major decision point in the management of advanced melanoma is the determination of BRAF mutational status. The clinical development and approval of BRAF and MEK inhibitors has been based on the treatment of a patient population selected based on the expression of mutant BRAF at position V600. This is because preclinical studies predict that BRAF inhibitors are ineffective when BRAF mutations are not present, and there is even data that they may be detrimental by inducing paradoxical MAPK activation (see the following) and increased cancer progression.[205–208] Therefore, the decision to use BRAF inhibitors should be based on a positive testing for a BRAF V600 mutation (either V600E or V600K, with currently less clear benefits in other BRAF mutations).

BRAF mutation needs to be tested from DNA obtained from a melanoma biopsy or resection. It is best to test a metastatic lesion than an archival primary lesion as it cannot be assured that the metastases come from that particular primary lesion. The tumor DNA is usually obtained from formalin-fixed paraffin-embedded tissue blocks, and the assay laboratories usually isolate the genomic DNA from this fixed tissue. The actual BRAF mutation test can be performed using the mutation-specific PCR tests, such as the FDA-approved companion diagnostic assays for the use of vemurafenib, dabrafenib, or trametinib, or by less sensitive Sanger sequencing. In addition, assay panels that provide results from multiple hot-spot single nucleotide mutations have been developed, as well as assays based on next generation sequencing of a panel of several hundred genes that are commonly associated with cancer.[60]

Surgery for Distant Metastases (Stage IV)

Patient Selection and Prognostic Factors

Selected patients may benefit from surgery for distant metastatic (stage IV) melanoma. The benefit can be palliative in some patients and may be curative in rare cases. There are numerous clinical scenarios in which surgery may be considered, and it is not possible to address all of them here. However, it is useful to consider some of them.

Cases in Which the Benefit of Surgery Is Clear

- Anemia due to occult bleeding from intestinal metastasis
- Bowel obstruction due to small bowel metastasis
- Cutaneous or subcutaneous metastasis with ulceration, pain, or impending ulceration
- Lymph node metastasis with neurologic symptoms
- Symptomatic brain metastasis
- Life-threatening hemorrhage from metastasis

Melanoma frequently metastasizes to the gastrointestinal tract. It usually originates as an intramural lesion but grows into the lumen and through the serosa with time. These usually present as anemia due to occult gastrointestinal bleeding or as intermittent small bowel obstruction due to intussusception (Fig. 94.13). They are difficult to diagnose by CT scan in the absence of symptoms. PET/CT is probably the best study now available. However, it may miss small lesions. Nonetheless, when a patient presents with gastrointestinal blood loss or obstruction associated with a small bowel (or other gastrointestinal) metastasis of melanoma, operation is usually indicated. If the tumor involves the mesenteric nodes and is matted, then it may not be feasible or appropriate to resect the entire tumor, but enteroenteric bypass of the obstruction will be palliative. Resection of most or all small bowel metastases can manage bleeding and obstruction effectively. If there is a single small bowel metastasis, then a simple resection and reanastomosis is appropriate (Fig. 94.14). However, if there are numerous small bowel metastases, then excision of large lesions with reanastomosis is appropriate, but small lesions may be excised by partial-diameter excision and stapled (or sewn) closure. If the patient can be rendered surgically free of disease, then there may be long-term survival >5 years in as many as 25% of patients and mean survival >2 years.[209]

Cutaneous, subcutaneous, and nodal metastases are not usually a cause of death, but they can be a cause of substantial morbidity. As they grow, they develop substantial inflammation in the overlying skin (see Fig. 94.12) and without resection may often ulcerate. Because such lesions usually can be resected under local anesthesia with minimal morbidity, it is reasonable to offer resection.

Extensive lymph node metastasis with neurologic symptoms is commonly an issue in the axilla, where tumor growth may compress or invade the brachial plexus and axillary vein. Patients with extensive axillary recurrence with neurologic symptoms and patients with other nodal disease and neurologic symptoms should be considered for radical resection of the involved nodal basin. The morbidity of surgery usually is much less than the morbidity of the tumor left untreated. Major risks of tumor growth include paralysis or major neurologic dysfunction of the extremity, intractable lymphedema, disabling pain, and unresectability.

Brain metastasis is a particularly ominous sign in terms of future survival, which can usually be measured in months. However, some patients with isolated brain metastasis can have long-term control after surgical resection or stereotactic radiation therapy. For patients with symptomatic brain metastases, the presentation with acute cognitive deficits can be dramatic. Steroid therapy should be instituted immediately (4 mg orally every 6 hours per day initially). However, if this fails, or if the presentation is particularly acute with impending herniation, then surgical resection of the brain metastasis can be therapeutic.

Melanoma can metastasize to nodes, adrenal glands, or other sites and then develop spontaneous hemorrhage. Sometimes such bleeding can be trivial, but in some cases, there can be massive hemorrhage into the tissues, with associated hypovolemia. In such cases, resection of the hemorrhagic mass may diminish future risk of bleeding, decrease pain, and delay death.

New effective systemic therapies,[151] including CTLA4 blockade or mutant BRAF inhibition, as well as blockade of PD-1/PD-L1, may be alternatives for managing patients with metastases that are too extensive to resect, but a multidisciplinary team assessment is advised to weigh the short-term risks of delaying surgery against the possibility of major systemic tumor regression with those therapies. In cases where systemic therapy induces partial responses, surgical resection of residual gastrointestinal disease may be feasible to render the patient clinically free of disease.

Cases in Which the Benefit of Surgery Is Likely

- Solitary asymptomatic visceral metastasis resectable with minimal morbidity

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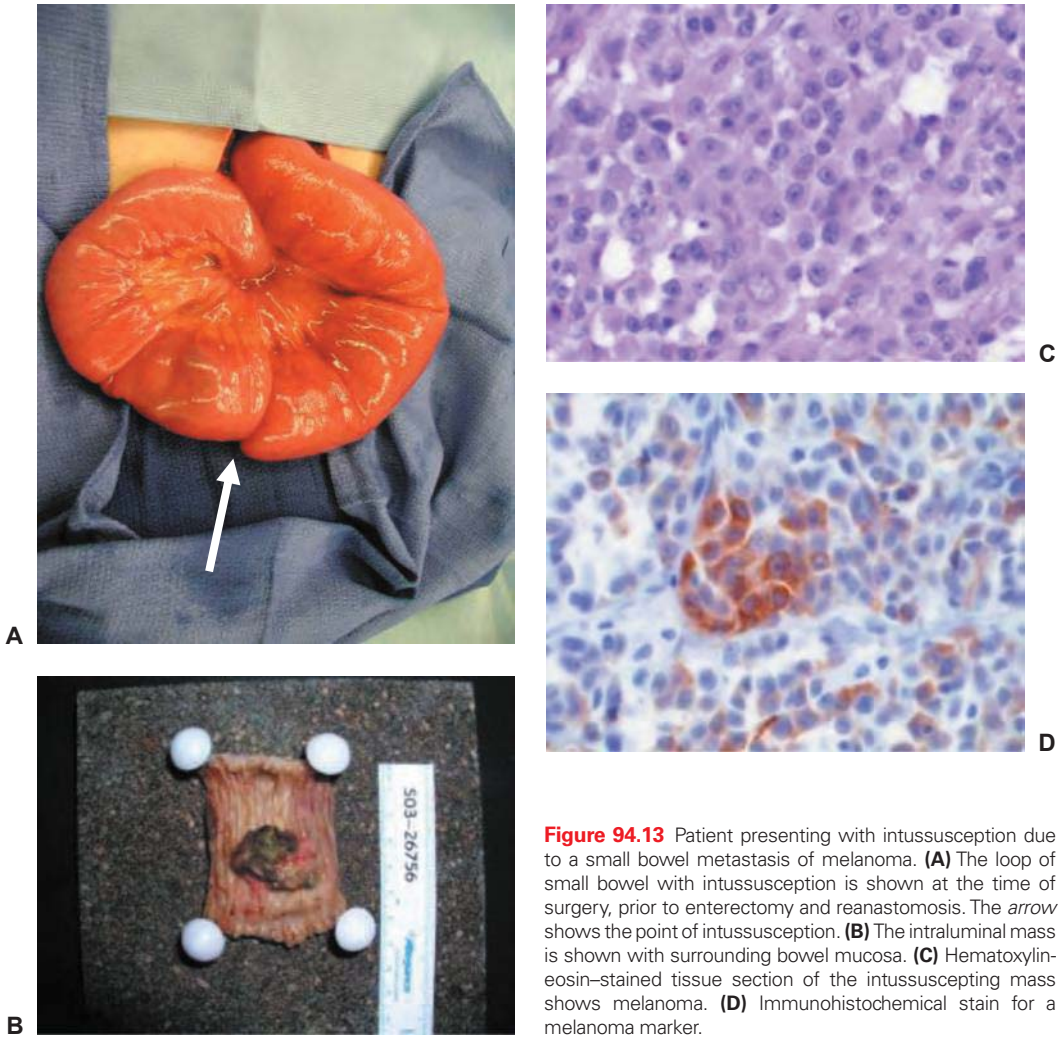

Figure 94.13 Patient presenting with intussusception due to a small bowel metastasis of melanoma. **(A)** The loop of small bowel with intussusception is shown at the time of surgery, prior to enterectomy and reanastomosis. The *arrow* shows the point of intussusception. **(B)** The intraluminal mass is shown with surrounding bowel mucosa. **(C)** Hematoxylin-eosin–stained tissue section of the intussuscepting mass shows melanoma. **(D)** Immunohistochemical stain for a melanoma marker.

- Bony metastasis with pain or joint involvement, unresponsive to radiation
- Solitary brain metastasis without symptoms
- Large, asymptomatic nodal metastasis with concurrent low-volume systemic disease

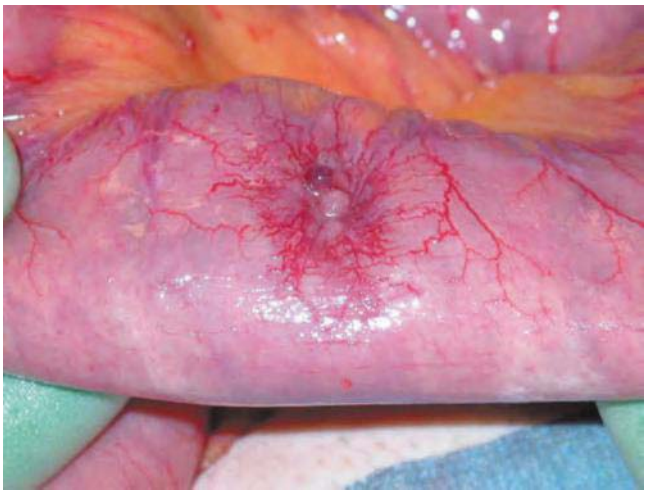

Figure 94.14 Small bowel metastasis of melanoma with extension through the bowel wall and with extensive neovascularity.

- Extensive skin and soft tissue metastases in the absence of visceral metastases
- Isolated growing metastasis in the setting of stable or regressing metastases after therapy

In general, in a patient with solitary visceral metastasis, if excision can be accomplished with minimal morbidity, the excision can be both therapeutic and diagnostic. The OS for patients with one or several distant metastases resected coupled with experimental melanoma vaccine therapy has been associated with 5-year survival rates in the 40% to 60% range.[210] Another reasonable option for the patient with a single (or few) resectable distant and/or visceral metastases is to enroll in an experimental therapeutic trial or to take an approved systemic therapy in the hope of clinical response but with the additional benefit of having about 3 months of observation time to be sure that no other new visceral lesions appear prior to resection of the lesion in question.

Bone metastases can cause pain and fracture. Radiation therapy is usually the first choice for therapeutic intervention if significant pain exists. If patients are at risk of impending fracture, orthopedic stabilization should be considered before radiation. However, if the lesion does not respond to radiation or is solitary, resection with bone grafting or joint replacement can be considered. Current success rates with such therapy are high, but the period of postoperative recovery can be extended, and so careful patient selection is indicated.

An asymptomatic solitary brain metastasis that is amenable to resection can often be removed surgically with minimal morbidity and often with approximately a 3-day hospital stay. Stereotactic

radiosurgery (e.g., gamma knife) is often the first choice for treatment of such lesions, but surgery is another reasonable option and probably will have benefit, especially if the solitary brain lesion is >2 to 3 cm diameter, in which case stereotactic radiosurgery may be less effective and surgery may be a preferred option.

In patients with multiple metastases, systemic therapy may be associated with partial clinical responses with progressive growth of one or more lesions while the remainder are stable or shrinking and asymptomatic. In that case, patients may benefit from resecting the one or several tumor deposits that are progressing. This will not be curative, but it may lead to a more prolonged period of good quality of life, with minimal perioperative morbidity.

Cases in Which Some Patients May Benefit from Surgery but Risk and Benefit Are Closely Balanced

- More than one visceral metastasis, without symptoms
- Multiple lung nodules
- Bilateral adrenal metastases
- Extensive skin and soft tissue metastases in the setting of visceral disease

A more difficult decision is whether to treat patients with surgery when they have multiple asymptomatic visceral metastases, such as multiple lung nodules, bilateral adrenal metastases, or extensive skin metastases in the presence of visceral disease. These are generally situations in which surgery is not recommended as the treatment of choice, but there are anecdotes of such patients enjoying prolonged disease-free survival after such surgery, and so it is worth considering in very selected patients. Situations that may push the patient and the clinician toward such an aggressive surgical approach include (1) prior failure of systemic therapy, (2) a young patient for whom perioperative morbidity is not a major concern, and (3) disease sites that are particularly amenable to surgery through limited surgery (e.g., multiple lung nodules amenable to thoracoscopic lobectomy).

Adjuvant Therapy for Resected Stage IV Melanoma

There is no standard adjuvant therapy after resection of metastatic melanoma (stage IV). Interferon has not been thoroughly evaluated in this setting. Therefore, observation remains the standard management of patients in this setting. Investigational vaccines, GM-CSF, CTLA4 blockade, PD-1 blockade, and other experimental therapies are being evaluated for these patients. ECOG 4697, a US intergroup clinical trial testing the potential benefit of GM-CSF as an adjuvant therapy for resected stage IIIB-IV melanoma, demonstrated no impact on survival overall.[192] Several ongoing adjuvant therapy clinical trials for stage III melanoma are open to enrollment to this patient population and are testing the potential benefits of adjuvant therapy with ipilimumab (ECOG 1609), vemurafenib single agent, or dabrafenib and trametinib in combination.

Treatment of Unresectable Metastatic (Stage IV) Melanoma

Progress in Treatment for Metastatic Melanoma

Clinical translation of preclinical scientific knowledge has resulted in the rapid advancement of new therapies active in patients with metastatic melanoma. This contrasts sharply with the lack of significant progress for many years when attempting to treat melanoma with nonspecific agents, in particular chemotherapy, and performing combination studies with low active components. In this context, the OS and PFS was analyzed in 70 US cooperative group single-arm phase 2 clinical trials performed between 1975 and 2005 (termed the Korn meta-analysis) that had been deemed to not be promising for further development.[211] From this meta-analysis, individual-level and trial-level data were obtained for patients enrolled onto 42 phase 2 trials. Prognostic factors for OS were performance status, presence of visceral disease, sex, and whether the trial excluded patients with brain metastases. The Korn meta-analysis has provided the minimum benchmarks of OS and PFS at defined time points to compare to new clinical trials in patients with metastatic melanoma. The recent clinical trials with immune checkpoint inhibitors and BRAF inhibitor–based targeted therapies have improved this grim panorama (Table 94.8), and there are reasons to anticipate that treatment options for advanced melanoma will continue to improve. The ability to understand mechanisms of response and resistance to BRAF inhibitor–based therapies and immune checkpoint blockade at a molecular level, and the rapid advancement of the knowledge brought through by the scientific community's renewed interest in melanoma, predicts further improvements in the development of effective therapies for this disease.

TABLE 94.8

Summary of Best Clinical Endpoints with Selected Therapies for the Treatment of Advanced Melanoma

Improved overall survival	Ipilimumab (vs. gp100 peptide vaccine) Vemurafenib (vs. dacarbazine) Trametinib (vs. dacarbazine)
Improved progression-free survival	Dabrafenib (vs. dacarbazine) Nab-paclitaxel (vs. dacarbazine) Dabrafenib + trametinib (vs. dabrafenib)
Improved response duration	T-Vec (vs. granulocyte macrophage–colony-stimulating factor)
Validated antitumor activity	High dose interleukin-2 TIL ACT Carboplatin-paclitaxel Dacarbazine Fotemustine
Promising new agents and combinations	Anti-PD-1 antibodies: nivolumab, MK3475, MEDI Anti-PD-L1: BMS559, MPDL8032A BRAF and MEK inhibitor combination Anti-PD-1 and anti-CTLA4 combination
Agents and combinations not supported by data	Nistosurea combination chemotherapy: CVD, biochemotherapy Darmuth regimen and tamoxifen-chemotherapy combinations Thalidomide and thalidomide-chemotherapy combinations Sorafenib and sorafenib-chemotherapy combinations Elesclomol-chemotherapy combinations Peptide vaccines

TIL ACT, tumor infiltrating lymphocyte adoptive cell transfer therapy; PD, programmed death; CTLA, cytotoxic T lymphocyte associated-antigen; CVD, cisplatin-vinblastin-dacarbazine.

Anti–Cytotoxic T-Lymphocyte Antigen 4 Blocking Antibodies

Approaches such as inactivated tumor vaccines, dendritic cell vaccines, or immune-stimulating cytokines like interferon-α and IL-2 are aimed at turning on T cells against cancer. This has led to tumor responses in a minority of patients, but with the remarkable feature that these tumor responses tend to be durable (counted in years) in most cases. This feature, together with the lack of significant activity of standard therapy approaches for melanoma, has maintained the interest in this mode of therapy for advanced

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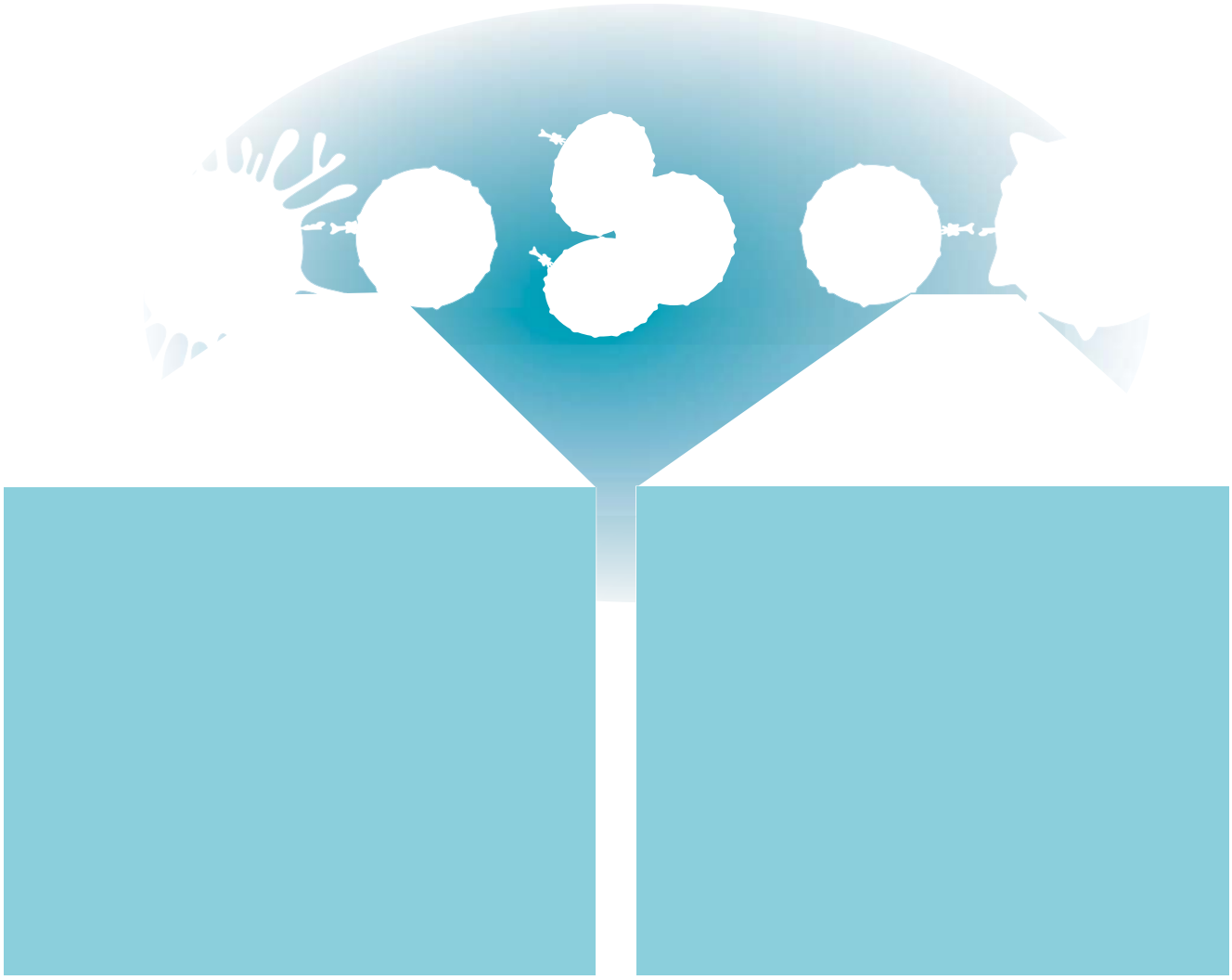

Figure 94.15 Schematic representation of the mechanism of action of anti–cytotoxic T lymphocyte–associated protein 4 (*CTLA-4*) and anti–programmed cell death protein (PD)-1 monoclonal antibodies. Cytotoxic T lymphocyte–associated protein 4 is a negative regulatory signal that limits activation of T cells upon ligation with cluster of differentiation 80 or cluster of differentiation 86 costimulatory molecules expressed by antigen-presenting cells, within the priming phase of a T-cell response in lymph nodes. PD-1 is expressed by T cells upon chronic antigen exposure and results in negative regulation on T cells upon ligation with PD-L1, which is primarily expressed by peripheral tissues including in the tumor microenvironment. The PD-1/PD-L1 interaction happens in the effector phase of a T-cell response. Its blockade with antibodies to PD-1 or PD-L1 results in the preferential activation of T cells with specificity for the cancer. MHC, major histocompatibility complex; TCR, T-cell receptor. (From Ribas A. Tumor immunotherapy directed at PD-1. *N Engl J Med* 2012;366:2517–2519.)

melanoma. Immune responses against cancer are usually kept under negative regulatory control by a series of physiological breaks (checkpoints; Fig. 94.15). Negative immune regulatory checkpoints are induced after T-cell activation. These include CTLA4, which when engaged by the costimulatory molecules B7.1 (CD80) or B7.2 (CD86) results in a dominant negative regulation of T cells by competing with the CD28-positive costimulatory receptor. When an activated T cell arrives to the peripheral tissues, including melanoma metastases, it can be turned off by the PD-1/PD-L1 interaction. PD-1 is a negative regulatory checkpoint receptor that is expressed by T cells upon activation and chronic antigen exposure, as in cancer. Its main peripheral ligand, PD-L1, is expressed by cancer and stromal cells mainly upon exposure to T cell–produced interferons. Therefore, it represents a mechanism of acquired immune resistance that allows melanoma to hide from activated T cells.[212] Release of both the CTLA4 and PD-1 checkpoints has resulted in objective and durable immune-mediated tumor responses in patients with advanced melanoma, and it is an area of active clinical research and drug development.

Ipilimumab (Yervoy, Bristol-Myers Squibb, New York, NY) is a fully human monoclonal antibody (IgG1) that blocks CTLA4. The FDA approved ipilimumab at a dose of 3 mg/kg administered at 3-week intervals for four doses for the treatment of unresectable or metastatic melanoma in 2011. This approval was based on a randomized clinical trial of ipilimumab compared to a gp100 peptide vaccine, or in combination, in patients with previously treated metastatic melanoma.[195] 676 HLA-A*0201–positive patients with unresectable stage III or IV melanoma who had been previously treated with systemic therapy for metastatic disease were randomly assigned, in a 3:1:1 ratio, to ipilimumab plus gp100 (403 patients), ipilimumab alone (137 patients), or gp100 alone (136 patients). Ipilimumab was administered at a dose of 3 mg per kilogram every 3 weeks for up to four treatments (induction). Eligible patients could receive re-induction therapy. The primary end point of median OS with ipilimumab alone was 10.1 months as compared with 6.4 months among patients receiving gp100 alone (HR = 0.66; $p = 0.003$). No difference in OS was detected between the ipilimumab groups (HR with ipilimumab plus gp100 = 1.04; $p = 0.76$),

TABLE 94.9

Comparison of Selected Toxicities by Ipilimumab, Vemurafenib, Dabrafenib, and Trametinib in Phase 3 Monotherapy Trials[16,25,26,41]

	Hodi et al. 2010[195]		Chapman et al. 2011[229]		Hauschild et al. 2012[232]		Flaherty et al. 2012[236]	
	Ipilimumab		Vemurafenib		Dabrafenib		Trametinib	
Toxicity grades	All grades	Grade 3-4	All grades	Grade 3-4	Grade 2	Grade 3-4	All grades	Grade 3-4
Fatigue	42	5	33	2	5	5	26	4
Pyrexia	12	0	18	0	8	3		
Skin rash	19	1	36	8	NA	NA	57	8
Acneiform dermatitis							19	1
Photosensitivity			30	3				
Pruritus	24	0	22	1				
Palmoplantar disesthesia			7		6	2		
Hyperkeratosis			20	1	12	1		
Alopecia			35	<1			17	<1
cuSCC/KA				18	—	6		
Nausea	35	2	30	7	1	0	18	1
Diarrhea/colitis	29	8	25	<1			43	0
Endocrine	8	2						
Hepatic	4	0						
Arthralgia			49	6	0	1		
Peripheral edema			15				26	1
Hypertension							15	12

NA, not available; cuSCC/KA, cutaneous squamous cell carcinoma and keratoacanthoma.

and the median OS was 10.0 months among patients receiving ipilimumab plus gp100 (HR for death = 0.68; $p < 0.001$). Grade 3-4 immune-related adverse events occurred in 10% to 15% of patients treated with ipilimumab, the most common being colitis, skin rash, and endocrinopathies (Table 94.9). There were 14 deaths related to ipilimumab (2.1%), and 7 were associated with immune-related adverse events. This was the first randomized clinical trial demonstrating an improvement in OS in patients with metastatic melanoma (Table 94.10).

There was a second randomized clinical trial with OS improvement using ipilimumab.[196] This was a frontline trial comparing ipilimumab (10 mg/kg) plus dacarbazine (850 mg/m^2 of body surface area), or dacarbazine (850 mg/m^2) plus placebo, in patients with previously untreated metastatic melanoma. A total of 502 patients were randomized in a 1:1 ratio, with the study drugs given at weeks 1, 4, 7, and 10. Patients with stable disease or an objective response and no dose-limiting toxic effects were eligible to receive ipilimumab every 12 weeks thereafter as maintenance therapy. The primary end point of OS was significantly improved in the group receiving ipilimumab plus dacarbazine compared to dacarbazine plus placebo (11.2 months versus 9.1 months). Survival rates at 1 year (47.3% versus 36.3%), 2 years (28.5% versus

TABLE 94.10

Main Efficacy Endpoints Taking from the Experimental Arm of Randomized Trials Leading to the Approval of Ipilimumab, Vemurafenib, Dabrafenib, and Trametinib

	Hodi et al. 2010[195,a]		Chapman et al. 2011[229,b]		Hauschild et al. 2012[232,b]		Flaherty et al. 2012[236,b]	
	Ipilimumab	gp100	Vemurafenib	Dacarbazine	Dabrafenib	Dacarbazine	Trametinib	Dacarbazine or Paclitaxel
Response rate	11%	1.5%	48%	5%	50%	6%	22%	8%
Median PFS	2.9 mo	2.8 mo	5.3 mo	1.6 mo	5.1 mo	2.7 mo	4.8 mo	1.5 mo
HR PFS	0.64		0.26		0.30		0.45	
Median OS	10.1 mo	6.4 mo	NR	NR	NR	NR	NR	NR
HR OS	0.66		0.37		0.61		NR	

PFS, progression-free survival; HR, hazard ratio; OS, overall survival; NR, not reported.
[a] Enrolled previously treated patients with advanced melanoma with no BRAF restriction.
[b] Enrolled patients with BRAF mutant advanced melanoma who had not been previously treated with a systemic therapy.

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17.9%), and 3 years (20.8% versus 12.2%) were significantly improved (HR for death = 0.72; p <0.001). Grade 3 or 4 adverse events occurred in 56.3% of patients treated with ipilimumab plus dacarbazine, as compared with 27.5% treated with dacarbazine and placebo (p <0.001). The most frequent toxicities in the experimental combination group were increases in transaminases. No drug-related deaths or gastrointestinal perforations occurred in the ipilimumab–dacarbazine group.

The FDA approval of ipilimumab comes with a black box warning due to the potential for severe and occasionally fatal immune-mediated adverse reactions. The most common are enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathies like hypophysitis and thyroiditis. The recommendation is to permanently discontinue ipilimumab infusions and initiate systemic high-dose corticosteroid therapy for severe immune-mediated reactions.

Re-induction with ipilimumab after the first four infusions without serious side effects is an option for patients with stable disease sustained for at least 3 months or a prior confirmed partial or complete response. Among 31 patients given re-induction with ipilimumab, a complete or partial response or stable disease was achieved in 13%, 37.5%, and 65.2%, respectively.[213]

Several studies are testing the effects of combinations based on ipilimumab. E1608 is a phase 2 randomized trial that tested the combination of ipilimumab with GM-CSF with the goal of analyzing if this combination improved OS.[214] A total of 245 patients were randomized to ipilimumab (10 mg/kg every 3 weeks intravenously for four courses, then every 12 weeks) plus GM-CSF (250 μg subcutaneously on days 1 to 14 of 21-day cycles) or ipilimumab alone. With a median follow-up of 13.3 months, the response rate and PFS was not different between both arms. In a planned interim analysis, the primary end point of median OS was improved with the combination. One-year OS with the combination was 67.9% (59%, 76%), while it was 51.2% (42.6%, 61.3%) for ipilimumab alone (stratified log rank p_1 = 0.016, p_2 = 0.033; HR = 0.65). Grade 3–5 toxicities were lower with the combination compared to ipilimumab alone (45% compared to 57%, p_2 = 0.078). Therefore, ipilimumab plus GM-CSF may increase OS and decrease toxicities compared to ipilimumab alone. However, this combination remains investigational unless the benefit is confirmed in further clinical trials.

Another anti-CTLA4 antibody has been developed clinically in patients with metastatic melanoma, tremelimumab (by Pfizer and MedImmune-Astra-Zeneca, New York, NY). This fully human IgG2 monoclonal antibody went through phase 1, 2, and 3 testing in melanoma,[215–217] but failed to demonstrate improvement in OS compared to dacarbazine.[216] Potential contributing factors were the study design, the treated population, the dosing regimen, and the postrandomization use of ipilimumab in the control arm.[218]

The main feature of therapy with anti-CTLA4 antibodies is the long durability of tumor responses in patients with an objective tumor response, as exemplified by a patient with metastasis to the lung and liver who initially received ipilimumab and then tremelimumab in May of 2001[219] and continues in response over a decade later. In many instances, it is difficult to assess objective responses as these may appear late after the therapy and go through a process of apparent clinical progression when using standard response evaluation criteria. This has led to the proposal of alternate response evaluation criteria tailored to this mechanism of action, termed the immune-related response criteria.[220,221]

Interleukin-2

The intravenous administration of high-dose IL-2 (aldesleukin) was approved for the treatment of patients with metastatic melanoma in 1998 mainly thanks to its ability to mediate durable complete responses in patients with widespread metastatic disease. The administration of IL-2 represented the first demonstration that purely immunotherapeutic maneuvers could mediate the regression of metastatic cancer.[222,223] IL-2 has no direct effect on cancer cells, and all of its antitumor activity is a function of its ability to modulate immunologic responses in the host.

The FDA-approved regimen for the treatment of patients with metastatic melanoma using IL-2 involves the use of an intravenous bolus infusion of 600,000 to 720,000 IU/kg every 8 hours to tolerance using two cycles separated by approximately 10 days (maximum of 15 doses per cycle). Results of this treatment are evaluated at 2 months after the first dose, and if tumor is regressing or stable, a second course is then administered.

In the report of the original 270 patients treated at 22 different institutions that was the basis of the approval of IL-2 by the FDA, a 16% objective response rate was obtained, with 17 complete responses (6%) and 26 partial responses (10%).[224] At the last full analysis of these 270 patients, the median duration of response for complete responders had not been reached but exceeded 59 months, and disease progression was not observed in any patient who responded for more than 30 months. An analysis of patients treated from 1988 to 2006 in the Surgery Branch, National Cancer Institute, reported 13 complete responders, only 2 of which had recurred with the remainder ongoing at 1 to 21 years.[225] Thus, IL-2 appears to be one of a very small group of systemic treatments capable of curing patients with a metastatic solid cancer.

Because of the side effects associated with high-dose bolus IL-2 administration, this treatment is generally restricted to patients younger than the age of 70 years with an ECOG performance status of ≤2 and in patients who do not have active systemic infections or other major medical illness of the cardiovascular respiratory or immune system. Because IL-2 often causes transient renal and hepatic toxicity, eligibility criteria generally require normal serum creatinine and serum bilirubin levels. Patients with any history of systemic ischemic heart disease or pulmonary dysfunction should undergo stress testing and pulmonary function tests before initiating therapy, and patients with significant abnormalities should not be included.

The administration of high-dose bolus IL-2 is different than the administration of most cancer therapeutics in that dosing is continued every 8 hours until patients reach grade 3 or 4 toxicity that is not easily reversible by supportive measures. The toxicities of IL-2 administration are transient, with virtually all returning to baseline after IL-2 administration is stopped. Thus, patients are often treated despite creatinine levels that increase to the 2- to 3-mg/dL range because of confidence that renal function will return to normal after cessation of IL-2 administration. Thus, there are no set doses that patients receive, and there is no correlation between the number of doses seen and the likelihood of achieving a response as long as patients receive dosing to tolerance based on physical findings and laboratory measurements.

Administration of Interleukin-2 Plus Vaccine

In 1998, Rosenberg et al.[226] reported an increase in objective response to IL-2 when administered in conjunction with a heteroclitic gp100:209–217(210M) melanoma peptide vaccine. An updated analysis showed a response rate of 12.8% to IL-2 alone compared to a 25.0% response rate to IL-2 plus immunization with this peptide (p = 0.01). A prospective randomized trial in patients with metastatic melanoma of IL-2 alone or in conjunction with this peptide in 185 patients[227] reported centrally reviewed response rates of 6% and 16%, respectively (p = 0.02), with an increase in PFS in the vaccine arm (p = 0.008) and a strong trend toward an increase in survival (17.8 versus 11.1 months, p = 0.06).

BRAF Inhibitors

Vemurafenib (Zelboraf, Roche, Basel, Switzerland) and dabrafenib (Tafinlar, GlaxoSmithKline) are two BRAF inhibitors approved by the FDA, European Medicines Agency, and other regulatory bodies with high antitumor activity in patients with $BRAF^{V600}$ mutant metastatic melanoma mediated by the inhibition of oncogenic MAPK signaling.[151,228] They are both classified as type I BRAF kinase inhibitors because they block the enzymatic activity of BRAF in the activated, mutated conformation, as opposed to

type II RAF inhibitors that work only in the inactive conformation, such as sorafenib.[228] In a phase 3 trial in which patients with $BRAF^{V600E}$ metastatic melanoma were treated with vemurafenib versus dacarbazine, there was a large early improvement in OS, leading to an early closure of the clinical trial.[229] This was a phase 3 randomized clinical trial comparing vemurafenib (960 mg orally twice daily) with dacarbazine (1,000 mg/m^2 of body surface area intravenously every 3 weeks) in 675 patients with previously untreated, metastatic melanoma with the $BRAF^{V600E}$ mutation. A planned interim analysis after 98 deaths led to the closing of this trial. In this interim analysis, vemurafenib was associated with a relative reduction of 63% in the risk of death and 74% in the risk of either death or disease progression, as compared with dacarbazine (p <0.001 for both comparisons). The independent data and safety monitoring board recommended crossover from dacarbazine to vemurafenib for patients under study. Response rates were 48% for vemurafenib and 5% for dacarbazine. At 6 months, OS was 84% (95% CI, 78 to 89) in the vemurafenib group and 64% (95% CI, 56 to 73) in the dacarbazine group. Common adverse events associated with vemurafenib were arthralgia, rash, fatigue, alopecia, keratoacanthoma or squamous cell carcinoma, photosensitivity, nausea, and diarrhea (see Table 94.9); 38% of patients required dose modification because of toxic effects.

Secondary cutaneous squamous cell carcinomas and keratoacanthomas are the most common grade 3-4 toxicities with vemurafenib, which occur in 20% of the patients treated, often with RAS mutations and usually the first 2 to 3 months of therapy.[230] The pathogenesis of their development is due to the inhibition of wild-type BRAF within a dimmer with CRAF, which results in increased MAPK signaling through the paradoxical transactivation of CRAF in the setting of strong RAS-GTP signaling in that cell.[208,231] This is the so-called paradoxical activation of the MAPK pathway with BRAF inhibitors. These skin squamoepithelial proliferative lesions are usually treated with local excision and do not require changing the doses of the BRAF inhibitor.

Dabrafenib was tested in a phase 3 trial in patients with previously untreated stage IV or unresectable stage III $BRAF^{V600E}$ mutation–positive metastatic melanoma.[232] Patients were randomly assigned (3:1) to receive dabrafenib (150 mg twice daily, orally) or dacarbazine (1,000 mg/m^2 intravenously every 3 weeks). A total of 250 patients were randomized either to dabrafenib (187 patients) or dacarbazine (63 patients). The primary end point of median PFS was 5.1 months for dabrafenib and 2.7 months for dacarbazine (HR = 0.30; 95% CI, 0.18 to 0,51; p <0.0001) (see Table 94.10). Treatment-related adverse events (grade 2 or higher) occurred in 100 (53%) of the 187 patients who received dabrafenib and in 26 (44%) of the 59 patients who received dacarbazine. The most common adverse events with dabrafenib were skin-related toxic effects, fever, fatigue, arthralgia, and headache (see Table 94.9). An update reported of this phase 3 trial with a longer follow-up showed a median OS in the dabrafenib arm of 18 months compared to 15 months with dacarbazine.[233]

Overall, both vemurafenib and dabrafenib used as single agents showed similar effects in terms of objective response rate and PFS. The incidence of clinically significant photosensitivity is higher with vemurafenib compared to dabrafenib, and the incidence of clinically significant pyrexia is higher with dabrafenib compared to vemurafenib (see Table 94.8).

MEK Inhibitors

MEK inhibitors block signaling in the MAPK pathway downstream from BRAF. These agents effectively inhibit cellular proliferation and tumor growth in BRAF mutant melanoma, although they may also have some activity in NRAS mutant disease.[234,235] Trametinib (Mekinist, GlaxoSmithKline) was approved by the FDA and other regulatory bodies in 2013 based on the results of a phase 3 trial in patients who had metastatic melanoma with a V600E or V600K BRAF mutations.[236] A total of 322 patients were randomly assigned to either trametinib (2 mg orally once daily) or intravenous dacarbazine (1,000 mg/m^2 of body surface area) or paclitaxel (175 mg/m^2) every 3 weeks. The primary end point of median PFS was 4.8 months in the trametinib group and 1.5 months in the chemotherapy group (HR = 0.45; 95% CI, 0.33 to 0.63; p <0.001) (see Table 94.10). At 6 months, OS was 81% in the trametinib group and 67% in the chemotherapy group despite crossover (HR = 0.54; 95% CI, 0.32 to 0.92; p = 0.01). Rash, diarrhea, and peripheral edema were the most common toxic effects in the trametinib group (see Table 94.9) and were managed with dose interruption and dose reduction; asymptomatic and reversible reduction in the cardiac ejection fraction and ocular toxic effects occurred infrequently. Due to the higher incidence of side effects and lower efficacy (see Tables 94.9 and 94.10), the use of BRAF inhibitors is usually preferred over the use of MEK inhibitors for the treatment of patients with BRAF mutant advanced melanoma.

Mechanisms of Resistance to BRAF Inhibitors

Progression on therapy with no evidence of tumor response (innate resistance) to BRAF inhibitors is rare, present in approximately 15% of patients. However, progressive growth after a period of tumor response (acquired resistance) is common with a median PFS of 6 to 7 months. Mechanisms of acquired resistance are diverse and can be categorized between the ones that reactivate the MAPK pathway and the mechanisms that lead to a MAPK pathway–independent signaling that substitutes for the blocked driver oncogenic signal. MAPK reactivating mechanisms are more common and have the common theme of reactivating oncogenic signaling through MEK and ERK. Specific mechanisms reported to date in patient-derived samples include truncations in the BRAF protein resulting in increased kinase activity,[237] amplifications of the mutant BRAF gene,[238] secondary mutations in NRAS or MEK,[239–241] or overexpression of COT.[242] The mechanisms leading to MAPK-redundant pathway activation are less well characterized and most may represent mechanisms of adaptive resistance to provide an alternative survival pathway. These include the overexpression or overactivation of receptor tyrosine kinases like the platelet-derived growth factor receptor beta[239,243] or the insulin-like growth factor receptor 1,[244] leading oncogenic signaling through the PI3K-AKT pathway.

In a study of 100 biopsies taken from patients receiving BRAF inhibitors,[245,246] MAPK reactivation mechanisms were detected among disease progression tissues in 64% of samples; among these, *RAS* mutations (37%), mutant *BRAF* amplification (30%), and alternative splicing (20%) were most common. This study also detected genetic alterations in the PI3K-AKT pathway among progressive tissues in 26% of samples. Furthermore, increasing evidence suggests that multiple mechanisms of resistance may develop in the same patient when melanoma relapses after therapy with BRAF inhibitors. In 20% of patients, at least two mechanisms of resistance were detected in the same melanoma in the same or different progressive tumor sites. When studying the genetic features of multiple temporally and geographically distinct baseline and progressive metastatic lesions from an individual patient, it revealed distinct drivers of resistance via both divergent and convergent evolution and evidence of genomic diversification associated with an altered mutational spectra. Therefore, *BRAF* mutant melanomas acquire BRAF inhibitor resistance via diverse molecular alterations, which indicate MAPK and PI3K-AKT pathway addiction.[246] The finding of multiple genetic mechanisms of escape in the same patient implies that the use of upfront, co-targeting of the escape pathways may be an essential strategy for durable responses.

Combination of Targeted Therapies for BRAF Mutant Melanoma

As the most common core pathway mechanism of resistance to single-agent BRAF inhibitor therapy is mediated by the reactivation of the MAPK pathway through MEK,[246,247] combined therapy with a BRAF and a MEK inhibitor may result in a greater initial tumor response and prevent MAPK-driven acquired resistance mecha-

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nisms. A randomized phase 2 trial tested two dosing regimens of combined dabrafenib and trametinib, or dabrafenib alone.[248] The rate of complete or partial response with combination therapy was 76%, as compared with 54% with monotherapy ($p = 0.03$). Median PFS in the combination was 9.4 months, as compared with 5.8 months in the monotherapy group (HR = 0.39; 95% CI, 0.25 to 0.62; $P < 0.001$). Pyrexia was the most common toxicity in the combined therapy group compared with the monotherapy group (71% versus 26%). The combination had the additional benefit of blocking the paradoxical activation of the MAPK pathway induced by BRAF inhibitors, and thus decreasing toxicities compared with BRAF inhibitor monotherapy such as squamous cell carcinomas and hyperkeratotic skin lesions. Other BRAF and MEK inhibitor combinations are being evaluated in clinical trials with promising activity and a distinct safety profile, including the combination of the BRAF inhibitor vemurafenib with the MEK inhibitor cobimetinib, and the BRAF inhibitor LGX818 with the MEK inhibitor MEK162. Several phase 3 trials that compare the BRAF/MEK inhibitor combination with a BRAF inhibitor as monotherapy are ongoing. Therefore, the combination of BRAF and MEK inhibitors is likely to result in improved initial responses through better oncogenic BRAF inhibition, more durable responses by preventing mechanisms of acquired resistance, and decreased toxicities by inhibiting paradoxical MAPK activation with BRAF inhibitors. But the final results of the phase 3 trials will be needed to fully evaluate the benefits of combined therapy compared to single-agent BRAF inhibitors.

When acquired resistance to a BRAF inhibitor has been established, the sequential use of a MEK inhibitor after stopping therapy with the BRAF inhibitor does not result in secondary tumor responses.[249] However, in agreement with preclinical models, there are secondary responses when adding a MEK inhibitor to continued therapy with a BRAF inhibitor in patients progressing on single-agent BRAF inhibitors. In some instances, a secondary response can be achieved with the reintroduction of therapy with a BRAF inhibitor in patients who previously progressed on this therapy and had been off therapy for a period of time.[250]

KIT Inhibitors

Mutations in the KIT receptor occur infrequently (2% to 3% of unselected cases of metastatic melanoma) and are more prevalent in mucosal and acral melanomas.[48] Expression of Kit protein by immunohistochemical staining for CD117 is not sufficient to select for sensitivity to Kit inhibitors, and testing for KIT alterations needs to be performed by DNA sequencing. Imatinib (Gleevec, Novartis, Basel, Switzerland) has modest activity in patients with metastatic melanoma, and KIT mutations in the juxtamembrane domain (L596, V559), as well as K642E, are present.[50,51] The overall durable response rate was 16% (95% CI, 2 to 30) among 51 patients with KIT mutations or genetic amplification, with a median time to progression of 12 weeks.[16] Other drugs such as dasatinib (Sprycel, Bristol-Myer Squibb) and sunitinib (Sutent, Pfizer) also appear to have activity in KIT mutant melanoma.[52–54] Therefore, KIT inhibitors have modest activity in patients with KIT mutant metastatic melanoma.

Single-Agent Chemotherapy

Melanoma is regarded as a relatively chemotherapy-refractory tumor. The specific mechanisms underlying resistance are not well known, but likely derive from the inherent resilience of melanocytes, which have to be naturally resistant to apoptotic death when exposed to UV radiation from the sun. In particular, DNA repair enzymes as well as the expression of efflux pumps for xenobiotics are more highly expressed in melanoma compared with many other cancers.

Dacarbazine, an imidazole carboxamide [5-(3,3-dimethyl-l-triazeno)-imidazole-4-carboxamide], is a classic alkylating agent. It was first evaluated in clinical trials in melanoma in the late 1960s and approved by the FDA for the treatment of metastatic melanoma in 1974 on the basis of a response rate of approximately 20%,[251] but this response assessment predates modern stringent response evaluation criteria. In the majority of trials, dacarbazine was administered intravenously at daily doses of 200 mg/m^2 for 5 days every 3 or 4 weeks; however, 1,000 mg/m^2 given once every 3 or 4 weeks has been the standard regimen in recent trials. The most common toxicities are myelosuppression and nausea. The severity of myelosuppression rarely requires the use of growth factor support, and the advent of potent antiemetics has significantly improved the tolerability of this agent. Of note, an adequately powered randomized trial of dacarbazine or any other systemic therapy compared with best supportive care has never been undertaken in advanced melanoma.

Temozolomide is an orally available prodrug that is metabolized into the active form MTIC [5-(3-methyltriazen-1-yl) imidazole-4-carboxamide], which is closely related to dacarbazine.[252] MTIC penetrates into the cerebrospinal fluid.[253] Because it is an oral therapy, temozolomide is also more amenable to protracted dosing schedules than dacarbazine, particularly an advantage when combined with fractionated radiation therapy. Single-arm phase 2 trials demonstrated activity in patients with metastatic melanoma, but more modest activity was noted for patients with brain metastases treated with this agent.[254] EORTC 18032 was a phase 3 randomized clinical trial in which a dose-intense schedule of temozolomide was administered (150 mg/m^2 daily for 7 of every 14 days), compared with dacarbazine (1,000 mg/m^2 intravenously every 28 days). This trial randomized 859 patients and sought a 23% improvement in OS as the primary end point. However, OS was not significantly different between the two arms (HR = 1.0; $p = 1.0$). Response rate was superior in the temozolomide group (14.4% versus 9.8%, $p = 0.05$), but PFS was not (HR = 0.92 in favor of temozolomide; $p = 0.092$).[255] Therefore, there is little evidence for the use of temozolomide compared to using dacarbazine in patients with metastatic melanoma.

Fotemustine is a nitrosourea approved by European regulatory bodies for the treatment of advanced melanoma. It is administered by intravenous infusion (100 mg/m^2) weekly for 3 weeks followed by a 4- to 5-week break, with continued administration every 3 weeks for stable or responding patients.[256] A total of 229 patients were randomized to fotemustine or dacarbazine, seeking to demonstrate a 17% absolute difference in objective response rate. The observed difference in response rate (13% for fotemustine versus 6% for dacarbazine) was not statistically significant. Median time to progression was similar for the two arms. Overall survival, a secondary end point, was not significantly improved with fotemustine compared to dacarbazine (median, 7.3 versus 5.6 months).

Nab-paclitaxel is an albumin nanoparticle-bound paclitaxel that was demonstrated to have an improved PFS over dacarbazine in a phase 3 randomized open-label trial in chemotherapy-naïve patients with stage IV metastatic melanoma with no brain metastasis and LDH ≤2 upper limit of normal. Patients received either nab-paclitaxel 150 mg/m^2 on days 1, 8, and 15 every 4 weeks, or dacarbazine 1,000 mg/m^2 every 3 weeks. A total of 529 patients were randomized to nab-paclitaxel ($n = 264$) or dacarbazine ($n = 265$) between April 2009 and June 2011. In the intent-to-treat population, the primary end point of PFS was improved in favor of the nab-paclitaxel group (4.8 and 2.5 median months in the nab-paclitaxel and DTIC arm, respectively; HR = 0.792; 95.1% CI, 0.631 to 0.992; $p = 0.044$). Interim OS was 12.8 with nab-paclitaxel and 10.7 months with dacarbazine (HR = 0.831; 99.9% CI, 0.578 to 1.196; $p = 0.094$). The most common grade 3 or higher treatment-related adverse events was neuropathy with nab-paclitaxel and neutropenia with dacarbazine. The median time to neuropathy improvement was 28 days. Further follow-up to assess potential effects in OS will be needed to determine the role of nab-paclitaxel in patients with advanced melanoma.

Combination Chemotherapy

Over the past 20 years, several uncontrolled clinical trials suggested the benefits of combination chemotherapy regimens, such

as cisplatin, vinblastine, and dacarbazine (CVD) and the so-called Dartmouth regimen (cisplatin, carmustine, dacarbazine, and tamoxifen). A phase 3 trial was conducted among 240 patients comparing the Dartmouth regimen with single-agent dacarbazine with the goal of detecting a 50% improvement in OS.[257] The median OS was similar between the two arms (7.7 months for Dartmouth versus 6.3 months for dacarbazine; $p = 0.52$), and 1-year survival rates were also very similar (23% for Dartmouth versus 28% for dacarbazine; $p = 0.38$). The response rate was not significantly higher for the combination regimen (17% for Dartmouth versus 10% for dacarbazine; $p = 0.09$) and was substantially lower than the reported response rate in the smaller, single-institution studies. The Dartmouth regimen was associated with significantly more severe neutropenia, anemia, nausea, and vomiting. In addition, several randomized trials refuted the concept that tamoxifen substantially modulates the efficacy of chemotherapy in metastatic melanoma. CVD was tested in the context of ECOG 3695, in which CVD was the control-arm therapy for 201 patients with metastatic melanoma. The response rate was 12% and median PFS was 3.1 months, suggesting a very low activity.[258] Overall, there is little evidence of benefit with these combination chemotherapy regimens for the treatment of patients with metastatic melanoma.

The combination of carboplatin and paclitaxel has been tested in melanoma, initially as a combination chemotherapy with sorafenib that was then shown to have benefits as a chemotherapy combination without sorafenib. This was based on the results of two randomized clinical trials. E2603 was a US intergroup double-blind, randomized, placebo-controlled phase 3 study that enrolled 823 patients to carboplatin-paclitaxel or the same combination with the addition of sorafenib.[259] At final analysis, the median OS was 11.3 months (95% CI, 9.8 to 12.2 months) for carboplatin-paclitaxel and 11.1 months (95% CI, 10.3 to 12.3 months) for carboplatin-paclitaxel-sorafenib. Median PFS was 4.9 months for carboplatin-paclitaxel-sorafenib and 4.2 months for carboplatin-paclitaxel. Response rate was 20% for carboplatin-paclitaxel-sorafenib and 18% for carboplatin-paclitaxel. This study established a benchmark for the carboplatin-paclitaxel regimen in first-line therapy of metastatic melanoma.

The PRISM study randomized 270 patients with previously treated metastatic melanoma to the same two regimens.[260] The median PFS was 17.9 weeks for carboplatin-paclitaxel and 17.4 weeks for the sorafenib plus carboplatin-paclitaxel arm. Response rate was 11% with carboplatin-paclitaxel versus 12% with the addition of sorafenib. Together, these studies demonstrate that sorafenib has no role in this combination and that carboplatin-paclitaxel has activity in patients with metastatic melanoma.

Biochemotherapy

For most of the 1990s and 2000s, biochemotherapy was considered by many melanoma clinicians as a treatment option due to the lack of other alternatives and the reported high response rates in uncontrolled, mostly single-institution clinical trials. However, over 20 randomized clinical trials have failed to demonstrate an improvement in OS when administering biochemotherapy compared to a control arm, although many have suggested some benefit in terms of PFS or response rate. A meta-analysis of 18 trials involving 2,621 patients provided evidence that biochemotherapy improves response rates over single-agent chemotherapy or cytokine therapy, but this does not appear to translate into a survival benefit.[261] The definitive testing of biochemotherapy has come from two large cooperative group trials.

In E3695, the combination of CVD with interferon and IL-2 administered concurrently was compared with CVD in patients with metastatic melanoma.[258] Accrual was stopped after 416 patients were enrolled because of a preplanned futility analysis. OS was not improved (8.4-month median for biochemotherapy versus 9.1 months for CVD). PFS was superior (5-month median for biochemotherapy versus 3.1 months for CVD) but not significantly so. Response rate was not significantly higher in the biochemotherapy arm (17% versus 12%).

In EORTC 18951, cisplatin, dacarbazine, and interferon-α were administered to all patients, with "decrescendo" IL-2 administered only to one cohort.[262] A total of 363 patients were accrued, with all receiving dacarbazine 250 mg/m^2 daily for 3 days, cisplatin 30 mg/m^2 daily for 3 days, and interferon-α2b, 10 MU/m^2 daily for 5 days. The experimental arm also received IL-2 for 4 days after completion of the chemotherapy. The dose per day was fixed, but the duration of the infusion was lengthened each day. Survival rate at 2 years was the primary end point, and an improvement from 10% to 20% was sought. The observed difference in 2-year survival (18% for the IL-2–containing arm versus 13% for the arm without IL-2) was not statistically significant. PFS and response rates were not significantly different between the two treatments. In conclusion, there is little supportive evidence to justify the use of biochemotherapy in the management of patients with metastatic melanoma.

EXPERIMENTAL IMMUNOLOGIC THERAPIES FOR METASTATIC MELANOMA

Anti-PD-1 and Anti-PD-L1

Early clinical testing of antibodies blocking PD-1, an inhibitory T-cell receptor belonging to the CD28 superfamily of immune-regulatory receptors, or blocking its main ligand PD-L1 (also known as B7-H1 or CD274), demonstrate a high rate of durable tumor responses.[263–265] PD-1 downregulates T-cell function by blocking T-cell receptor signaling upon binding to its ligands, PD-L1 or PD-L2 (also known as B7-DC or CD273). PD-L1 is expressed by inflamed tissues and cancer mainly in response to interferons, and confers peripheral tolerance from endogenous antigens. PD-L2 is expressed primarily by antigen-presenting cells.[212]

In a phase 1 trial, patients with advanced melanoma, non–small-cell lung cancer, castration-resistant prostate cancer, or renal cell or colorectal cancer were treated with the anti-PD-1 antibody nivolumab at a dose of 0.1 to 10.0 mg/kg of body weight every 2 weeks.[264] Response rate was 28% among patients with melanoma (26 of 94 patients). A maximum tolerated dose was not defined at the range of dosing levels tested. Common treatment-related adverse events included fatigue, rash, diarrhea, pruritus, decreased appetite, and nausea. Overall, grade 3 or 4 treatment-related adverse events were observed in 41 of 296 patients (14%). Drug-related serious adverse events occurred in 32 of 296 patients (11%). Drug-related adverse events of special interest included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis. An update analysis of the nivolumab phase 1 clinical trial reported a median OS over 16 months, with more than 40% of patients alive after 3 years of treatment with a long-term favorable safety profile.[266]

In another phase 1 clinical trial, patients with advanced melanoma, with and without prior treatment with ipilimumab, received the anti-PD-1 antibody MK-3475 (transiently known as lambrolizumab) intravenously at 10 mg/kg every 2 or 3 weeks or at 2 mg/kg every 3 weeks.[265] A total of 135 patients with advanced melanoma were treated. The confirmed response rate evaluated by central radiologic review per response evaluation criteria in solid tumors (RECIST) 1.1, across all dose cohorts, was 38% (95% CI, 25 to 44), with the highest confirmed response rate observed in the 10 mg/kg every 2 weeks cohort (52%; 95% CI, 38 to 66). Response rate was not different between patients with and without prior ipilimumab treatment. Responses were durable in the majority of patients. Most adverse events were low grade, and common adverse events attributed to treatment were fatigue, skin rash, pruritus, and diarrhea.

A phase 1 trial has tested the combination of anti-PD-1 therapy with nivolumab with the CTLA4-blocking antibody ipilimumab.[267] Nivolumab and ipilimumab was administered every 3 weeks for four doses followed by nivolumab alone every 3 weeks for four doses to 53 patients with metastatic melanoma. The objective response rate

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(according to modified WHO criteria) was 40%. At the maximum doses that were associated with an acceptable level of adverse events (nivolumab at a dose of 1 mg/kg of body weight and ipilimumab at a dose of 3 mg/kg), 53% of patients had an objective response. Grade 3 or 4 adverse events related to therapy occurred in 53% of patients, but were qualitatively similar to previous experience with monotherapy and were generally reversible.

Adoptive Cell Transfer Therapy

Adoptive cell transfer (ACT) therapy refers to an immunotherapy approach for the treatment of cancer that involves the infusion to the tumor-bearing host of cells with antitumor activity that can recognize cancer antigens and result in the destruction of cancer cells. Although it is still experimental, ACT has emerged among the most effective treatments for patients with metastatic melanoma; 50% to 70% of patients with metastatic melanoma experience objective cancer regressions by RECIST when treated with ACT.[268,269]

ACT has a variety of advantages compared with other forms of cancer immunotherapy.[270,271] T-lymphocytes, once identified as cancer reactive, can be expanded to large numbers in vitro using cytokine growth factors. Thus, patients can be administered very large numbers of cells, often much larger than can be naturally generated in vivo. These antitumor lymphocytes can be activated in vitro to express appropriate effector functions such as the ability to lyse tumor cells and secrete cytokines. Secreted cytokines can have a variety of secondary antitumor effects at the cancer site such as the destruction of surrounding blood vessels, the direct lysis of tumor cells, and providing chemokine signals to attract additional effector cell types, such as activated macrophages, to the tumor site. Perhaps most important, when using ACT, it is possible to modify the host to enhance the ability of the infused cells to establish, grow, and function in vivo. The ability to immunosuppress the host prior to cell infusion is unique to ACT. Immunosuppression can counteract the impact of T-regulatory cells that can suppress cellular immune reactions as well as remove other endogenous lymphocytes that compete with the infused cells for homeostatic cytokines such as IL-7 and IL-15, which are necessary for antitumor T-cell expansion in vivo.[270]

A critical step in the development of effective ACT for human cancer was the demonstration in 1987 that lymphocytes infiltrating into deposits of human metastatic melanoma could be grown in IL-2. Tumor-infiltrating lymphocytes (TIL) with antitumor activity could be generated from approximately 70% of patients with metastatic melanoma. Using these human TILs, over 50 different antigenic epitopes have been identified in patients with melanoma, including antigens such as MART1 and gp100 that are widely shared among melanomas from different individuals.

The first report of ACT in humans in 1988[271] and extended in 1994[272] used the transfer of autologous TIL followed by the administration of high-dose IL-2. A major improvement in human ACT occurred when immunosuppressive regimens were administered prior to cell infusions, and this change led to a new generation of ACT clinical protocols.[269,273] A schematic of ACT treatment in humans with metastatic melanoma developed in the Surgery Branch of the National Cancer Institute is shown in Figure 94.16,

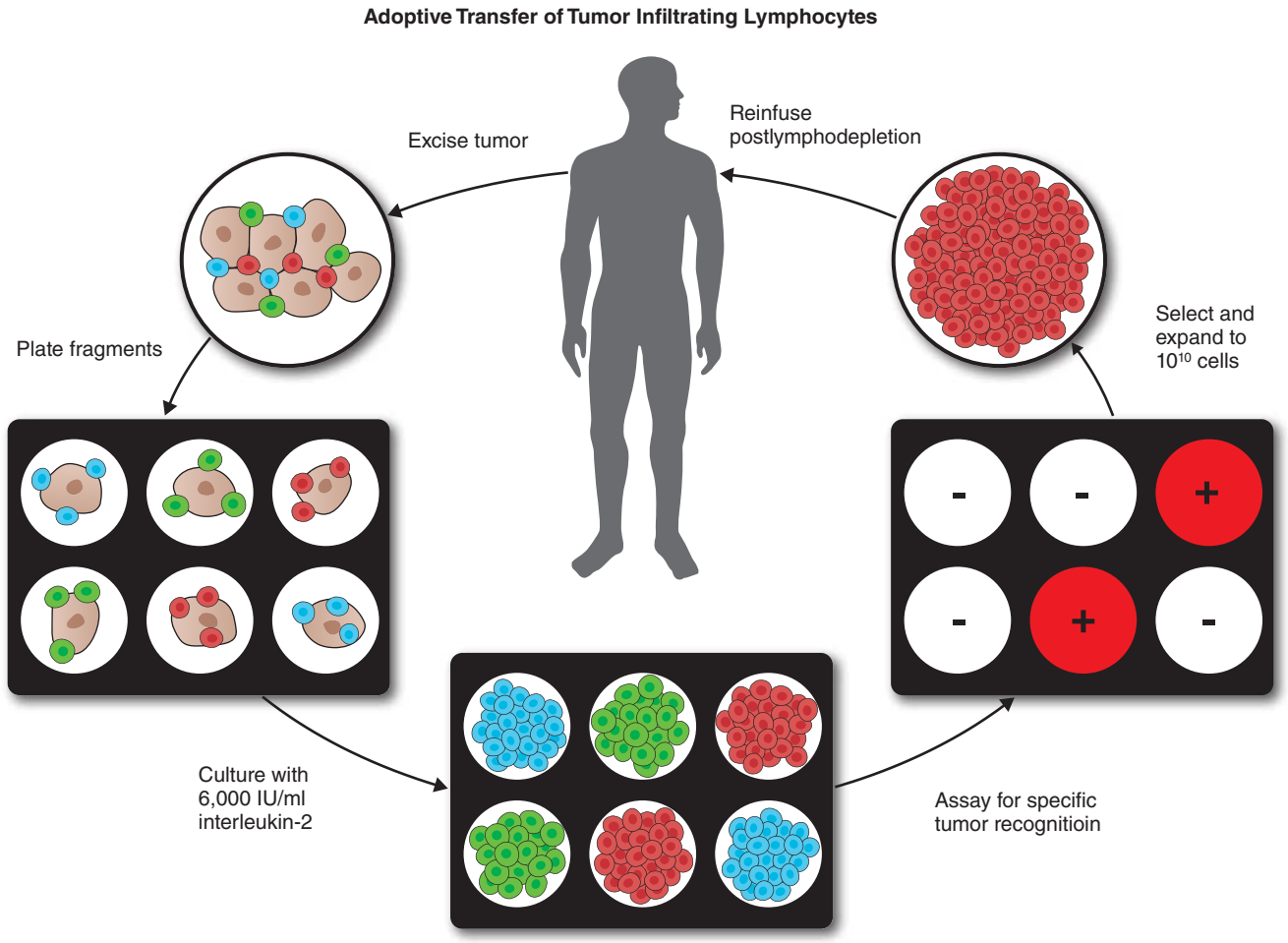

Figure 94.16 A schematic representation of the adoptive transfer of tumor-infiltrating lymphocytes into patients following a lymphodepleting preparative regimen.

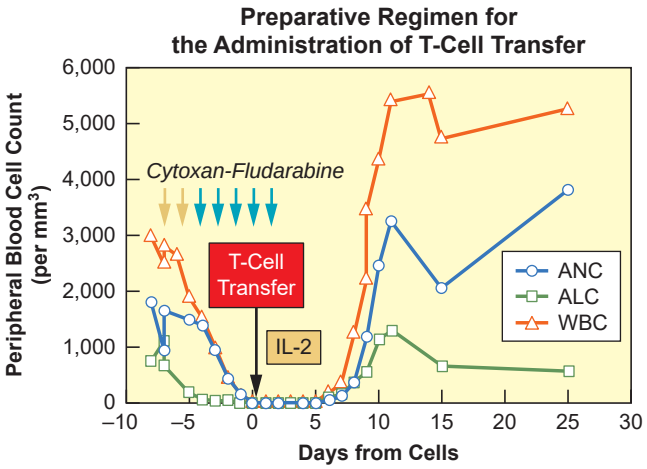

Figure 94.17 The preparative regimen administered prior to the administration of T-cell transfer with typical recovery of neutrophils (*ANC*), lymphocytes (*ALC*), and white blood cells (*WBC*).

and the preparative regimen prior to cell transfer is shown in Figure 94.17. In this treatment, metastatic melanoma deposits are resected and used to generate cultures of TILs with antitumor activity. When cultures reach from 5 to 10 × 10^{10} cells, they are infused into patients following an immunosuppressive preparative regimen. The first trial of this approach used a nonmyeloablative preparative regimen consisting of 60 mg/kg of cyclophosphamide for 2 days followed by 5 days of fludarabine at 25 mg/m^2. On the day following the last dose of fludarabine, the TILs were administered intravenously and IL-2 was then administered for 2 to 3 days at 720,000 IU/kg intravenously every 8 hours. An objective response rate by standard RECIST criteria was seen in 21 of 43 patients (49%)[268] (Fig. 94.18).

Because animal models demonstrated that more profound lymphodepletion was associated with higher antitumor effects, two additional clinical trials were performed in 25 patients each who received this cyclophosphamide–fludarabine chemotherapy plus 2 Gy or 12 Gy of whole-body irradiation. Objective tumor regressions were seen in 13 of 25 (52%) and in 18 of 25 (72%) patients, respectively, including 10 complete regressions (40%) in the latter trial.[268] In these trials, only 1 of 20 complete responders has recurred, with the others ongoing at 63 to 108 months[269] (Fig. 94.19). These results, although still experimental and available in only a few centers, represent the most effective treatments for patients with metastatic melanoma.[268,269] Factors associated with clinical response included higher telomere lengths, increased numbers of CD27+CD28+ T cells, and increased in vivo persistence at 1 month of the transferred TILs. Among a small subset of 11 patients who had previously been treated with anti-CTLA4, survival and response rate appeared to be higher.

Recent studies using the adoptive transfer of autologous peripheral lymphocytes transduced with genes encoding antitumor T-cell receptors directed against the melanoma/melanocyte antigens MART-1 and gp100 have also shown objective responses in patients with metastatic melanoma,[274,275] though toxicities directed against melanocyte in the eyes and ears limits the application of this gene therapy approach. Analysis of the antigens recognized by TILs associated with complete cancer regression indicate that these transferred cells recognize unique mutations present in each patient's melanoma.[276]

Recently, adoptive transfer of autologous lymphocytes transduced with genes encoding T-cell receptors against the NY-ESO-1 cancer-testis antigen have also mediated regressions in patients with metastatic melanoma.[277]

RADIATION THERAPY FOR METASTATIC MELANOMA (STAGE IV)

The Role of Radiation Therapy in the Management of Distant Metastatic Disease

In general, patients with one to two sites of metastatic melanoma, good performance status, and long interval from diagnosis of the primary lesion should be considered for surgical resection. Patients with widespread metastatic disease may be managed with systemic immunotherapy, targeted therapy, or chemotherapy, with palliative radiation to symptomatic areas of progressive disease. From a radiotherapy perspective, patients with distant metastasis of melanoma are generally managed similar to patients with distant metastases of other solid tumors, with the only main area of controversy being the role of whole-brain radiation therapy (WBRT) for patients with melanoma brain metastases. Patients with widespread systemic disease and short life expectancies should be treated with short courses of high dose per fraction radiation.

Cell Transfer Therapy

Treatment	Total	PR	CR	OR (%)
		Number of patients (duration in months)		
No TBI	43	16 (37%) (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (12%) (114+, 112+, 111+, 97+, 86+)	21 (49%)
200 TBI	25	8 (32%) (14, 9, 6, 6, 5, 4, 3, 3)	5 (20%) (101+, 98+, 93+, 90+, 70+)	13 (52%)
1200 TBI	25	8 (32%) (21, 13, 7, 6, 6, 5, 3, 2)	10 (40%) (81+, 78+, 77+, 72+, 72+, 71+, 71+, 70+, 70+, 19)	18 (72%)

(20 complete responses: 19 ongoing at 70 to 114 months)

Figure 94.18 The objective response by RECIST criteria of patients receiving cell transfer therapy using either the nonmyeloablative preparative regimen (No TBI) or with addition of 200 or 1,200 cGy total whole body irradiation (TBI). TBI, total body irradiation.

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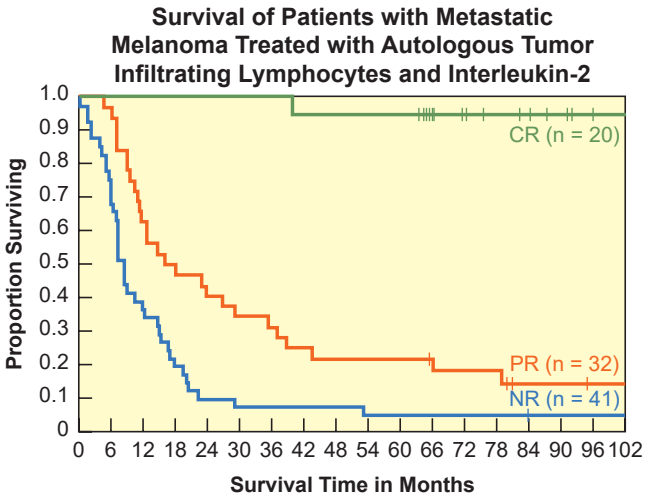

Figure 94.19 Survival curves of the 93 patients receiving autologous tumor-infiltrating lymphocytes and interleukin-2 following lymphodepleting preparative regimens. Twenty of the ninety-three patients achieved a complete response, and only one has recurred with the remaining complete responders continually disease free beyond 60 months.

Radiation Therapy for Brain Metastases

Carella et al.[278] retrospectively analyzed 60 patients from two RTOG studies performed in the 1970s with cerebral metastases from malignant melanoma to determine the response to whole-brain irradiation and reported that the palliative and survival benefit of WBRT for melanoma patients was comparable to those found for all other primary tumors. However, the median survival times for the two studies were short, only 10 and 14 weeks, and response was measured as symptomatic improvement, which may have been at least partially due to corticosteroids given to reduce cerebral edema. In a retrospective study from the MD Anderson Cancer Center reporting on 87 patients treated for metastatic melanoma to the brain with WBRT with total doses of at least 30 Gy, it was concluded that the frequent use of corticosteroids made it difficult to assess palliative benefit from the radiation, although some patients may have derived benefit as approximately 50% of patients were able to discontinue corticosteroids on the completion of radiation treatments. However, despite this potential benefit, the median survival was only 19 weeks.[279]

Several prospective clinical trials with objective imaging criteria have been conducted to assess tumor response to WBRT alone or with concurrent chemotherapy (temozolomide, fotemustine) in patients with melanoma brain metastases.[254,258,280] These studies suggest that WBRT has limited activity in the treatment of malignant melanoma metastatic to the brain and should be reserved for patients with widespread systemic metastases or diffuse brain metastases that are not amenable to surgical resection or stereotactic radiosurgery.

Stereotactic radiosurgery delivers high doses of radiation in a single fraction to cerebral lesions that are generally <3 cm in diameter and do not involve the brainstem. Yu et al.[281] reported one of the largest retrospective studies to date from the University of California, Los Angeles, consisting of 122 consecutive patients with 332 intracranial melanoma metastases who underwent gamma knife radiosurgery with a median prescribed dose of 20 Gy (range, 14 to 24 Gy). One-third of the patients also received WBRT. The overall median survival was 7 months from radiosurgery and 9.1 months from the onset of brain metastasis. In multivariate analysis, WBRT did not improve survival, and freedom from subsequent brain metastasis depended on intracranial tumor volume.

The RTOG trial 9508 was a phase 3 randomized trial that enrolled 333 patients with one to three brain metastases with multiple histologies (4% of patients had melanoma) to receive WBRT to a total dose of 37.5 Gy in 15 fractions over 3 weeks with and without a radiosurgery boost, with the dose depending on the tumor volume (tumors ≤2 cm received 24 Gy, tumors >2 cm and ≤3 cm received 18 Gy, and tumors >3 cm and ≤4 cm received 15 Gy).[282] Patients with a single brain metastasis had statistically improved survival with a radiosurgical boost compared with those without a boost (6.5 versus 4.9 months, respectively), and the authors concluded that WBRT and stereotactic radiosurgical boost should be the treatment of choice for patients with solitary unresectable brain metastasis. Given the historic data suggesting limited benefit from WBRT for patients with melanoma brain metastasis, the ECOG initiated study E6397, which was a phase 2 trial of radiosurgery for one to three newly diagnosed brain metastases for renal cell carcinoma, melanoma, and sarcoma without WBRT.[283] The dose prescription was based on tumor size and was similar to the RTOG 9508 trial. Thirty-one eligible patients were accrued to this study. The median survival was 8.3 months. The intracranial failure rate in and outside the radiosurgically treated volume at 3 months was 19% and 16%, and at 6 months it was 32% and 32%, respectively. The authors concluded that delaying WBRT may be appropriate for some subgroups of patients with radioresistant tumors, but routine avoidance of WBRT should be approached judiciously given the high intracranial failure rates.

In summary, current data would support the following treatment recommendations for patients with brain metastases from melanoma. All patients with symptomatic cerebral edema should be administered corticosteroids initially. Patients with good performance status and no or minimal systemic disease and a solitary resectable brain lesion should undergo resection. Similar patients with an unresectable brain lesion or up to five small metastatic lesions should be treated with stereotactic radiosurgery, and both groups should be considered for WBRT or close observation with serial imaging. Patients with poor performance status, diffuse systemic disease, and more than five brain lesions have a poor overall prognosis and should be considered for palliative WBRT.

Radiation Therapy for Vertebral Metastases

Patients with vertebral metastases causing spinal cord compression with a reasonable life expectancy of >1 to 2 months should be treated with corticosteroids and surgical decompression if they are operative candidates, with subsequent postoperative radiotherapy as opposed to palliative radiation alone, as this has been shown to result in superior preservation of neurologic function.[284] Patients who are not operative candidates should be considered for radiation therapy. Recent advancements in radiation therapy planning and delivery have led to the development of stereotactic body radiation therapy (SBRT) for lesions in multiple organ sites (lung, liver, and spine). With SBRT, patients are treated with one to five fractions of high-dose and highly conformal radiation isodose distributions.[285] Patients who have limited systemic disease burden who are not considered surgical candidates, or patients with spinal cord compression from disease progression following palliative conventional radiation therapy, should be considered for SBRT.

Current Radiation Research for Melanoma: Interactions with Immune Therapy

Radiation has been reported to have immunomodulatory effects in melanoma animal models thought to be secondary to cell death and inflammation leading to enhanced antigen presentation and antigen-specific cellular immunity, which has been termed the abscopal effect.[286] The two main strategies of integration of radia-

tion into immune-based treatment strategies include local tumor irradiation resulting in enhanced tumor-antigen presentation and antigen-specific cellular immunity,[286,287] and total body irradiation–induced host lymphodepletion resulting in enhanced efficacy of adoptive T-cell transfer-based immunotherapy.[288] Recent case reports have documented systemic responses triggered after localized radiation therapy in patients receiving the anti-CTLA4 antibody ipilimumab.[289,290] These cases have triggered a renewed interest in the prospective testing of radiation therapy combined with immunotherapy in prospective clinical trials.

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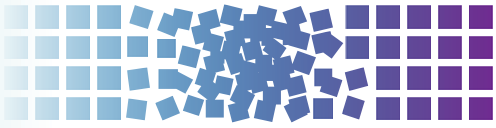

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INTRODUCTION

Many hereditary cancer predisposition syndromes are associated with cutaneous findings. Identification of unique dermatologic features provides an opportunity to distinguish hereditary cancer syndromes with similar associated internal malignancies. Although skin findings are an important diagnostic tool for a number of cancer syndromes, including Cowden syndrome, Birt–Hogg–Dubé, hereditary leiomyomatosis renal cell carcinoma, and others (Table 95.1),[1–18] this section will focus on skin cancer as well as tumor syndromes with cutaneous findings that are not included elsewhere in this book, including hereditary melanoma, basal cell nevus syndrome (BCNS), and neurofibromatosis type 1 (NF1), and neurofibromatosis type 2 (NF2).

The identification of dermatologic abnormalities and their association with internal malignancies often require thorough observation from clinicians. A consultation with a dermatologist may be helpful to identify specific dermatologic abnormalities. In some cases, biopsy and pathology may be necessary for a diagnosis.

GENETIC COUNSELING

Genetic counseling for hereditary skin diseases is similar to the process for other cancer predisposition syndromes. The genetic counseling process generally includes a detailed family and medical history, risk assessment, discussion of benefits, and limitations of available genetic testing, including possible test results, discussion of medical management, and implications for family members.[19] Dermatologic evaluation and review of pathology records pertaining to the cutaneous findings may provide clarification on specific dermatologic observations. Consultation with a dermatologist and/or other specialist who is knowledgeable about hereditary syndromes is often essential to a clinical evaluation. When possible, reviewing the medical records of family members is also helpful to confirm dermatologic diagnoses, as reports of some skin findings in family members may contain some inaccuracies.[20]

HEREDITARY SKIN CANCER AND THE NEUROFIBROMATOSES

In addition to a few known single-gene disorders associated with skin cancers, confounding environmental factors, including solar ultraviolet radiation, as well as other genetic factors also are known to be associated with a varying degree of skin cancer risk. Separately, other hereditary tumor and cancer predisposition syndromes, such as NF1 and NF2, contain benign cutaneous features as common and sometimes predominant findings. General characteristics of a hereditary cancer predisposition syndrome include multiple tumors or cutaneous features in one individual, multiple affected family members, and individuals or families with related tumors, cancers, or unique physical characteristics. In some cases, young age at onset may also suggest a higher likelihood of a hereditary syndrome.

Hereditary Melanoma

Approximately 10% of melanoma cases are attributed to hereditary predisposition. Hereditary melanoma has been associated with mutations in two genes, cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and cyclin-dependent kinase 4 (*CDK4*). Mutations in *CDK4* are rare and have been identified in only a few hereditary melanoma families.[21] Of families with hereditary melanoma, defined as three or more diagnoses of melanoma in one family, approximately 20% to 40% will have a detectable mutation in *CDKN2A*.[22]

CDKN2A and *CDK4* both function as tumor suppressors. *CDKN2A* encodes two transcripts: p16 and p14ARF through alternate reading frames. The majority of *CDKN2A* mutation-carrying families have been found to have mutations that affect the p16 protein. Mutations affecting the function of p14ARF are reportedly rare in cutaneous melanoma families.[23]

Phenotype

Hereditary melanoma has also been referred to as familial atypical mole melanoma syndrome.[24] Although the presence of atypical moles has been associated with an increased risk for melanoma, it has not been identified as a strong predictor of *CDKN2A* mutation status.[25,26]

The penetrance of *CDKN2A* mutations has been observed to be dependent on geography. This is likely due to varying environmental and other genetic factors across geographic regions. A study of *CDKN2A* carriers selected based on positive personal and family history of melanoma observed the melanoma risk for *CDKN2A* mutation carriers to be 58% in Europe, 76% in the United States, and 91% in Australia.[27] In a population-based study of patients with melanoma, the penetrance of *CDNK2A* mutations was observed to be lower (28% risk for melanoma by the age of 80 years).[28] Variants in the melanocortin 1 receptor (*MC1R*) gene have been associated with increased *CDKN2A* penetrance.[29] The prevalence of *MC1R* has been observed to differ with ethnic background and is one example of a genetic factor influencing melanoma risk that varies by geographical region.[30]

In addition to melanoma, other cancers have also been observed in increased frequency in *CDKN2A* mutation carriers. Most notably, an increased risk for pancreatic cancer has been reported in some *CDKN2A* mutation–carrying families.[31] Less commonly, an increased risk for other cancers, including neural system tumors, nonmelanoma skin cancers, uveal melanoma, and head and neck cancers, has also been reported in individuals with *CDKN2A* mutations.[31,32]

In the United States, which is an area of moderate to high melanoma incidence, genetic counseling for hereditary melanoma has been generally recommended in families in which (1) three or more relatives are affected with melanoma, (2) one individual has three or more primary melanomas, or (3) both pancreatic cancer and melanoma are present in one family (Table 95.2).[15] Early age at onset in the absence of a family history of melanoma is not highly suggestive of a *CDKN2A* mutation.[33,34]

TABLE 95.1

Summary of Hereditary Cancer Syndromes with Cutaneous Features

	Cutaneous Features	Internal Tumor Site
Benign cutaneous features prominent		
Cowden syndrome[1,2]	Trichilemmoma, palmoplantar keratoses, oral mucosal papillomas, cutaneous facial papules, lipomas, macular pigmentation of the glans penis	Breast, thyroid, uterus
Birt–Hogg–Dubé[3]	Fibrofolliculomas, trichodiscomas, angiofibromas, perifollicular fibromas, acrochordons	Kidney
Childhood cancer syndrome (homozygous Lynch syndrome)[4]	Neurofibromas, CALMs	Hematologic, neural system, colon, small intestine, urinary tract
Hereditary leiomyomatosis renal cell carcinoma[5]	Cutaneous leiomyomas	Kidney, uterus
Multiple endocrine neoplasia type 2B[6]	Mucosal neuromas of the lips/tongue	Thyroid, adrenal gland, gastrointestinal tract
NF1[7,8]	Neurofibromas (cutaneous and subcutaneous), CALMs, freckling (inguinal, axillary), hypopigmented macules, cutaneous angiomas xanthogranulomas, glomus tumors, hyperpigmentation	Brain, spine, peripheral nervous system, optic pathway, small intestine, neuroendocrine, breast
NF2[7,9]	CALMs (usually one to three), plaque lesions, intradermal schwannomas, subcutaneous schwannomas, cutaneous neurofibromas (uncommon)	Brain, spine, peripheral nervous system, optic pathway
Peutz–Jeghers syndrome[10]	Mucocutaneous pigmentation	Breast, stomach, small intestine, colon, pancreas, ovary, testicle
Tuberous sclerosis complex[11]	Hypomelanotic macules, facial angiofibromas, shagreen patches, fibrous facial plaques, ungual fibromas	Brain, kidney, heart, neuroendocrine
Benign cutaneous features sometimes present		
Multiple endocrine neoplasia type 1[12]	Facial angiofibromas, collagenomas, lipomas	Pituitary, pancreas, parathyroid, gastroenteropancreatic tract
Familial adenomatous polyposis[13]	Lipomas, fibromas, and epidermal cysts	Colon, thyroid, small intestine, liver, brain, pancreas, ampulla of Vater
Skin cancer prominent		
BCNS[14]	Basal cell carcinoma	Brain, ovary, heart
Hereditary melanoma[15]	Melanoma, dysplastic nevi	Pancreas
Xeroderma pigmentosum[16]	Melanoma, basal cell and squamous cell carcinoma, severe sunburn, lentigos, xerosis, erythema, actinic keratoses, poikiloderma	Oral cavity
Skin cancer sometimes present		
Hereditary breast and ovarian cancer syndrome[17]	Melanoma	Breast, ovary, prostate, pancreas
Lynch syndrome[18]	Sebaceous neoplasms, keratoacanthomas	Colon, uterus, stomach, ovary, hepatobiliary tract, urinary tract, small intestine, brain

CALM, café-au-lait macule; NF1, neurofibromatosis type 1; NF2, neurofibromatosis type 2; BCNS, basal cell nevus syndrome.

TABLE 95.2

Referral Criteria for Hereditary Melanoma Genetic Counseling

Three or more relatives on the same side of the family with melanoma
Three or more primary melanomas in one individual
Pancreatic cancer and melanoma on the same side of the family

From Leachman SA, Carucci J, Kohlmann W, et al. Selection criteria for genetic assessment of patients with familial melanoma. *J Am Acad Dermatol* 2009;61:677e1–677e14.

Genetic Testing

Clinical testing for *CDKN2A* and *CDK4* is available in the United States at several commercial laboratories. However, some of the laboratories offering hereditary melanoma testing perform analysis of only *CDKN2A*, given the relatively low-frequency *CDK4* mutations reported.

The utility of genetic testing for *CDKN2A* mutations remains a source of debate. This is partly due to the relatively low frequency of *CDKN2A* mutations in families with melanoma. In addition, many individuals with a personal and/or family history of melanoma are under close surveillance and aware of risk-reduction recommendations; therefore, genetic test results would not alter

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TABLE 95.3

***CDKN2A* Genetic Testing Results and Medical Management Recommendations**

Test Result	Medical Management
CDKN2A mutation positive	Melanoma surveillance: ■ Clinical skin examination with dermatologist every 4–6 mo ■ Biopsy should be performed on suspected lesions ■ Avoid prolonged direct sunlight and use sun-protective clothing and sunscreen ■ Monthly self-skin examinations ■ Inform at-risk relatives Pancreatic cancer surveillance[a]: ■ Recommended for individuals with a family history of pancreatic cancer and may be considered in other cases ■ Refer to gastroenterologist for discussion of pancreatic screening options ■ Inform at-risk relatives
CDKN2A variation of unknown significance	Etiology of the melanoma remains unknown: ■ Consider if genetic testing is indicated for other affected relatives ■ Proband and family remain at increased risk for melanoma ■ Screening recommendations should be based on personal and family history
CDKN2A mutation negative	No mutation previously identified in family: ■ Etiology of the melanoma remains unknown ■ Consider if genetic testing is indicated for other affected relatives ■ Proband and family remain at increased risk for melanoma ■ Screening recommendations should be based on personal and family history Mutation previously identified in family: ■ Proband and family remain at increased risk for melanoma, although the risk is lower than for relatives who carry a *CDKN2A* mutation ■ Screening recommendations should be based on personal and family history

[a] To date, pancreatic cancer surveillance has not been proven to be effective at improving pancreatic cancer outcome.

clinical management.[25] Also, the role of pancreatic cancer surveillance in *CDKN2A* carriers remains under investigation. Some studies have suggested that knowledge of *CDKN2A* mutation status improves short-term compliance to risk-reducing behaviors.[35,36] However, information regarding the long-term impact of *CDKN2A* testing is limited at this time. The possible genetic test results for an individual undergoing *CDKN2A* genetic testing are shown in Table 95.3.

Individuals with a *CDKN2A* mutation have a 50% chance of passing the mutation on to their children.

Medical Management

CDKN2A mutation carriers, or individuals at 50% risk to be a carrier, should be monitored carefully for melanoma through clinical and self-examinations (see Table 95.3). In addition, *CDKN2A* carriers are recommended to avoid prolonged direct sunlight and utilize sun-protective clothing and sunscreen.[25,37]

Individuals who test negative for a familial *CDKN2A* mutation may also have an increased risk for melanoma. However, this risk has been observed to be lower than the melanoma risk for *CDKN2A* mutation carriers.[28]

As noted in Table 95.3, *CDKN2A* mutation carriers, especially those with a family history of pancreatic cancer, are candidates for pancreatic cancer surveillance and should discuss the risks, benefits, and limitations of screening with a gastroenterology specialist.[38] However, to date, the effectiveness of pancreatic surveillance remains under investigation.[39]

Basal Cell Nevus Syndrome

BCNS, also known as Gorlin syndrome or nevoid basal cell carcinoma syndrome, is an autosomal dominant syndrome associated with cutaneous findings, including basal cell carcinoma, as well as skeletal system, nervous system, and ocular abnormalities.[40] Although BCNS has complete penetrance, the expression is variable.[41]

BCNS is thought to be relatively uncommon, and the incidence of BCNS has been estimated to be 1:30,827 to 1:57,000.[42] The variable expression may cause difficulty in diagnosing BCNS.

BCNS has been associated with mutations in the *patched gene 1* (*PTCH1*) gene. *PTCH1* functions as a tumor suppressor in the sonic hedgehog (Shh) pathway, which is also involved in embryonic development.[43] Chromosomal abnormalities of 9q22.3 region, which includes *PTCH1*, have been reported in a few individuals with features of BCNS as well as other features, including short stature, developmental delay, and seizures.[44] Rarely, mutations in other genes, including *SUFU* and *PTCH2*, have also been reported in individuals with features of BCNS.[45,46]

Phenotype

The phenotype of BCNS is variable, and some characteristics are present at different life stages. Therefore, it is important to obtain a complete medical history, including physical examination and dermatologic, cardiac, and gynecologic examinations as well as radiologic studies to confirm a diagnosis of BCNS.

The clinical manifestations of BCNS include the following.

Skin

Basal Cell Carcinoma. Approximately 50% to 75% of individuals with BCNS will develop basal cell carcinomas.[47] Typically, basal cell carcinomas develop in the late teens through the 30s, but some published reports have indicated the detection of basal cell carcinomas in early childhood in individuals with BCNS. The presence of basal cell carcinomas is also dependent on other factors, including skin type and radiation exposure, including sun exposure.[40,41]

Noncancerous Cutaneous Features. The majority of individuals with BCNS will have multiple nevi present by adulthood.[40] In addition, BCNS is associated with an increased prevalence of facial milia, dermoid cysts, and skin tags. Palmar and plantar pits are also a common feature of BCNS and usually are evident by early adulthood.[40]

Skeletal

Skeletal abnormalities, including rib and spinal abnormalities, are reported with increased frequency in BCNS. The majority of individuals with BCNS are reported to have macrocephaly.[48]

Central Nervous System

Ectopic Calcification. Ectopic calcification, particularly of the falx celebri, has been reported as a common finding in individuals with BCNS.[48]

Brain Tumor. Although other types of brain tumors have been reported in individuals with BCNS, medulloblastoma, typically desmoplastic type, is the most common.[49] Approximately 5% of individuals with BCNS are diagnosed with medulloblastoma, usually around 2 years of age.

Other Features

Jaw Keratocysts. Approximately 75% of affected individuals with BCNS develop multiple jaw keratocysts.[50]

Characteristic Facial Features. Facial features characteristic of BCNS, including macrocephaly, bossing of the forehead, coarse facial features, and facial milia, have been observed in approximately 60% of BCNS cases.[14]

In addition to these features, congenital malformations such as cleft lip/palate, polydactyly, and eye anomalies have also been reported as features of BCNS.[40]

Additional associated tumors including cardiac and ovarian fibromas have also been reported to occur with increased frequency in BCNS.[51,52]

Diagnosis and Genetic Testing

A diagnosis of BCNS was initially based on clinical criteria; however, the availability of molecular testing has identified mutations in individuals with a more variable phenotype. The First International Colloquium on BCNS concluded that the clinical criteria should be used to consider a suspected diagnosis of BCNS rather than as diagnostic criteria.[53] The colloquium recommends that a suspected diagnosis of BCNS be considered in individuals with an identified *PTCH1* mutation and one major clinical criterion, individuals who express two major criteria, and individuals with one major and two minor criteria (Table 95.4).

Genetic testing for the *PTCH1* gene is clinically available. Approximately 50% to 85% of individuals with clinical features of BCNS will have a detectable mutation in the *PTCH1* gene through gene sequencing analysis. Deletions and duplications of the *PTCH1* gene have also been reported.[54]

Approximately 20% to 30% of individuals with BCNS are de novo, meaning that neither parent carries the associated gene mutation.[14] Individuals affected with BCNS have a 50% chance of having an affected child. In cases where a mutation has been identified, testing is an option for at-risk family members. In addition, both preconception genetic diagnosis and prenatal testing are available for known *PTCH1* mutations.

Medical Management

Because of the many variable symptoms of BCNS, individuals with BCNS should be referred to an appropriate specialist depending on the symptoms.

TABLE 95.4

Clinical Criteria for Suspected Diagnosis of Basal Cell Nevus Syndrome

Major criteria

- Early-onset/multiple basal cell carcinoma
- Odontogenic keratocyst of the jaw (<20 y of age)
- Palmaroplantar pitting
- Calcification of the falx cerebri
- Medulloblastoma (usually desmoplastic)
- First-degree relative with BCNS

Minor criteria

- Rib anomalies
- Skeletal malformations and radiologic changes
- Macrocephaly
- Cleft lip/palate
- Ovarian/cardiac fibroma
- Lymphomesenteric cysts
- Ocular abnormalities

BCNS, basal cell nevus syndrome.
From Bree AF, Shah MR. Consensus statement from the first international colloquium on basal cell nevus syndrome (BCNS). *Am J Med Genet A* 2011; 155A:2091–2097.

Basal Cell Carcinoma. Early diagnosis is important for management and to limit cosmetic damage. Surgery, oral retinoids, topical therapies, and photodynamic therapy have all been utilized with varying degrees of success for individuals with BCNS.[47]

Medulloblastoma. Consideration of developmental assessment and physical examination every 6 months is an option for children during infancy and early childhood. Imaging for medulloblastoma surveillance is not currently recommended.[14]

Jaw Keratocysts. Clinical examinations and imaging are recommended for individuals with BCNS, starting during childhood. These tumors may sometimes be detected during routine dental examinations.[55]

Ovarian and Cardiac Fibromas. Affected individuals with cardiac fibromas should be referred to a cardiologist. Ovarian fibromas also warrant a specialty referral and may require surgery, ideally with the aim of preserving fertility.[56]

Radiation Exposure. Given the known increased risk for basal cell carcinoma, it is recommended that individuals with BCNS avoid sun exposure. In addition, it is recommended that other radiation exposure also be avoided if possible, including radiation as treatment for medulloblastoma.[49]

Neurofibromatosis Type 1

NF1 is one of the most common genetic disorders, affecting an estimated 1:2,500 to 1:3,000 individuals at birth.[7] Formerly known as von Recklinghausen disease or peripheral neurofibromatosis, manifestations of the disease affect multiple areas of the body, including, but not limited to, the central and peripheral nervous systems, skin, eyes, skeleton, gastrointestinal system, and the cardiovascular system. Historically, observations of patients with NF1 date back to the 13th century, but the disorder was first formally described in 1882 by Friedrich von Recklinghausen.[7,57,58]

NF1 is a completely penetrant autosomal dominant condition with widely variable expression, both within and between families.[59] No ethnic, racial, or sex predilection has been observed.[57] NF1 is caused by mutations in the NF1 gene on 17q11.2. The

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protein product of NF1 is neurofibromin, a GTPase-activating protein that is expressed across many tissue types and in particularly high levels within neurocutaneous tissue. It acts as a negative regulator of intracellular Ras signaling pathways involved in cell growth and proliferation.[7,60,61] More recently, NF1 has also been linked to the development of skeletal muscle.[62]

Phenotype

In 1987, the National Institutes of Health developed clinical diagnostic criteria for NF1 (Table 95.5) on which diagnosis of the disease is most often based.[59] The disease usually presents in childhood, beginning with skin findings, which are often present by 1 year of age. In general, the clinical manifestations of NF1 are age dependent: By the age of 6 years, approximately 90% of individuals with NF1 meet diagnostic criteria; by 8 years of age, 97% meet criteria, and virtually all meet the criteria by the time they are 20 years old.[59]

Skin

Among the numerous and variable clinical manifestations of NF1, cutaneous findings feature prominently and can even be the sole basis for a diagnosis of NF1. The following skin findings are hallmark features of NF1, and each is a component of the diagnostic criteria (see Table 95.5).

Café-Au-Lait Macules. Café-au-lait macules (CALM) are the most common and often the earliest presenting feature of NF1. CALMs may be congenital and are observed in almost all patients with NF1 within the first year of life. They often become larger and more numerous through adolescence and may fade as an adult.[57]

Intertriginous Freckling. Skinfold freckling, or Crowe sign, is a cardinal feature of NF1.[59] Freckling occurs most often in the axillary and inguinal regions of the body and is exhibited by up to 90% of patients, usually beginning in childhood. Freckling may also be found in other areas of the body including beneath the breasts in females, on the neck, above the eyelids, around the mouth, and on the trunk in adults.[58]

Neurofibromas. The hallmark feature of NF1, neurofibromas can develop in almost any part of the body, including on or just below the surface of the skin. Cutaneous neurofibromas vary in size (<1 mm to large/disfiguring) and number; they are soft and fleshy, and may be raised or flat, ranging in color from blue/purple to brown to flesh colored. Subcutaneous neurofibromas are firm, tender nodules that are often visible beneath the skin. Cutaneous and/or subcutaneous neurofibromas usually manifest later than CALMs and freckling, either later in childhood or in early adolescence.[58,59]

Less common, nondiagnostic cutaneous features of NF1 include hyperpigmentation, which may be generalized or appearing in conjunction with affected body areas in segmental NF1, glomus tumors, hypopigmented macules (usually on the trunk), xanthogranulomas, cutaneous angiomas, and pruritus.[7,57,60]

TABLE 95.5

National Institutes of Health Diagnostic Criteria: Neurofibromatosis Type 1

Clinical diagnosis of NF1 can be made for an individual exhibiting any two (or more) of the following:

- Six or more café-au-lait macules:
  - ≥5 mm prepubertal
  - ≥15 mm postpubertal
- Two or more neurofibromas of any type, or one or more plexiform neurofibromas
- Freckling in the axillary or inguinal region
- Optic glioma
- Two or more Lisch nodules (iris hamartomas)
- A distinctive osseous lesion such as sphenoid dysplasia or tibial pseudoarthrosis
- A first-degree relative with NF1 as defined by the above criteria

NF1, neurofibromatosis type 1.
From Evans DG, Raymond FL, Barwell JG, et al. Genetic testing and screening of individuals at risk of NF2. *Clin Genet* 2012;82:416–424.

Neurologic

Tumors of the central and peripheral nervous systems are prevalent among individuals with NF1. These include spinal neurofibromas, peripheral nerve sheath tumors, plexiform neurofibromas, and astrocytomas. In addition, optic pathway gliomas (OPG) are slow-growing tumors occurring among 15% to 20% of patients, usually by the age of 6 years. OPGs are symptomatic in only 5% of individuals, in which case they are most often diagnosed by the age of 3 years.[7,57,60]

A variety of nontumor neurologic manifestations are reported among individuals with NF1. These include learning disabilities, which occur in 60% or more of children with NF1, decreased IQ (occasionally <70), attention-deficit/hyperactivity disorder, and other behavior difficulties. Unidentified bright objects (UBO) are a characteristic magnetic resonance imaging (MRI) finding in NF1. The clinical significance of UBOs is not known, but some evidence correlates UBO prevalence with severity of cognitive and behavioral difficulties.[58,59] Seizure disorders and multiple sclerosis also occur at a higher frequency in NF1, and Chiari type I malformation, aqueductal stenosis, and macrocephaly have all been reported.[7,59]

Eye

Lisch nodules, or melanocytic iris hamartomas, are asymptomatic eye findings present in most individuals with NF1, usually by the age of 5 to 10 years. Lisch nodules are pathognomonic for NF1 and are most reliably detected by an experienced ophthalmologist by slit-lamp examination.[7,8,60] Glaucoma, choroidal abnormalities, and ptosis are less common but have all been reported in patients with NF1.[7]

Skeletal

Bony growths and other abnormalities of the bone are key features of NF1. Diagnostic bone findings include thinning of the long-bone cortex (with or without pseudoarthrosis) and sphenoid wing dysplasia. In addition, there is an increased frequency of short stature, scoliosis, and, more recently noted, osteopenia and osteoporosis among individuals with NF1.[7,57,60]

Cardiovascular

Cardiovascular complications occur at a higher frequency among patients with NF1 and include congenital heart disease (pulmonary stenosis, coarctation of the aorta), hypertension, cerebrovascular disease, and renal artery stenosis.[7,63] Pulmonary stenosis is more prevalent among patients with classic NF1 but may also be found as part of a variant phenotype that combines features of NF1 and Noonan syndrome.[59]

Other Features

Respiratory Complications. Respiratory complications include restrictive lung disease caused by compression from neurofibroma and metastases from malignant peripheral nerve sheath tumors.[7]

Neurofibromatosis Type 1–Associated Malignancies. The overall increased risk of cancer in NF1 patients is 2.7-fold, and the cumulative risk for patients older than 50 years is 20%.[61] Malignant peripheral nerve sheath tumors are the most common cancerous tumors in NF1. Other malignancies include chronic myelogenous

leukemia, astrocytoma, rhabdomyosarcoma, gastrointestinal stromal tumors, carcinoid tumors (small intestine), pheochromocytomas (although usually not malignant), and breast carcinoma.[61] There are also a few reports of higher rates of melanoma seen in NF1; however, this association remains controversial.[57]

Diagnosis and Genetic Testing

Up to 50% of individuals with NF1 have no family history and represent de novo mutations. The *NF1* gene has one of the highest spontaneous mutations rates, about 1:10,000, and more than 500 pathogenic mutations have been identified.[61] The reason for the high mutation rate is not fully understood, but may be due in part to the large size of the gene.[59]

Although diagnosis of NF1 is almost always made on a clinical basis using the established criteria, genetic testing is available and can be useful, particularly in certain situations. Mutations are identifiable in 95% of individuals who meet the NF1 clinical diagnostic criteria.[64] In young children with no family history who do not yet meet the diagnostic criteria, genetic testing may aid in differentiating NF1 from other disorders with phenotypic overlap such as Legius syndrome, familial café-au-lait spots, and NF2. Genetic testing may also help identify rare variant forms of the disease that do not satisfy the National Institutes of Health criteria. In families with a previously identified mutation, prenatal diagnosis and prenatal testing are available. A common challenge in prenatal counseling and testing for NF1 arises from the variability and unpredictability of the disease presentation.[7,65]

Given the wide variability in expression of the disease even among members of the same family, it stands to reason that very few genotype–phenotype correlations have been described. It has been noted that individuals carrying a deletion of an entire NF1 allele (approximately 4% to 5% of cases) are likely to exhibit a more severe phenotype, including a greater number of cutaneous neurofibromas, often occurring at younger ages. Cognitive abnormalities are also more frequent and severe, and somatic overgrowth, large hands and feet, and dysmorphic facial features have been reported.[63] In addition, individuals with a three-base-pair in-frame deletion of exon 17 of NF1 may exhibit the common nontumor cutaneous features of NF1, without cutaneous or surface plexiform neurofibromas.[63]

In addition to the high rate of spontaneous mutations, another challenge associated with genetic counseling for NF1 is the high rate of mosaicism. Approximately 40% to 50% of cases are segmental, or mosaic, representing postzygotic NF1 mutations. In these cases, recurrence risks can be difficult to predict; however, they are usually estimated to be $<1\%$ unless the germline is affected. Indeed, there are cases of individuals with segmental NF1 bearing children with constitutional disease.[63]

Medical Management

Management of NF1 requires multidisciplinary input and, ideally, should be overseen by practitioners experienced in caring for patients with neurofibromatosis.[58,60]

Recommended surveillance for children with NF1 may vary somewhat by center, but typically includes annual physical and ophthalmologic examination until the age of 8 years. Between the ages of 8 and 18 years, examinations every other year may be sufficient.[58,60,64] Blood pressure monitoring should take place at least annually because of the risk of pheochromocytoma and renal artery stenosis.[58,64] In addition, annual neurologic examinations are advisable, with consideration of neuroimaging in the presence of any abnormal findings.[58] In addition, ongoing developmental and neuropsychological evaluation is recommended to assess cognitive function and to identify learning disabilities.[8,57]

Screening by way of MRI, electroencephalogram, and/or X-ray may be dictated by symptoms, clinical findings, and/or personal and family history. For certain findings, more frequent monitoring may be indicated and, in some cases, treatment may be available.

Plexiform Neurofibroma. Perform MRI every 6 to 12 months to monitor growing lesions. Depending on the location of the lesion, surgical debulking may be possible but is often incomplete, resulting in regrowth.[8,60,64]

Optic Pathway Gliomas. Once identified, MRI is used to monitor OPGs. Quarterly ophthalmologic evaluation is suggested for the first year, followed by annual examination of patients for at least 3 years or until the age of 8 years. Evaluation by endocrinology may be recommended. For symptomatic OPGs, chemotherapy treatment is available, but radiotherapy is not recommended.[8,57]

Malignant Peripheral Nerve Sheath Tumor. Monitor individuals with plexiform neurofibroma for increased size and pain, as well as changes in tumor texture; monitor unexplained neurologic changes. If possible, complete surgical resection is desired, but should be followed by radiation therapy if it is not complete.[8,64]

Cutaneous Neurofibromas. Surgical removal of neurofibromas may be possible when necessary for cosmetic or pain-related reasons.[8,57]

As necessary, referrals should be made to a variety of specialties, including cardiology, nephrology, plastic surgery, otolaryngology, and gastroenterology.

Neurofibromatosis Type 2

NF2 was first described by J. H. Wishart in 1822, at least 50 years prior to von Recklinghausen's description of NF1. Although there is relatively little overlap in the clinical phenotype of the two conditions, NF2 is much less common and was, until relatively recently, often mistaken as a variant form of NF1. It was not until 1987 when linkage studies attributed the conditions to two different genes on different chromosomes that the diseases were formally recognized as separate. Although more common than it was once thought to be, the estimated incidence of NF2 is approximately 1/10 of that of NF1, or 1:30,000 to 1:40,000.[66]

NF2 is inherited in an autosomal dominant manner and is virtually 100% penetrant by the age of 60 years.[9] It is caused by mutations in the NF2 gene on chromosome 22q12. The product of the *NF2* gene is the protein known as merlin (moesin–ezrin–radixin-like protein) or schwannomin, and it is thought to function in cell membrane protein organization, cellular adhesion, and negative regulation of cellular growth, proliferation, and motility.[60,64] The specific mechanism of the tumor suppressor function of merlin has not yet been fully elucidated and is an area of active investigation.[60]

A key difference between NF1 and NF2 relates to cutaneous findings, which, in NF2, may aid in diagnosis but are not diagnostic in and of themselves. The cardinal feature of NF2 is vestibular schwannoma, which arise bilaterally on the eighth cranial nerve in almost all cases of the disease.[7]

Phenotype

Contrary to the name of the disorder, schwannomas and meningiomas, not neurofibromas, are the most prominent tumor types found in NF2.[9,67] Individuals with NF2 most often present between 20 and 30 years of age with hearing loss (frequently unilateral) related to the presence of a vestibular schwannoma. Tinnitus, dizziness, and imbalance are also common adult symptoms at presentation.[9] Although children may also develop similar symptoms, they are more likely to present with less common features of NF2, making examination of other systems the key to accurate diagnosis. In these cases, neurologic examination, eye examination, and careful examination of the skin become crucial.[64] Several sets of NF2 diagnostic criteria exist, and the criteria may still be evolving[68]; however, currently, the most widely used criteria set is the Manchester Diagnostic Criteria, shown in Table 95.6.[60]

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TABLE 95.6

Diagnostic Criteria for Neurofibromatosis Type 2

Manchester Diagnostic Criteria for NF2

- Bilateral vestibular schwannoma
  or
- First-degree family member with NF2 and unilateral vestibular schwannoma, or any two of meningioma, glioma, neurofibroma, schwannoma, posterior subcapsular lenticular opacities
  or
- Unilateral vestibular schwannoma and any two of meningioma, glioma, neurofibroma, schwannoma, posterior subcapsular lenticular opacities
  or
- Multiple meningiomas (≥2) and unilateral vestibular schwannoma or any two of glioma, neurofibroma, schwannoma, cataract

NF2, neurofibromatosis type 2.

Skin

Although not hallmarks of the disease, the cutaneous manifestations of NF2 are prevalent and can be detected in up to 70% of cases.[7] As with NF1, skin findings include CALMs; however, when CALMs are present in NF2, it is generally fewer, about one to three per person. Individuals with NF2 may also exhibit plaque lesions on the surface of the skin, intradermal schwannomas, subcutaneous schwannomas, and, very rarely,[7,57,60] cutaneous neurofibromas.[7,9]

Neurologic

Tumors. Bilateral vestibular schwannomas occur in 90% to 95% of patients with NF2.[7] Although malignancy is rare, the location of growth is a common cause of increased morbidity, often causing progressive hearing loss and balance issues.[58] Schwannomas of other cranial nerves are not uncommon among patients with NF2[9]; in addition, spinal and peripheral nerve schwannomas often develop.[64] Meningiomas are the second most common tumor type, found in 58% to 75% of patients with NF2. Both cranial and spinal meningiomas can be found in NF2.[64] More rare, but also observed, are spinal and brainstem ependymomas, as well as spinal and cranial astrocytoma.[7]

Peripheral Neuropathy. The majority of patients with NF2 will develop peripheral neuropathy within their lifetime, often in childhood presenting as a hand or foot drop, or a palsy. Neuropathy is sometimes but not always related to tumor compression.[9]

Eye

Subcapsular lenticular opacities are a key diagnostic feature of NF2. They are found in 60% to 81% of patients and may develop into cataracts.[9,58] Additional ocular findings include epiretinal membranes, or thin translucent or semitranslucent sheets of fibrous tissue, which usually do not decrease visual acuity.[9] In addition, retinal hamartomas appear in 6% to 22% of patients with NF2 and can cause a loss of visual acuity.[9]

Diagnosis and Genetic Testing

In patients with suspected NF2 and a positive family history (two or more family members affected), genetic testing reveals mutations in ≥90%. However, approximately 50% of individuals with NF2 represent de novo mutations in the *NF2* gene. In isolated cases of classic NF2 with no known family history, mutations are identified in approximately 60% to 72%. In families with an identified mutation, presymptomatic genetic testing of at-risk family members is important for management of the disease. Prenatal genetic testing and preimplantation genetic diagnosis are also available.[69]

Somatic mosaicism is observed in roughly 33% of individuals with de novo cases of NF2, and identification of these individuals often relies on confirming the presence of the same mutation in tissue from two distinct NF2-related lesions.[67] Finally, for some mutations, genotype–phenotype correlation data are available.[9]

Medical Management

In general, as with NF1, it is best if patients with NF2 are able to be followed by experienced practitioners in a comprehensive clinic setting. Screening recommendations may include initiation of MRI screening for vestibular schwannomas at the age of 10 years, as symptoms of the tumors are rare in younger patients. When present, growth of vestibular schwannomas is best measured by tumor volume using MRI.[60] Head and spinal MRI is the primary screening tool and should be performed every 2 years for at-risk children younger than 20 years with no symptoms or tumors. After the age of 20 years, the tumors grow slower and screening can be decreased to every 3 to 5 years.[69] Annual ophthalmologic examination is recommended from infancy in at-risk or affected individuals. In addition, the following annual examinations, initiated in infancy, may be recommended: Neurologic examination and audiology with auditory brainstem evoked potentials.[9]

When it is possible, surgery is the primary mode of treatment for NF2 tumors, with the intent of improving quality of life and maintenance of function. Surgery is not always possible and, in some cases, radiation therapy may be used as an alternative. Overall, patients with NF2 have a shorter life expectancy.[60]

CONCLUSION

Dermatologic examinations, when combined with a thorough personal and family medical history, play an important role in the diagnosis of many cancer predisposition syndromes. Although some cutaneous features are strongly indicative of a specific diagnosis, others may be less common or less strongly associated with a particular syndrome; therefore, it remains important to consider these findings in the context of a patient's complete medical and family history. The current availability of molecular testing for many hereditary syndromes has significantly advanced the ability to distinguish and confirm a suspected clinical diagnosis. In addition to the syndromes listed in this chapter, it is important to note that other cancer predisposition syndromes may also have cutaneous components, and with the advancement of molecular testing, additional syndromes are likely to be identified in the future.

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96 Molecular Biology of Central Nervous System Tumors

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GLIOMAS

Gliomas account for 26.4% of all primary brain tumors, with malignant gliomas (grade III or IV) accounting for 19.9% of all primary brain tumors.[1] The World Health Organization (WHO) classifies gliomas by the cells they morphologically resemble (astrocytes, oligodendrocytes, or a mixture) and groups the tumors into four grades based on histology and aggressiveness.[2] High-grade gliomas (WHO grades III and IV) have a dismal prognosis, with the median survival for grade IV astrocytomas (glioblastoma multiforme [GBM]) less than 15 to 20 months.[3] Low-grade gliomas (WHO grades I and II) are heterogeneous in terms of their prognosis and likelihood to progress to high-grade gliomas.

Adult Low-Grade Gliomas

What drives some adult low-grade gliomas to progression whereas others remain indolent is an area of active investigation. Grade I gliomas, the majority of which are pilocytic astrocytomas, are histologically benign tumors with low potential for malignant progression that primarily occur in the pediatric population and are discussed in detail in the pediatric low-grade glioma section, which follows. Histologically, WHO grade II gliomas can be divided into tumors that arise from astrocytes (diffuse astrocytomas), oligodendrocytes (oligodendrogliomas), or tumors with elements of both cellular populations (oligoastrocytomas).[2] All three histologic subtypes have frequent neomorphic driver mutations affecting the R132 residue of isocitrate dehydrogenase 1 (IDH1),[4] a mutation that generates the oncometabolite 2-hydroxyglutarate (2HG).[5] Ultimately, the IDH1 R132 mutation drives gliomagenesis through epigenetic dysregulation, including DNA CpG hypermethylation (G-CIMP phenotype)[6] and alterations in histone methylation.[7–9] Despite sharing the IDH1 R132 driver mutation, grade II oligodendrogliomas have improved median overall survival (11.6 years for grade II oligodendrogliomas versus 5.6 years for grade II astrocytoma),[10] and a much lower rate of progression to a high-grade glioma (45% for oligodendroglioma versus 74% for astrocytoma).[1] The survival and progression benefit seen in IDH1 mutant grade II gliomas is likely modified by comutations. Oligodendrogliomas commonly have a loss of heterozygosity (LOH) on chromosomes 1p and 19q,[11–13] which is usually the result of a single pericentromeric translocation event.[14] The 1p/19q loss in oligodendrogliomas frequently co-occurs with somatic mutations in capicua transcriptional repressor (*CIC*, located on chr1) or far upstream element (FUSE) binding protein 1 (*FUBP1*, located on chr19),[15] and *IDH1-CIC/FUBP1-1p/19q loss* gliomas have a median survival of 8 years.[16] In contrast, grade II astrocytomas commonly have somatic mutations in the chromatin modifier alpha thalassemia/mental retardation syndrome X-linked (*ATRX*),[16] mutations in tumor protein p53 (*TP53*),[4] and LOH at chr17 (where TP53 is found), and *IDH1-ATRX-TP53* gliomas have a median survival of 5 years.[16] This latter group is likely to progress to form secondary GBMs, a process mediated by epigenomic dysregulation and deletion of retinoblastoma 1 (*RB1*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*), and phosphatase and tensin homolog (*PTEN*).[17,18] Oligoastrocytomas have a combination of mutations and chromosomal LOH found in astrocytomas and oligoastrocytomas and a median survival of 6.6 years.[10]

Adult High-Grade Gliomas

Glioblastoma multiforme (WHO grade IV) is the most common malignant brain tumor, accounting for 15.6% of all primary brain tumors and 60% of all gliomas.[1] There are two major routes to GBM formation: *de novo* formation (primary GBM, 95% of cases), or progression from low-grade glioma (secondary GBM, 5% of cases) (Fig. 96.1).[17] Secondary GBMs occur in younger patients, result in improved survival, and bear *IDH1* mutations with common co-mutations in *ATRX* and *TP53* and deletion of *RB1*, *CDKN2A*, and *PTEN*.[16–21] In contrast, primary GBMs occur in older patients, have poor survival, and have dysregulation of three core pathways: p53, retinoblastoma (Rb), and receptor tyrosine kinase/Ras/phosphoinositide 3-kinase (PI3K) signaling (RTK/Ras/PI3K signaling).[17,20,22–24] A recent large-scale GBM next-generation sequencing study by The Cancer Genome Atlas (TCGA) found that the p53 pathway is somatically disrupted in 85.3% of GBMs, through p53 loss (27.9%), homozygous deletion of *CDKN2A* (57.8%), and amplification of *MDM1/2/4* (15.1%).[24] The Rb pathway is also impacted by frequent *CDKN2A* deletion, and other hits to Rb signaling have been seen via RB1 loss (7.6%) or amplification of cyclin-dependent kinase 4 or 6(*CDK4/6*) (15.5%), for a total of 78.9% alteration of Rb signaling.[24] Somatic alterations in receptor tyrosine kinases were observed in 67.3% of GBMs, most prominently in epidermal growth factor receptor (*EGFR*) (57.4%) and platelet-derived growth factor receptor alpha (*PDGFRA*) (13.1%).[24] Of GBMs showed either PTEN loss or PI3K mutation, and neurofibromin 1 (NF1) loss was seen in 10% of tumors.[24] In combination, the RTK/Ras/PI3K signaling was hit once in 89.6% of tumors and hit multiple times in 39% of tumors.[24] Additionally, 83.3% of GBMs were reported to have recurrent telomerase reverse transcriptase (*TERT*) promoter mutations (C228T or C250T), and these mutations are mutually exclusive with *ATRX* mutations.[25]

Based on gene expression profiling, GBMs cluster into four groups: classical (chromosome 7 amplification with chromosome 10 loss, deletion of *CDKN2A*), mesenchymal (*NF1* focal deletions or mutations), proneural (*IDH1* mutant or *PDGFRA* amplification), and neural (*EGFR* amplification with a neural expression signature).[23] These classifications have a utility for predicting response to therapy; for example, the classical subtype

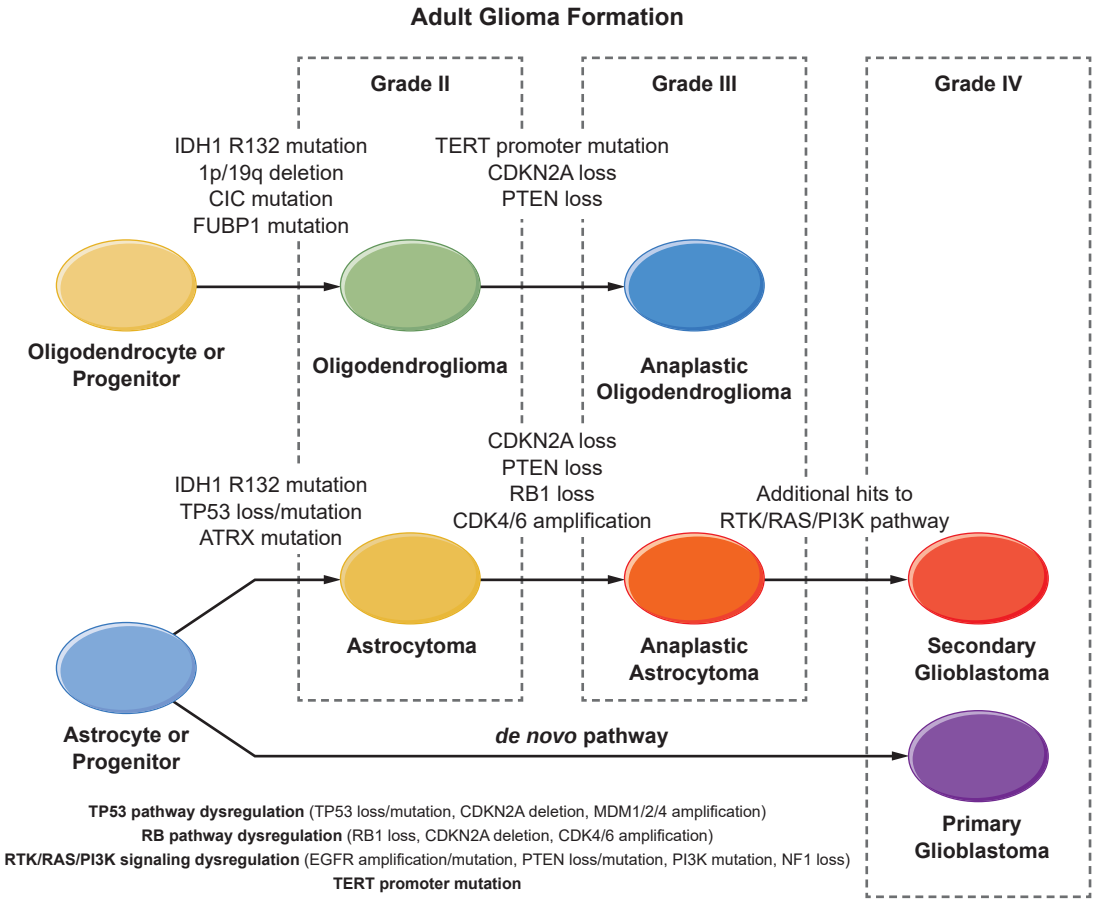

Figure 96.1 Driver events in adult gliomagenesis. Glioblastoma multiforme can either arise from progression of lower grade gliomas (secondary GBM) or *de novo* (primary GBM). IDH1, isocitrate dehydrogenase 1; CIC, capicua transcriptional repressor; FUBP1, far upstream element (FUSE) binding protein 1; TERT, telomerase reverse transcriptase; CDKN2A, cyclin-dependent kinase inhibitor 2A; PTEN, phosphatase and tensin homolog; TP53, tumor protein p53; ATRX, alpha thalassemia/mental retardation syndrome X-linked; RB1, retinoblastoma 1; CDK4/6, cyclin-dependent kinase 4/6; RTK, receptor tyrosine kinase; PI3K, phosphoinositide 3-kinase; EGFR, epidermal growth factor receptor; NF1, neurofibromin 1.

responds to more intensive therapy whereas the proneural subtype shows no benefit to this regimen.[23] Another prognostic indicator is the CpG island methylator phenotype (G-CIMP) demonstrated in IDH1 mutant proneural tumors, which had a significantly better survival (median of 150 weeks) compared to proneural G-CIMP–negative patients (median survival of 42 weeks) or other GBM subtypes (median survival 54 weeks).[6] Also clinically useful to predict response to therapy is O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation status,[19,26] because tumors with silenced *MGMT* are unable to remove the alkyl groups deposited on the O6 position of guanine by alkylating agents such as temozolomide.[27]

Anaplastic (grade III) gliomas, including anaplastic astrocytomas, anaplastic oligoastrocytomas, and anaplastic oligodendrogliomas, are not as well characterized genomically as GBMs. Clinically, they can arise without a prior history of low-grade glioma (presumed *de novo*) or through progression from low-grade gliomas (secondary anaplastic glioma). Grade III gliomas have a high risk of progression to GBM, although the rate of progression and prognosis varies by histology, with anaplastic astrocytomas having a 5-year overall survival of 26.5% and anaplastic oligodendroglioma having a 5-year overall survival of 50.7%.[1] *IDH1* mutations have been observed in 75% to 90% of grade III gliomas.[4,16,28] Anaplastic astrocytomas, accounting for 1.7% of primary brain tumors,[1] commonly have mutations in *ATRX*, *IDH1*, and loss of p53 as well as alterations to the Rb pathway (including RB1 loss, *CDKN2A* deletion, and *CDK4/6* amplification).[16,18,28] It is believed that additional hits in the RTK/Ras/PI3K pathway, including LOH at chr10q, lead to progression to frank glioblastoma.[17,18,21] Anaplastic oligodendrogliomas are rare (0.5% of primary brain tumors),[1] and the progression from grade II oligodendrogliomas (characterized by chr1p/19q loss and *IDH1-CIC/FUBP1* mutations) is likely mediated by additional deletion of *CDKN2A* and *PTEN*.[13] Like GBMs, recurrent *TERT* promoter mutations (C288T or C250T) have been identified in anaplastic gliomas, reported in 14.8% of anaplastic astrocytomas, 26.7% of anaplastic oligoastrocytomas, and 88.4% of anaplastic oligodendrogliomas.[25] The differences in *TERT* promoter mutations between the subtypes is probably due to the higher frequency of *ATRX* mutations in anaplastic astrocytomas, because *TERT* promoter mutations are mutually exclusive with *ATRX* mutations in GBMs and other tumors.[25]

Pediatric Low-Grade Gliomas

Pediatric low-grade gliomas (WHO grade I and II) are the most common brain tumors in children[1] and can be broadly divided into nondiffuse (e.g., pilocytic astrocytomas) and diffuse gliomas. Pilocytic astrocytomas (WHO grade I) are histologically benign tumors with a low probability of malignant progression that are usually pediatric, are primarily found in the cerebellar hemisphere (67%)[10], and are cystic.[2] The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway was originally implicated in driving the formation of pilocytic

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astrocytomas through studies of the hereditary tumor syndrome neurofibromatosis type 1, in which ~15% of patients with germline loss-of-function mutations in *NF1*, a negative regulator of Ras signaling, develop pilocytic astrocytomas in addition to café-au-lait spots and cutaneous neurofibromas.[29] Further investigation has revealed that MAPK/ERK pathway activation is crucial to sporadic pilocytic astrocytoma formation, with 90% of cerebellar pilocytic astrocytomas demonstrating a *KIAA1549-BRAF* (B-Raf proto-oncogene, serine/threonine kinase) fusion gene that results in constitutively active BRAF signaling via a truncation of the BRAF autoinhibitory domain.[30–33] Pilocytic astrocytomas can also have constitutive activation of MAPK/ERK through somatic *BRAF V600E* mutations, Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations, Raf-1 proto-oncogene, serine/threonine kinase (*RAF1*) fusions, and *NF1* loss-of-function mutations.[32,33] Approximately 20% of noncerebellar pilocytic astrocytomas lack the *KIAA1549-BRAF* fusion, and these fusion-negative tumors have recently been discovered to activate MAPK signaling through additional methods, including fibroblast growth factor receptor 1 (*FGFR1*) alterations (mutations, tyrosine kinase domain [TK] duplications, and gene fusions with transforming, acidic coiled-coil containing protein *1* [*TACC1*]),[32,33] neurotrophic tyrosine kinase, receptor, type 2 (*NTRK2*) fusions resulting in TK truncations that are predicted to induce constitutive dimerization,[33] and protein tyrosine phosphatase, non-receptor type 11 (*PTPN11*) hotspot mutations in tumors co-mutated for *FGFR1*.[33]

Pediatric diffuse gliomas, including diffuse astrocytomas, gangliogliomas, angiocentric gliomas, pleomorphic xanthoastrocytomas, oligodendrogliomas, and oligoastrocytomas,[32,34] differ from pilocytic astrocytomas in their diffuse growth pattern, anatomic location (generally supratentorial), and their propensity for malignant transformation.[2] In a recent study by Zhang et al.,[32] 52% (12 out of 23) of diffuse astrocytomas (WHO grade II) demonstrated ERK/MAPK signaling activation via *FGFR1/3* alterations (fusions or *FGFR1* TK duplication), *BRAF* alterations (*V600E* mutations or fusions), or *KRAS* activating mutation (*Q61H*). This same series identified common, recurrent *FGFR1* alterations in oligodendrogliomas (3 out of 5: *FGFR1* TK duplications) and oligoastrocytomas (6 out of 8, 4 *FGFR1* TK duplications; 1 fusion; 1 mutation).[32] A subset of ERK/MAPK activated diffuse astrocytomas (2 out of 12; 16.67%) had the recurrent H3 histone, family 3A (*H3F3A*) *K27M* mutation, an alteration that has been frequently described in pediatric glioblastomas.[32]

The majority of other diffuse low-grade gliomas also demonstrate MAPK/ERK activation. Pleomorphic xanthoastrocytomas (WHO grade II) are rare, supratentorial astrocytomas that are found in the pediatric population two-thirds of the time.[35] The 5-year overall survival is 81%, and malignant progression can occur but is generally rare.[36] A recent study showed that 7 out of 10 pleomorphic xanthoastrocytomas had *BRAF V600E* mutations.[32] Similarly, this study found 55.6% (5 out of 9) of gangliogliomas showed ERK/MAPK activation by *BRAF* alterations (*V600E* = 3; *BRAF* fusion production = 2).[32]

In contrast to pilocytic astrocytomas, about one-quarter of pediatric diffuse astrocytomas (6 out of 23; 26%) demonstrated alterations in v-myb avian myeloblastosis viral oncogene homolog (*MYB*), including gene fusion with protocadherin gamma subfamily A, 1 (*PCDHGA1*) or episome formation, and one case of v-myb avian myeloblastosis viral oncogene homolog-like 1 (*MYBL1*) rearrangement.[32] In an independent, copy number alteration study, Ramkissoon et al.[34] identified focal amplification resulting in tandem duplication/truncation of MYBL1 in 28% (5 out of 18) of pediatric diffuse astrocytomas. These MYB/MYBL1 alterations were not observed in pilocytic astrocytomas, although two out of two angiocentric gliomas from Zhang et al.[32] and an additional two angiocentric gliomas from Ramkissoon et al.[34] bore MYB or MYBL1 fusions, providing further implication of this pathway as specific to a subset of diffuse pediatric gliomas.

Pediatric High-Grade Gliomas

Although histologically similar to adult glioblastomas, a subset of pediatric glioblastomas have distinct genomic hits driving tumor formation that implicate epigenetic dysregulation in the formation of these tumors. In a study by Schwartzentruber et al.,[37] recurrent mutations in histone variant H3.3 (H3F3A K27M; G34R/V), commonly co-occurring with damaging mutations in the chromatin remodelers *ATRX* or death-domain associated protein (*DAXX*) and *TP53*, drove pediatric glioblastoma formation in 31% of pediatric GBMs. This percentage may be higher in diffuse intrinsic pontine gliomas (DIPG), where an independent study found that 78% of DIPG contained somatic *K27M* mutations in *H3F3A* or histone cluster 1, H3b (*HIST1H3B*), whereas *H3F3A G34R* was restricted to non–brain stem pediatric glioblastomas.[38] Recently, H3K27 K27M was characterized to be a dominant-negative mutation that results in a genome-wide reduction of H3K27me3 repressive marks due to altered binding with the polycomb repressive complex 2 (PRC2) and also causes global DNA hypomethylation.[39] In pediatric glioblastomas, these H3.3 mutations were mutually exclusive with the neomorphic IDH1 R132H,[8,37] which was rarely seen in pediatric GBM but has been shown to cause epigenomic dysregulation and DNA hypermethylation (G-CIMP) phenotype.[9] Similar to pediatric low-grade gliomas, the BRAF V600E mutation has been reported in ~10% of pediatric high-grade gliomas[40]; however, unlike the pediatric low-grade gliomas, this mutation commonly co-occurs with homozygous *CDKN2A/B* deletion.[41]

In terms of structural variation, pediatric glioblastomas differ from the classic, primary adult GBM findings of chr7 amplification (74% in adult versus 13% in pediatric) and chr10 loss (80% adult versus 35% pediatric).[42] However, pediatric glioblastomas have more frequent 1q gain (30% in pediatric versus 9% in adult), frequent focal amplification of *PDGFRA* in 12% of tumors, and frequent focal, homozygous deletion of *CDKN2A/B* in 19% of tumors.[42] The driver events for adult and pediatric gliomas are summarized in Table 96.1.

MENINGIOMAS

Meningiomas are the most common primary intracranial tumor, accounting for one-third of all primary brain tumors.[1] Thought to arise from the arachnoid layer of the meninges, meningiomas can be found throughout the neuraxis, and are notable for a wide variety of histologic subtypes (15 according to the 2007 WHO classifications).[43] Although 70% to 80% of meningiomas are benign (WHO grade I), the remaining subset can exhibit more aggressive behavior (WHO grade II and grade III).[43] Recent genomic studies have revealed that 80% of meningiomas can be neatly categorized into three clinically relevant, mutually exclusive genetic groups with differences in histology, anatomic location, and likelihood for malignant progression (Fig. 96.2).[44–46]

The first and largest group, *NF2/chr22 loss* tumors, is characterized by biallelic loss of the tumor suppressor neurofibromin 2 (merlin, NF2). One of the hallmarks of the inherited tumor syndrome neurofibromatosis type II is multiple meningiomas due to germline mutations in the tumor suppressor *NF2*,[47] and biallelic loss of NF2 drives ~50% of sporadic meningioma formation[48] and is a risk factor for malignant transformation. At least 75% of WHO grade II tumors have *NF2/chr22 loss*.[43,44] *NF2/chr22 loss* tumors are more likely to form in the meninges flanking the cerebral convexities, but when they grow in the skull base, the growth is restricted to the posterior and lateral portions of the skull.[44] *NF2/chr22 loss* is also commonly found in spinal meningiomas. Rarely, *NF2/chr22 loss* tumors also have a biallelic loss of the chromatin remodeling gene SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1 (*SMARCB1*).[49] Loss of the tumor suppressor SMARCB1, which is also found on chromosome 22, has been reported in various malignant rhabdoid

TABLE 96.1

Summary of Driver Events in Pediatric and Adult Gliomas

WHO Grade	Population	Diagnosis	Pathways Affected	Hallmark Mutations or Structural Abnormalities	Notes
I	Pediatric	Pilocytic astrocytoma	MAPK/ERK pathway	KIAA1549-BRAF fusion (90% of cerebellar)	Single hit to MAPK/ERK signaling
				BRAF V600E	
				KRAS	
				RAF1 fusions	
				NF1 loss of function	
				FGFR1 alterations (mutations, fusion with TACC1), co-mutations in PTPN11 hotspots	
II	Pediatric	Diffuse astrocytoma	MAPK/ERK pathway	FGFR1/3 alterations (fusions or FGFR1 TK duplication)	
				BRAF alterations (V600E mutations or fusions)	
				KRAS activation mutation (Q61H)	
			MYB	MYB, including fusion with PCDHGA1 or episome formation, and 1 case of MYBL1 rearrangement	
II	Adult	Diffuse astrocytoma	Epigenetic dysregulation	IDH1 R132H/C/S	More likely to progress to high grade glioma
			Chromatin remodeling	ATRX	
			TP53 pathway	Mutations in TP53, LOH at chr17	
		Oligodendroglioma	Epigenetic dysregulation	IDH1 R132H/C/S	
			Unknown	1p/19q loss	
				CIC	
				FUBP1	
		Oligoastrocytoma	Epigenetic dysregulation	IDH1 R132H/C/S	
				Combination of genetic events seen in astrocytes and oligodendrocytes	
III	Adult	Anaplastic astrocytoma	Epigenetic dysregulation	IDH1 R132H/C/S	Additional hits in RTK/RAS/P13K pathway drive progression to frank glioblastoma
			Telomere maintenance	TERT promoter mutations	
			Chromatin remodeling	ATRX mutations	
			p53 pathway	TP53 mutations and LOH at chr17	
				CDKN2A loss	
			Rb pathway	RB1 loss	
				CDKN2A loss	
				CDK4/6 amplification	
IV	Pediatric	GBM	Chromatin remodeling	H3F3A K27M; G34R/V	
				ATRX mutations	
				DAXX mutations	
			p53 pathway	TP53	

(continued)

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TABLE 96.1

Summary of Driver Events in Pediatric and Adult Gliomas *(continued)*

WHO Grade	Population	Diagnosis	Pathways Affected	Hallmark Mutations or Structural Abnormalities	Notes
IV	Adult	Secondary GBM	Epigenetic dysregulation	IDH1 R132H/C/S	Progressed from lower grade gliomas
			Chromatin remodeling	ATRX mutations	
			p53 pathway	TP53 mutations, LOH at chr17	
			Rb pathway	Loss of RB1	
				Loss of CDKN2A	
			PI3K	Loss of PTEN	
		Primary GBM	TP53 pathway	TP53 loss	
				CDKN2A loss	
				MDM1/2/4 gain	
			RB pathway	CDKN2A loss	
				RB1 loss	
				CDK4/6 amplification	
			Receptor tyrosine kinase	EGFR amplification	
				PDGFRA amplification	
			PI3K	PTEN loss	
				PI3K mutation	
			RAS	NF1 loss	
			Telomere maintenance	TERT	

BRAF, B-Raf proto-oncogene, serine/threonine kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog; RAF1, v-raf-1 murine leukemia viral oncogene homolog 1; TACC1, transforming, acidic coiled-coil containing protein 1; PTPN11, protein tyrosine phosphatase, non-receptor type 11; FGFR1/3, fibroblast growth factor receptor 1/3; TK, tyrosine kinase domain; MYB, v-myb avian myeloblastosis viral oncogene homolog; PCDHGA1, protocadherin gamma subfamily a, 1; MYBL1, v-myb avian myeloblastosis viral oncogene homolog-like 1; IDH1, isocitrate dehydrogenase 1; ATRX, alpha thalassemia/mental retardation syndrome X-linked; TP53, tumor protein p53; CIC, capicua transcriptional repressor; FUBP1, far upstream element (FUSE) binding protein 1; TERT, telomerase reverse transcriptase; CDKN2A, cyclin-dependent kinase inhibitor 2A; RB1, retinoblastoma 1; CDK4/6, cyclin-dependent kinase 4/6; H3F3A, H3 histone, family 3A; DAXX, death-domain associated protein; PTEN, phosphatase and tensin homolog; EGFR, epidermal growth factor receptor; PDGFRA, platelet-derived growth factor receptor alpha; PI3K, phosphoinositide 3-kinase; NF1, neurofibromin 1.

tumors[50,51] as well as in families with multiple meningiomas with schwannomatosis.[52]

The second group of meningiomas, *TRAF7* mutant tumors, have a lower risk of malignant transformation.[44] Somatic mutations in tumor necrosis factor (TNF) receptor-associated factor 7 (*TRAF7*), a proapoptotic N-terminal RING and zinc finger domain protein with E3 ubiquitin ligase activity, have been reported in ~25% of meningiomas (including 27% of grade I tumors). *TRAF7* mutant tumors are commonly comutated for AKT/PI3K/mammalian target of rapamycin (mTOR) pathway members, most notably the recurrent v-akt murine thymoma viral oncogene homolog 1 (AKT1) E17K mutation, which activates PI3K signaling and is reported in 14% of grade I meningiomas.[44,45] A second *TRAF7* subgroup is defined by comutations in the transcription factor Krupple-like factor 4 (KLF4),[44,46] an important regulator of development and one of four Yamanaka factors capable of reprogramming differentiated cells into an induced pluripotent stem cell state.[53] The recurrent KLF4 K409Q mutation, seen in 12% of WHO grade I meningiomas, is in the DNA-binding domain, suggesting that the mutation may alter binding to the consensus sequence.[44,46] Noted for their increased risk of postoperative peritumoral edema[54], 100% of secretory meningiomas are comutated for *KLF4 K409Q* and *TRAF7*[44,46]. *TRAF7* mutant tumors are typically found in the midline of the skull base, especially the anterior skull base, although they can grow in the meninges flanking the frontal lobe.[44]

The third major group of meningiomas is the sonic Hedgehog (SHH) group. Approximately 3% of benign meningiomas have mutations in smoothened, frizzled class (SMO) that activate SHH signaling.[44,45] Interestingly, tumors with *SMO L412F* (n = 5) all localized to the medial anterior fossa.[44] Germline loss-of-function mutations in suppressor of fused homolog (Drosophila) (*SUFU*), which is downstream of SMO and inhibits SHH signaling, have been reported in a family with multiple meningiomas.[55]

MEDULLOBLASTOMAS

Arising in the cerebellum, medulloblastomas (WHO grade IV) are the most common malignant brain tumors in children. These invasive embryonal tumors commonly metastasize to the leptomeninges via the cerebrospinal fluid system, gaining access by extending through the fourth ventricle.[2] The past decade of research has revealed that medulloblastoma is not a homogeneous disease; rather, these tumors can be divided into four subgroups with marked differences in transcriptional profiles, somatic copy number alterations, underlying driver mutations, cell of origin, anatomic location, prognosis, tendency to metastasize, and response to targeted therapies (Fig. 96.3).

The first hints of the existence of different molecular subgroups came from studies of hereditary tumor syndromes. The SHH pathway was implicated in driving medulloblastoma and nevoid basal

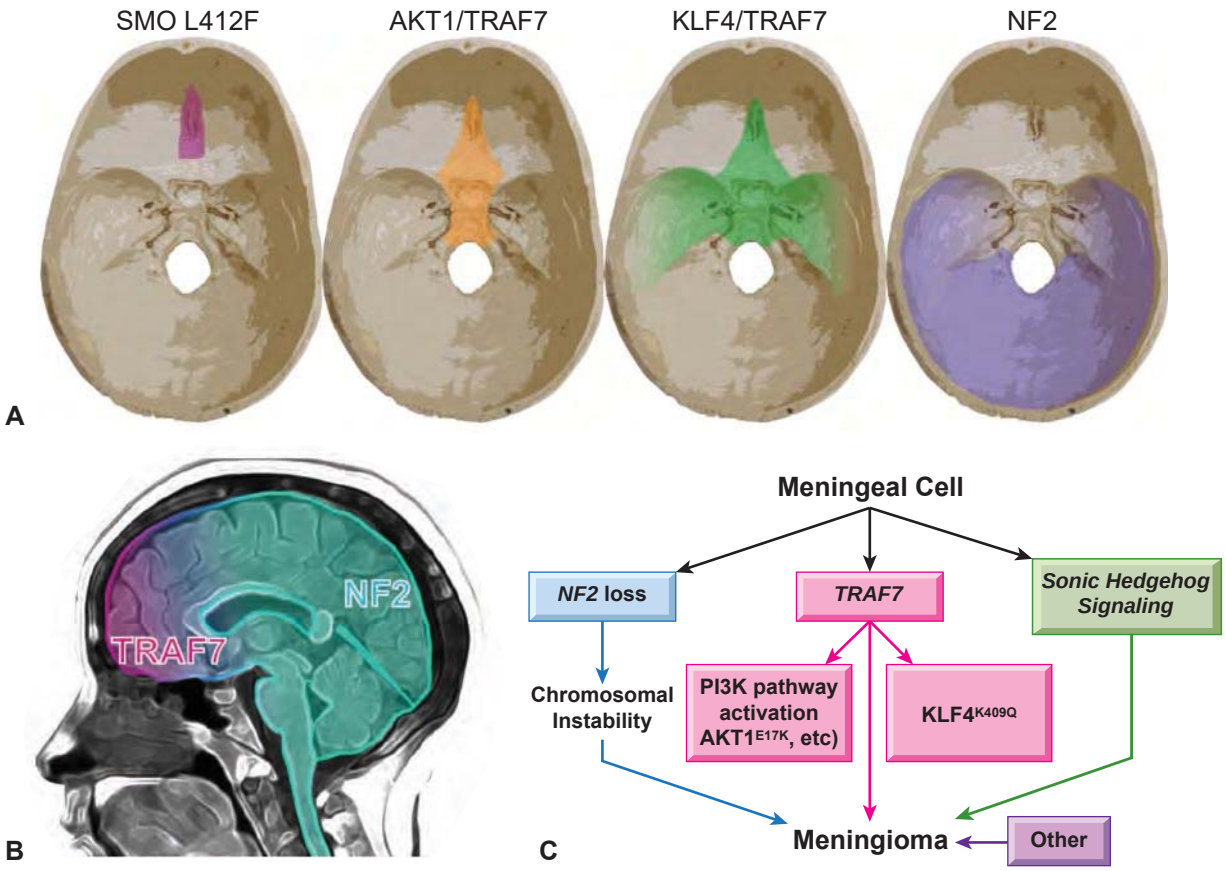

Figure 96.2 Driver events in meningioma formation. **(A)** For grade 1 meningiomas, growth of *NF2/chr22 loss* meningiomas is restricted to the posterior and lateral skull base, whereas non-*NF2* mutant tumors grow in the midline of the skull base. **(B)** For meningiomas flanking the convexities, TRAF7 mutant tumors are found anteriorly. **(C)** Summary of meningioma driver events and known pathway activations. NF2, neurofibromin 2; TRAF7, TNF receptor-associated factor 7; AKT1, v-akt murine thymoma viral oncogene 1; KLF4, Kruppel-like factor 4 (gut); SMO, smoothened, frizzled class receptor.

cell carcinoma formation in patients with Gorlin syndrome, who have activated SHH signaling due to a germline loss of patched 1 (PTCH1).[56,57] The WNT pathway was implicated in driving medulloblastoma formation in patients with Turcot syndrome (now called familial adenomatous polyposis), a subset of whom had medulloblastomas in addition to inherited colonic polyposis as a result of germline loss-of-function adenomatous polyposis coli (*APC*) mutations.[58]

Candidate gene studies identified somatic mutations in SHH or WNT pathway genes were found in sporadic medulloblastomas,[59,60]

A **Medullablastoma Subtype Characteristics**

Group	Progenitor Cell	Growth	Prognosis	Characteristic mutations	Structural Events
WNT	Lower rhombic lip	4th ventricle, with dorsal brain stem infiltration	Good	*CTNNB1* (91%), *DDX3X*, *SMARCA4*, *MLL2*, *TP53*	Monosomy 6
SHH	Cerebellar granule neuron precursors of the external granule layer	Cerebellar hemispheres	Intermediate	*PTCH1*, *SUFU*, *SMO*, *TP53*, *MLL2*, *DDX3X*	Genome instability, including frequent amplification of *MYCN* and *GLI2*, deletion of *PTCH1*
Group 3	Cerebellar granule neuron precursors of the external granule layer, neural stem cells	~30% metastatic at diagnosis (47% for infants)	Poor		Genome instability, including *MYC* amplification, *MYC/PVT1* fusions, somatic copy number alterations in TGFB signaling members including amplification of *OTX2*
Group 4	Unknown	~30% metastatic at diagnosis (36% for infants)	Intermediate	*KDM6A*	Genome instability, including *SNCAIP* tandem duplication, *MYCN* amplification

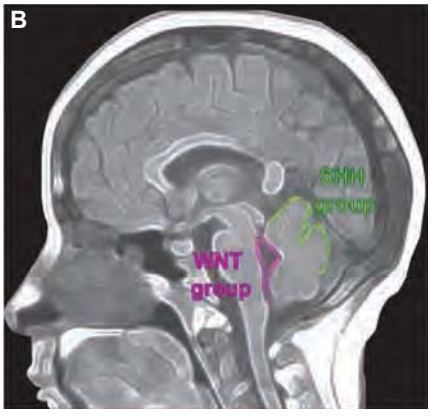

Figure 96.3 Medulloblastoma subtype characteristics. **(A)** Table summarizing the genomic underpinnings, anatomic location, and presumed progenitor cell for the four medulloblastoma subtypes. **(B)** WNT group medulloblastomas arise in the fourth ventricle, whereas SHH group medulloblastomas arise in the cerebellar hemispheres. CTNNB1, catenin (cadherin-associated protein), beta 1, 88kDa; DDX3X, DEAD (Asp-Glu-Ala-Asp) box helicase 3, X-linked; SMARCA4, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4; MLL2, lysine (K)-specific methyltransferase 2D; TP53, tumor protein p53; PTCH1, patched 1; SUFU, suppressor of fused homolog (*Drosophila*); SMO, smoothened, frizzled class receptor; KDM6A, Lysine (K)-Specific Demethylase 6A; MYCN, v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog; GLI2, GLI family zinc finger 2; MYC, v-myc avian myelocytomatosis viral oncogene homolog; OTX2, orthodenticle homeobox 2; SNCAIP, synuclein, alpha interacting protein.

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but the development of microarray-based transcriptional profiling and next-generation sequencing have clarified four medulloblastoma subgroups with differential pathway activations and underlying genomic changes. The WNT group (~10% of medulloblastomas), initially identified by unsupervised hierarchical clustering of gene expression profiles,[61] is characterized by somatic activating mutations in beta-catenin (*CTNNB1*) in 91% of cases.[62–65] *CTNNB1* is commonly comutated with DEAD (Asp-Glu-Ala-Asp) box helicase 3, X-linked (*DDX3X*) in 50% of cases, and this group is also defined by frequent mutations in the chromatin modifier SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (*SMARCA4*, 26.3%), lysine (K)-specific methyltransferase 2D (*KMT2D* or *MLL2*, 12.5%), and *TP53* (12.5%).[62–65] In terms of structural variation, this group has monosomy chromosome 6 but an otherwise stable genome.[66] Elegant mouse modeling experiments have provided compelling evidence that WNT medulloblastomas originate from lower rhombic lip progenitor cells, and WNT subtype medulloblastomas grow within the fourth ventricle and infiltrate the dorsal brain stem.[67] The WNT group has the best prognosis, with a 95% overall 5-year survival.[68] These tumors rarely recur.[69]

The SHH group (~30% of medulloblastomas) shows increased expression of SHH signaling,[70] and commonly bears somatic mutations (*PTCH1*, *SUFU*, *SMO*),[61–64] or somatic copy number alterations (amplification of v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (*MYCN*) and GLI Family Zinc Finger 2 (*GLI2*); deletion of *PTCH1*)[66] impacting SHH pathway members. In addition to Gorlin syndrome, patients with germline *TP53* mutations (Li-Fraumeni syndrome) can develop SHH medulloblastomas by chromothripsis-mediated amplification of SHH oncogenes,[71] and *TP53* mutations or deletions are seen in 13.6% of SHH tumors.[62,64–66] The subset of SHH medulloblastomas that bear *TP53* mutations or deletions, have a particularly a poor prognosis, with a 5-year overall survival of 41% in *TP53* mutant SHH cases versus 81% for *TP53* wild-type SHH cases.[72] Like the WNT group, the SHH tumors also bear mutations in *MLL2* (12.9%) and *DDX3X* (11.7%).[62–65] However, SHH tumors are more genomically unstable in terms of their structural variation and have frequent focal copy number changes in SHH signaling (18%), p53 signaling (9.4%), and/or RTK/PI3K signaling (10%).[66] In contrast to WNT medulloblastomas, mouse SHH medulloblastomas arise from external granule layer progenitor cells, and in humans are found in the cerebellar hemispheres.[67] SHH tumors have a worse prognosis than WNT tumors, with a 5-year survival of ~75%.[65,70] They are also more likely to recur than WNT tumors, and the recurrence is generally local (versus metastatic).[73] Clinical use of SHH inhibitors (e.g., SMO inhibitors) has shown initial but transient response.[74,75]

The remaining two groups of medulloblastomas, group 3 and group 4, currently bear generic names while the underlying biology is clarified. Both groups demonstrate genomic instability and tend to present with metastases at diagnosis in ~30% of cases.[70] Group 3 tumors (~25% of medulloblastomas) have high *MYC* amplification and express a gene signature consistent with photoreceptors/GABAergic (gamma-aminobutyric acid) neurons.[76,77] A recent somatic copy number alterations (SCNA) analysis of >1,000 medulloblastomas revealed that SCNAs found in group 3 are enriched for transforming growth factor beta (TGFB) signaling, including frequent amplification of orthodenticle homeobox 2 (*OTX2*), which is a TGFB target during neurodevelopment.[66] Mutually exclusive with *OTX2* amplification are group 3 tumors with *MYC* amplification, a large subset of which harbor recurrent *MYC/PVT1* gene fusions as a result of chromothripsis on chromosome 8.[66] The 5-year overall survival for group 3 tumors is ~50%.[65,70] Group 4 tumors (~35%) express a neuronal gene signature. Compared to group 3 tumors, group 4 tumors do not have *MYC* amplification, but have *MYCN* amplification in 6.3% of tumors (which is also seen in ~8.2% of SHH tumors).[65,66] Damaging mutations in *KDM6A*, a histone H3K27 demethylase, were restricted to group 4 tumors, but only explain the formation of 12% of tumors.[62–66] Of group 4 tumors, 10.4% had a recurrent synuclein, alpha interacting protein (*SNCAIP*) tandem duplication, which plays a role in Lewy body formation in Parkinson disease.[66] The 5-year overall survival for group 4 tumors is 75%.[65,70]

EPENDYMAL TUMORS

Ependymomas are glial tumors that arise from cells lining the ventricular system. Interestingly, the anatomic location of ependymoma growth predicts prognosis, with infratentorial ependymomas in children bearing the worst prognosis. Emerging evidence subcategorizing ependymomas by anatomic location has identified differences in age of onset, prognosis, driver mutations, structural variants, and transcriptional profiles (Fig. 96.4). The candidate cancer stem cell of ependymomas is the radial glial cell,[78] which has region-specific patterns of gene expression that

A

Ependymoma Subtype Characteristics

Anatomical Location	Population	Genomic characteristic
Supratentorial	Adult	*CDKN2A* deletion, amplification of *EPHB2*
Infratentorial, medial	Pediatric	chr1q gain
Infratentorial, lateral	Young adult	NF2/chr22 loss
Spinal	Adult	NF2/chr22 loss

B

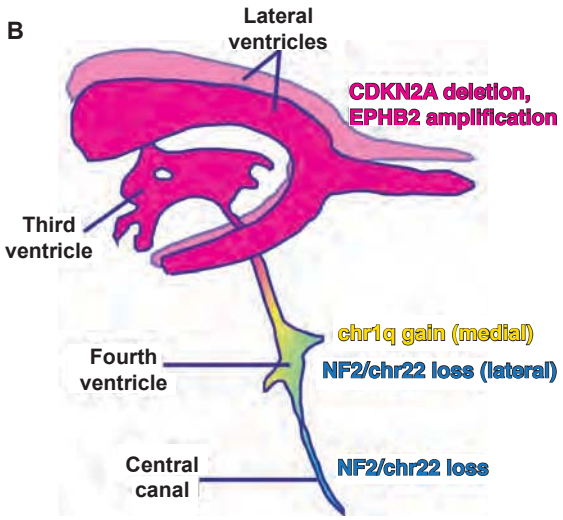

Figure 96.4 Anatomic location correlates with mutation and susceptible population for ependymomas. **(A)** Table summarizing the genomic driver events anatomic location and affected population for ependymomas. **(B)** Spinal ependymomas and lateral infratentorial ependymomas are characterized by *NF2/chr22 loss*, medial infratentorial ependymomas have chr1q gain, and supratentorial ependymomas have *CDKN2A* deletion and *EPHB2* amplification. NF2, neurofibromin 2; CDKN2A, cyclin-dependent kinase inhibitor 2A; EPHB2, EPH receptor b2.

match the expression profiles of CD133+ ependymal cells harvested from the correlating anatomic compartment (spinal cord versus infratentorial versus supratentorial).[79] For example, patients with the inherited tumor syndrome neurofibromatosis type II frequently have intramedullary spinal ependymomas but not cortical ependymomas, and up to 95% of adult sporadic spinal cord ependymomas have monosomy chr22.[80] In contrast, >90% of supratentorial ependymomas have deletion of *CDKN2A*, frequently with co-occurring focal amplification of EPH Receptor B2 (*Ephb2*). Posterior fossa ependymomas appear to be split into two groups by anatomic location.[81] Lateral posterior fossa tumors are more chromosomally stable, occur in younger patients, have 1q gain, are more likely to recur with metastasis, and have a poor prognosis.[81] In contrast, midline posterior fossa ependymomas have more widespread chromosomal instability, are older, and have chr22 loss.[81]

SUMMARY

In just a few short years, the use of next-generation sequencing technologies has ushered about a golden age of discovery for the molecular pathways driving the formation of central nervous system tumors. Tumors classifications, once based primarily on observable histopathologic findings, are increasingly refined to distinct, clinically relevant entities based on differences in gene mutations, genomic stability, epigenetic changes, differences in gene expression profiles, differences in the anatomic location of growth, differences in response to therapy, and differences in overall survival. The challenge of translating the emerging molecular classifications of brain tumors to successful individualized cancer therapy is daunting, but the major advances brought about by genomic characterization serve as an ideal starting point for hypothesis-driven clinical research.

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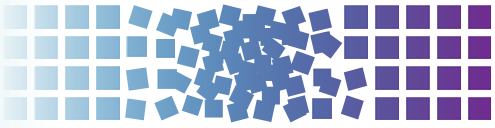
97 Neoplasms of the Central Nervous System



Susan M. Chang, Minesh P. Mehta, Michael A. Vogelbaum, Michael D. Taylor, and Manmeet S. Ahluwalia

EPIDEMIOLOGY OF BRAIN TUMORS

Incidence and Prevalence

The incidence and prevalence of brain and central nervous system (CNS) tumors is imprecisely documented because benign tumors were not required to be reported prior to 2003, and metastatic disease to the brain remains unreported. The major data sources for the United States include the Surveillance, Epidemiology, and End Results (SEER) program and the Central Brain Tumor Registry of the United States (CBTRUS).[1–2] The CBTRUS database from 2006 to 2010 reported 326,711 incident tumors (112,458 malignant and 214,253 nonmalignant), with an overall average annual age-adjusted incidence of 21.03 per 100,000. The overall incidence rate was 5.26 per 100,000 for children 0 to 19 years of age (5.14 per 100,000 for children less than 15 years), and 27.38 per 100,000 for adults (20+ years).[2] The median age at diagnosis is 59 years with approximately 7% of the cases in individuals less than 20 years of age. The distribution patterns of histologies within age groups differ substantially. The most frequently reported histology was nonmalignant meningioma, accounting for about one-third of all tumors. Of all brain and CNS tumors, 42% occurred in males. For the malignant histologies, 55% occurred in males, and in the nonmalignant histologies, 36% occurred in males. The aggregate average annual age-adjusted mortality is 4.25 deaths per 100,000 with considerable variation noted by state. Males were found to have a statistically higher mortality rate for brain and CNS than females in the United States population (5.19 versus 3.46 per 100,000). The estimated total number of new cases is 66,240 (22,810 malignant and 43,430 nonmalignant) for 2014.

In 1993, the World Health Organization (WHO) ratified a new classification, assuming that each tumor results from a specific cell type. Most registries do not contain detailed information regarding the distribution of various CNS tumors, as specified in the WHO classification.[3–4] Many of these tumors are radiographically and clinically diagnosed; examples include infiltrating pontine gliomas, vestibular schwannomas, skull-base meningiomas, and brain metastases. Specific CNS tumor types also differ in incidence based on anatomic location. Figure 97.1 presents a simplified distribution by subtype.

The increased utilization of cranial imaging for headaches, seizures, and trauma has led to an increase in the diagnosis of benign tumors. SEER suggests that between 1975 and 1987, there was a significant increase in the incidence of CNS tumors, which leveled off between 1991 and 2006. Because many patients with CNS tumors survive for several years, the prevalence exceeds the incidence. Overall prevalence rate of individuals with a brain tumor was estimated to be 209 per 100,000 in 2004 and 221.8 per 100,000 in 2010. The female prevalence rate (264.8 per 100,000) was higher than that in males (158.7 per 100,000). The averaged prevalence rate for malignant tumors (42.5 per 100,000) was lower than the prevalence for nonmalignant tumors (166.5 per 100,000). Estimates of the expected number of individuals living with primary brain tumor diagnoses in the United States was 612,770 in 2004 and 688,096 in 2010.[5]

Etiologic Factors

No agent has been definitively implicated in the causation of CNS tumors, and risk factors can be identified only in a minority. Commonly implicated associations described with other malignancies, such as diet, exercise, alcohol, tobacco, and viruses, are generally not considered to be significant for CNS tumors.[6]

Environmental Factors

Farmers and petrochemical workers have been shown to have a higher incidence of primary brain tumors. A variety of chemical exposures have been linked.[7] Ionizing and nonionizing radiation has been implicated, with the clearest association coming from the occurrence of superficial meningiomas in individuals receiving cranial or scalp irradiation, with the association being stronger for young children receiving low doses of irradiation for benign conditions.[8] Exposure to ionizing radiation is a known risk factor for a small percentage of astrocytomas, sarcomas, and other tumors.[9] There is a 2.3% incidence of primary brain tumors in long-term survivors among children given prophylactic cranial irradiation for acute leukemia, a fourfold increase over the expected rate.[10]

In addition, a retrospective study suggests an increased risk for developing gliomas in children undergoing computed tomography (CT) scans.[11] Exposure to dental x-rays performed at a time when radiation exposure was greater than currently used appears to be associated with an increased risk of intracranial meningioma.[12]

There are conflicting reports regarding nonionizing radiation emitted by cellular telephones.[13–19] Several investigators have reported meta-analyses of case control studies evaluating cell phone use and the development of a brain tumor. Kan et al.[16] reviewed nine studies (5,259 cases and 12,074 controls) and showed an overall odds ratio (OR) of 0.90 for cellular phone use and brain tumor development; the OR was 1.25 for long-term users. An OR of 0.98 for developing malignant and benign tumors of the brain as well as the head and neck was reported by Myung et al.[17] when collating 23 case control studies (12,544 cases and 25,572 controls). The International Commission for Non-Ionizing Radiation Protection Standing Committee on Epidemiology reviewed the epidemiologic evidence, and they concluded that there was not a causal association between mobile phone use and malignant gliomas, but for slow-growing tumors, the observation period was too short for conclusive statements.[18] A recent report of the INTERPHONE study, an international, population-based case control study, also did not find an increased risk of gliomas or meningiomas.[19] Glioma incidence has not followed the increase in cell phone use, but because of the potential for a lag in trends, continued surveillance on children who are exposed from an early age is warranted.[15]

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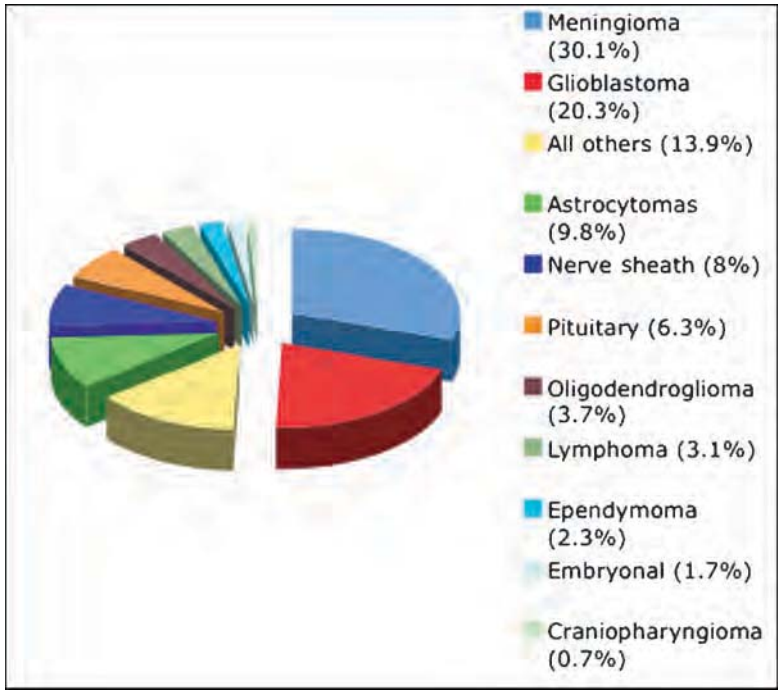

Figure 97.1 Proportionate distribution of the incidence of central nervous system neoplasms by histopathologic type, based on the Central Brain Tumor Registry of the United States database.

Viral Associations

Although certain canine and feline CNS tumors may have a viral association, the human evidence remains weak. Specifically, no increase in the risk of developing a brain tumor has been associated with previous polio vaccination, which discredits claims that simian virus 40, which contaminated older polio vaccine preparations, caused brain tumors.[20] The exception to this is primary CNS lymphoma, which has been shown to be associated with Epstein-Barr virus.[21] An increase in incidence of primary CNS lymphoma is most likely due to the increasing numbers of immunosuppressed patients in the setting of HIV and posttransplant use of immunosuppressants.[21–22]

The association between human cytomegalovirus (HCMV) infection and glioblastoma was first described by Cobbs et al.[23] in 2002. The presence of HCMV was also demonstrated in glioblastoma and in other gliomas.[24–25] HCMV may have tropism for microglia and CD133-positive glioma cancer stem cells and further work is needed to evaluate the role of this virus.[26]

Hereditary Syndromes

Neurofibromatosis type 1 (NF1) is an autosomal-dominant disorder associated with intra- and extracranial Schwann cell tumors. Optic gliomas, astrocytomas, and meningiomas also occur at higher frequency in NF1. NF2 is characterized by bilateral vestibular schwannomas and meningiomas. Systemic schwannomas also occur in NF2. Subependymal giant cell astrocytoma commonly occur in children with tuberous sclerosis, an autosomal-dominant disorder caused by mutation in the *TSC1* and *TSC2* genes. Other hereditary tumor syndromes affecting the CNS include Li-Fraumeni syndrome (germline mutation in one p53 allele; malignant gliomas); von Hippel-Lindau syndrome (germline mutation of the *VHL* gene; hemangioblastomas), and Turcot syndrome (germline mutations of the adenomatous polyposis gene; medulloblastoma).[27,28] The nevoid basal cell carcinoma syndrome (Gorlin syndrome) is associated with medulloblastomas (and possibly meningiomas) and represents mutations in the *PTCH* suppressor gene or other members of the Sonic hedgehog pathway.[29,30]

Meningiomas and schwannomas are more common in females; gliomas, medulloblastomas, and most other CNS tumors are more common in males. Meningiomas are more common in African Americans and gliomas and medulloblastomas are more common in Caucasians. It has been suggested that there is a lower incidence of meningiomas and a higher incidence of gliomas and vestibular schwannomas in higher socioeconomic groups.[31–34]

CLASSIFICATION

Primary CNS tumors are of ecto- and mesodermal origin and arise from the brain, cranial nerves, meninges, pituitary, pineal, and vascular elements. The WHO classification lists approximately 100 subtypes of CNS malignancies in seven broad categories (Table 97.1).[3,4,35] In spite of the low proliferation rate within the meninges, meningiomas are among the most common CNS tumors. Astrocytes are among the most mitogenically competent cells, and astrocytomas, also referred to interchangeably as *gliomas*, are among the more common primary CNS tumors. The precise cell of origin of gliomas, however, remains unclear.

The WHO classification can be reduced to a simpler working formulation, categorizing the neoplasms into tumors presumably derived from glia, neurons, or from cells that surround the CNS or form specialized anatomic structures. Glial cells are believed to give rise to astrocytomas, oligodendrogliomas, and ependymomas. Neuronal cells are involved in the development of medulloblastoma and primitive neuroectodermal tumors (PNET). In PNETs, anatomic location is pivotal; the transformation of cortical neuroblasts leads to cortical PNETs, retinal neuroblasts form retinoblastoma, and pineal neuroblasts form pineoblastomas. Specialized anatomic structures within the CNS give rise to pituitary adenomas, pineocytomas, chordomas, hemangioblastomas, germ cell tumors, and choroid plexus papillomas and carcinomas.

This working formulation is speculative, based on scant phenotypical and immunohistochemical evidence. For example, oligodendrogliomas are diagnosed based on cellular morphology, including prominent nuclei surrounded by a cytoplasmic halo with a characteristic "fried egg" appearance, and many have codeletions of 1p and 19q. However, no definitive markers for oligodendrogliomas currently exist; these tumors can stain both for glial fibrillary acidic protein, an astrocytic marker, and for synaptophysin, a presumptive neuronal marker.[36] A third of all gliomas have

TABLE 97.1

Classification of Tumors of the Central Nervous System: Selected from the 2007 World Health Organization Classification

1. Neuroepithelial tumors
   - Astrocytic tumors
     - a. Pilocytic astrocytoma
     - b. Subependymal giant cell astrocytoma
     - c. Pleomorphic xanthoastrocytoma
     - d. Diffuse astrocytoma
       - a. Fibrillary astrocytoma
       - b. Gemistocytic astrocytoma
       - c. Pro-oplasmic astrocytoma
     - e. Anaplastic astrocytoma
     - f. Glioblastoma
       - a. Giant cell glioblastoma
       - b. Gliosarcoma
     - g. Gliomatosis cerebri
   - Oligodendroglial tumors
     - a. Oligodendroglioma
     - b. Anaplastic oligodendroglioma
   - Ependymal tumors
     - a. Subependymoma
     - b. Myxopapillary ependymoma
     - c. Ependymoma
     - d. Anaplastic ependymoma
   - Choroid plexus tumors
     - a. Choroid plexus papilloma
     - b. Atypical choroid plexus papilloma
     - c. Choroid plexus carcinoma
   - Other neuroepithelial tumors
     - a. Astroblastoma
     - b. Chordoid glioma of the third ventricle
     - c. Angiocentric glioma
   - Neuronal and mixed neuronal-glial tumors
     - a. Dysplastic gangliocytoma of cerebellum (Lhermitte-Duclos)
     - b. Desmoplastic infantile astrocytoma/ganglioglioma
     - c. Dysembryoplastic neuroepithelial tumor
     - d. Gangliocytoma
     - e. Ganglioglioma
     - f. Anaplastic ganglioglioma
     - g. Central neurocytoma
     - h. Extraventricular neurocytoma
     - i. Cerebellar liponeurocytoma
     - j. Papillary glioneuronal tumor
     - k. Rosette-forming glioneuronal tumor of the fourth ventricle
     - l. Paraganglioma
   - Tumors of the pineal region
     - a. Pineocytoma
     - b. Pineoblastoma
   - Embryonal tumors
     - a. Medulloblastoma
     - b. Primitive neuroectodermal tumors
     - c. Atypical teratoid/rhabdoid tumor
2. Tumors of cranial/spinal nerves
   - a. Schwannoma (neurilemoma, neurinoma)
   - b. Neurofibroma
   - c. Perineuroma
   - d. Malignant peripheral nerve sheath tumor
3. Tumors of the meninges
   - A. Tumors of meningothelial cells
     - a. Meningioma
     - b. Fibrous
     - c. Psammomatous
     - d. Clear cell
     - e. Atypical
     - f. Anaplastic (malignant)
   - B. Mesenchymal tumors
     - a. Lipoma
     - b. Solitary fibrous tumor
     - c. Rhabdomyosarcoma
     - d. Malignant fibrous histiocytoma
     - e. Chondrosarcoma
     - f. Osteoma
     - g. Hemangioma
     - h. Hemangiopericytoma
     - i. Kaposi sarcoma
4. Lymphomas and hematopoietic neoplasms
   - a. Malignant lymphomas
   - b. Plasmacytoma
5. Germ cell tumors
   - a. Germinoma
   - b. Yolk-sac tumor
   - c. Choriocarcinoma
   - d. Teratoma
   - e. Mixed-germ cell tumors
6. Sellar tumors
   - a. Pituitary adenoma
   - b. Craniopharyngioma
7. Metastatic tumors

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morphologic characteristics of both astrocytoma and oligodendroglioma, leading some to separate gliomas based on their molecular and genetic characteristics.[37] Evidence that suggests that some oligodendrocytes derive from a neuronal lineage, whereas some neuron-derived tumors (embryonal tumors) can show significant areas of glial differentiation, highlights the uncertainty.[38,39] An alternative hypothesis is that all neuroepithelial cells are derived from a common precursor cell (i.e., a multipotent neural stem cell), and hence all neuroepithelial tumors are derived from neural stem cells or their committed progeny.[40] The recent discovery, isolation, and characterization of cancer stem cells from human brain tumors provides supportive evidence.[41] However, more recently it was shown in an animal experiment that gliomas can originate from differentiated cells in the CNS, including cortical neurons.[42]

Approximately 15% of all primary CNS tumors arise in the spinal cord, where the distribution of tumor types is significantly different from that in the brain. Tumors of the lining of the spinal cord and nerve roots predominate (50% to 80% of all spinal tumors); schwannomas and meningiomas are most common, followed by ependymomas. Primary gliomas of the spinal cord are uncommon.[43] In children, three-quarters of tumors are comprised of ependymomas, pilocytic astrocytomas, and other neuroepithelial neoplasms.[2]

ANATOMIC LOCATION AND CLINICAL CONSIDERATIONS

Intracranial Tumors

Intracranial tumors produce five categories of symptoms: those arising from increased intracranial pressure (ICP), seizures, physiologic deficits specific to location, higher order neurocognitive deficits, and endocrinologic dysfunction. A headache arises from irritation of the dura or intracranial vessels or due to elevated ICP from tumor bulk, edema, or obstruction of a cerebrospinal fluid (CSF) pathway. Slow-growing tumors may grow to a remarkably large size without producing headaches, whereas rapidly growing tumors can cause headaches early in their course. Small tumors can cause headaches by growing in an enclosed space that is richly innervated with pain fibers, such as the cavernous sinus, or by causing obstructive hydrocephalus. Nausea and vomiting, gait and balance alterations, personality changes, and slowing of psychomotor function or even somnolence may be present with increased ICP. Because ICP increases with recumbency and hypoventilation during sleep, early-morning headaches that awaken the patient are typical. Sometimes the only presenting symptoms are changes in personality, mood, or mental capacity or slowing of psychomotor activity. Such changes may be confused with depression, especially in older patients. Although fewer than 6% of first seizures result from brain tumors, almost one-half of patients with supratentorial brain tumors present with seizures. An adult with a first seizure that occurs without an obvious precipitating event should undergo magnetic resonance imaging (MRI).

Tumors are sometimes associated with location-specific symptoms. Frontal tumors cause changes in personality, loss of initiative, and abulia (loss of ability to make independent decisions). Posterior frontal tumors can produce contralateral weakness by affecting the motor cortex and expressive aphasia if they involve the dominant (usually the left) frontal lobe. Bifrontal disease, seen with "butterfly" gliomas and lymphomas, may cause memory impairment, labile mood, gait imbalance, and urinary incontinence. These symptoms may be related to alteration of normal cortex and white matter by the tumor itself, or by surrounding tumor-related edema. Improvement of symptoms after a short course of high-dose glucocorticoids is often an indicator of whether the findings are related to tumor-associated edema. In the case of CNS lymphoma, corticosteroids can have a cytotoxic effect also with a reduction in the tumor mass.

Temporal tumors might cause symptoms detectable only on careful testing of perception and spatial judgment, but can also impair memory. Homonymous superior quadrantanopsia, auditory hallucinations, and abnormal behavior can occur with tumors in either temporal lobe. Nondominant temporal tumors can cause minor perceptual problems and spatial disorientation. Dominant temporal lobe tumors can present with dysnomia, impaired perception of verbal commands, and ultimately fluent (Wernicke-like) aphasia. Seizures are more common from tumors in this location.

Parietal tumors affect sensory and perceptual functions. Sensory disorders range from mild sensory extinction or stereognosis, which are observable only by testing, to a more severe sensory loss such as hemianesthesia. Poor proprioception in the affected limb is common and is sometimes associated with gait instability. Homonymous inferior quadrantanopsia, incongruent hemianopsia, or visual inattention may occur. Nondominant parietal tumors may cause contralateral neglect and, in severe cases, anosognosia and apraxia. Dominant parietal tumors lead to alexia, dysgraphia, and certain types of apraxia. Occipital tumors can produce contralateral homonymous hemianopsia or complex visual aberrations, affecting perception of color, size, or location. Bilateral occipital tumors can produce cortical blindness.

Classic corpus callosum disconnection syndromes are rare in brain tumor patients, even though infiltrative gliomas often cross the corpus callosum in the region of the genu or the splenium. Interruption of the anterior corpus callosum can cause a failure of the left hand to carry out spoken commands. Lesions in the posterior corpus callosum interrupt visual fibers that connect the right occipital lobe to the left angular gyrus, causing an inability to read or name colors.

Thalamic tumors can cause local effects and also obstructive hydrocephalus. Either sensory or motor syndromes or, on the dominant side, aphasia is possible. *Thalamic* pain disorders or motor syndromes from basal ganglia involvement may also occur. The most common brainstem tumor is the pontine glioma, which presents most frequently with cranial nerve VI and VII palsies. Long tract signs usually follow, with hemiplegia, unilateral limb ataxia, gait ataxia, paraplegia, hemisensory syndromes, gaze disorders, and occasionally, hiccups. Tectal involvement causes Parinaud syndrome, peduncular lesions cause contralateral motor impairment, and obstruction of the aqueduct causes hydrocephalus.

Tumors in the medulla can have a fulminant course, including dysphagia, dysarthria, and deficits in cranial nerves IX, X, and XII. Involvement of the medullary cardiac and respiratory centers can result in a rapidly fatal course. Fourth ventricular tumors, because of their location, cause symptomatic obstructive hydrocephalus at a relatively small size, with associated disturbances of gait and balance. In addition, nausea and vomiting can be a symptom of a fourth ventricular mass. Rapidly enlarging lesions may end in cerebellar herniation.

Cerebellar tumors have variable localizing presentations. Midline lesions in and around the vermis cause truncal and gait ataxia, whereas more lateral hemispheric lesions lead to unilateral appendicular ataxia, usually worst in the arm. Abnormal head position, with the head tilting back and away from the side of the tumor, is seen often in children but rarely in adults. Mass lesions within or abutting the brain or spinal cord can cause displacement of vital neurologic structures. This can lead, in the brain, to herniation syndromes with respiratory arrest and death and, in the spine, to paraplegia or quadriplegia. A hemorrhage into a tumor can also cause acute neurologic deterioration. This is often associated with iatrogenic coagulopathies such as thrombocytopenia due to chemotherapy or anticoagulation therapy for deep venous thrombosis. Primary tumors that most often bleed de novo are glioblastoma and oligodendrogliomas; of the metastatic tumors, lung cancer, melanoma, renal cell cancer, thyroid cancer, and choriocarcinoma most often show hemorrhage.

Lumbar puncture should not be performed in any of the acute herniation syndromes or when herniation is imminent. In fact, a lumbar puncture should be avoided in the setting of significantly elevated ICP that is directly related to a tumor's mass effect or to obstructive hydrocephalus.

Spinal Axis Tumors

For the clinical presentation of tumors of the spinal axis to be understood, the local anatomy must be appreciated. A spinal tumor can produce local (focal) and distal (remote) symptoms, or both. Local effects indicate the tumor's location along the spinal axis, and distal effects reflect involvement of motor and sensory long tracts within the cord.

Distal symptoms and signs are confined to structures innervated below the level of the tumor. Neurologic manifestations often begin unilaterally, with weakness and spasticity, if the tumor lies above the conus medullaris, or weakness and flaccidity if the tumor is at or below the conus. Impairment of sphincter and sexual function occurs later unless the tumor is in the conus. The upper level of impaired long-tract function usually is several segments below the tumor's actual site. Local manifestations may reflect involvement of bone (with axial pain) or spinal roots, with radicular pain and loss of motor and sensory functions of the root or roots.

NEURODIAGNOSTIC TESTS

Magnetic Resonance Imaging

The imaging modality of choice for most CNS tumors is MRI, which can demonstrate anatomy and pathologic processes in detail.[44] CT is generally reserved for those unable (e.g., because of an implanted pacemaker, metal fragment, or paramagnetic surgical clips) or unwilling (e.g., because of claustrophobia) to undergo MRI. Because of the link of nephrogenic systemic fibrosis to the infusion of gadolinium-based contrast agents, there are new preventative guidelines regarding the administration of gadolinium in patients who may be at high risk.[45]

The most useful imaging studies are T1-weighted sagittal images, gadolinium (Gd)-enhanced and unenhanced T1 axial images, and T2-weighted axial images (Fig. 97.2). Contrast-enhanced MRI provides an improved ability to discern tumors from other pathologic entities, one tumor type from another, and putatively higher from lower grade malignancies. There are, however, limitations in anatomic MRI to definitively diagnose a mass lesion as a tumor.[46] Other confounding diagnoses include bacterial abscesses, inflammatory disease such as sarcoidosis, tumefactive demyelination, and acute ischemic disease. It is conventionally believed that most low-grade gliomas (except pilocytic astrocytomas and pleomorphic xanthoastrocytoma) do not enhance, but in reviewing imaging studies of patients enrolled in several clinical trials, it is apparent that this may not be so categorical, in that even low-grade gliomas may frequently contain areas of enhancement, raising the concern that these areas might represent high-grade or malignant transformation (Fig. 97.3).[47] In addition, some high-grade lesions may not have contrast enhancement on MRI. Imaging is also unable to discern different histologic subtypes; however, the presence of calcifications is typical of oligodendroglioma.

Neuraxis or Spinal Imaging

In the evaluation of spinal cord tumors, MRI is also the preferred modality, providing superb visualization of the spinal cord contour and (with gadolinium contrast) of most intrinsic tumors (such as ependymomas, astrocytomas, meningiomas, and schwannomas), as well as facilitating the diagnosis of leptomeningeal dissemination. Tumor cysts are readily identified on MRI, and spinal cord tumors can often be distinguished from syringomyelia. Ideally, neuraxis imaging should be performed before surgery. In the immediate postoperative period, spinal MRI scans may be difficult to interpret because arachnoiditis and blood products can mimic leptomeningeal metastasis. Delayed spinal MRI (more than 3 weeks after surgery) combined with an increased dose of gadolinium is a sensitive imaging study for leptomeningeal disease.

Newer Imaging Modalities

Newer MRI techniques include magnetic resonance spectroscopy, dynamic contrast-enhanced MRI, diffusion-perfusion MRI, and functional MRI.[48] In addition, metabolic imaging using positron-emission tomography using various tracers is being explored.[49] These newer techniques remain to be validated as biomarkers of biological behavior or clinical outcome. Posttreatment metabolic scans may help distinguish recurrence from treatment-related

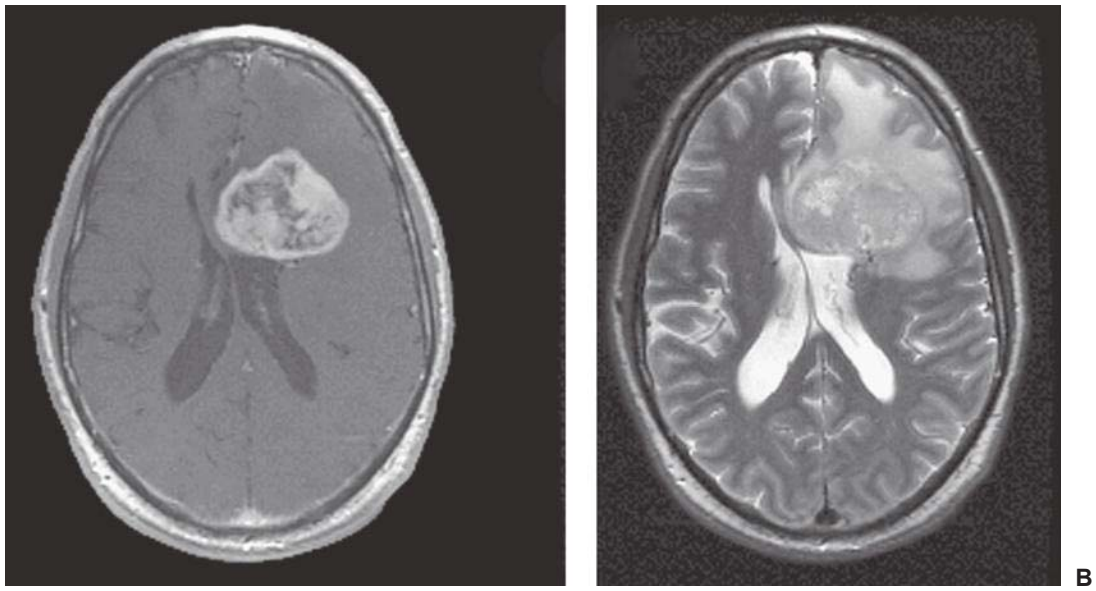

Figure 97.2 Magnetic resonance imaging of a patient with a malignant glioma demonstrates a large mass with heterogenous enhancement **(A)** and significant edema **(B)** on the T2-weighted sequences.

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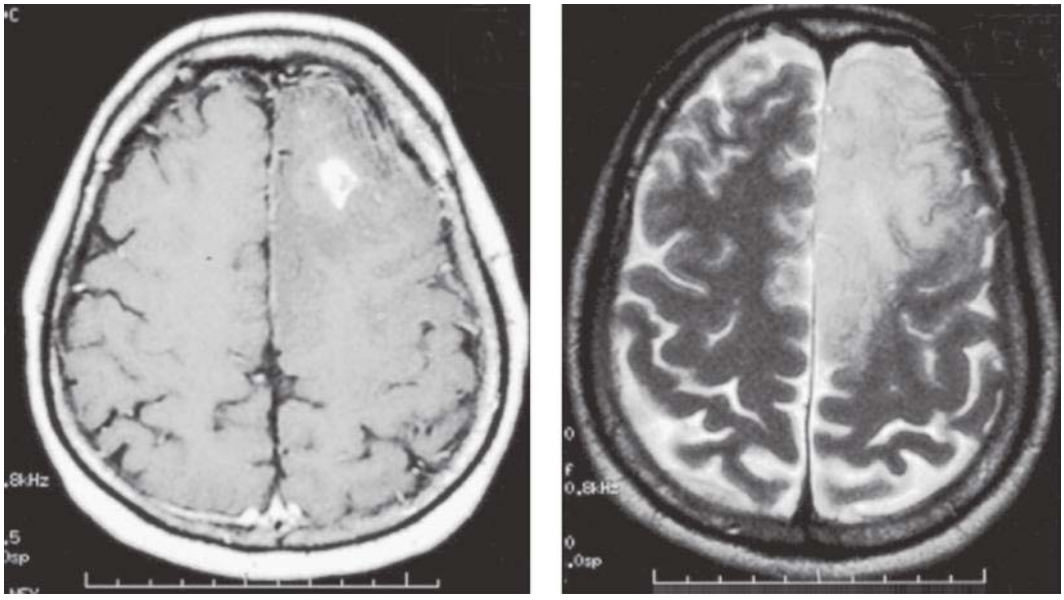

Figure 97.3 **A:** Low-grade astrocytomas often do not enhance and contrast-enhanced T1-weighted magnetic resonance sequences considerably underestimate the true infiltrative extent of these neoplasms. **B:** The fluid-attenuated inversion recovery (FLAIR) sequence is considerably more useful in appreciating the true extent of such neoplasms.

changes, although most modalities have a relatively high false negative rate. A modification of the standard MRI is quick brain MRI, which uses single-shot, fast-spin echo imaging to allow for adequate demonstration of ventricular anatomy and appropriate evaluation of shunt function.[50]

Pseudoresponse and Pseudoprogression

In malignant gliomas treated with combined modality therapy, it is speculated that 25% to 40% or even more may experience imaging changes relatively early in the course of therapy, usually within a few months, which appears consistent with radiographic progression. However, with time and without any therapy, many of these changes actually improve or even resolve (pseudoprogression), and in patients operated on with a presumptive diagnosis of tumor, the histopathology often reveals large areas of tumor necrosis.[51,52] With the advent of antiangiogenic therapies for malignant gliomas, rapid resolution of tumor enhancement is visualized on MRI, sometimes within days. This is consistent with the traditional definition of response, but in several instances, especially with time, even in the absence of contrast enhancement, tumor progression and clinical deterioration occurs, which is sometimes appreciated as T2 or fluid-attenuated inversion recovery (FLAIR) changes; this phenomenon is labeled as *pseudoresponse*.[53]

Cerebrospinal Fluid Examination

Typically, medulloblastoma, ependymoma, choroid plexus carcinoma, lymphoma, and some embryonal pineal and suprasellar region tumors have a high enough likelihood of spreading to justify CSF examinations to evaluate for malignant cells (cytology) and specific markers, such as human chorionic gonadotropin-β and α-fetoprotein.

CSF spread of a tumor may be associated with several possible findings, including CSF pressure above 150 mm H_2O at the lumbar level in a laterally positioned patient; elevated protein, typically greater than 40 mg/dL; reduced glucose (below 50 mg/mL); and tumor cells by cytologic examination. A high protein concentration with normal glucose levels and normal cytology is also seen with base of skull tumors, such as vestibular schwannoma, and with spinal cord tumors that obstruct the subarachnoid space and produce stasis of the CSF in the caudal lumbar sac. Sampling of the CSF in the immediate postoperative period may lead to false-positive results, however, and is best done before surgery or more than 3 weeks after surgery, as long as there is no uncontrolled raised intracranial pressure.

SURGERY

Preoperative Considerations

The major objectives of surgery are to maximally remove bulk tumor, reduce tumor-associated mass effect and elevated ICP, and provide tissue for pathologic analysis in a manner that minimizes risk to neurologic functioning. For some tumors, a complete resection can be curative. However, most brain tumors are diffusely infiltrative; for these, surgical cure is rarely possible. Nonetheless, surgery can rapidly reduce tumor bulk with potential benefits in terms of mass effect, edema, and hydrocephalus. Furthermore, there is mostly retrospective evidence for both high-grade and low-grade infiltrative gliomas for which maximizing the extent of bulk tumor removal is associated with a better outcome, albeit so long as new, permanent neurologic deficits are avoided.[54–57] The requirement for histopathologic confirmation of diagnosis is not necessary in certain well-defined situations, but a tissue diagnosis is still required to determine the appropriate treatment course in most circumstances. As molecularly targeted therapies become useful, tissue removal for molecular analysis will become more necessary to guide therapy. Pseudoprogression may make tissue-based confirmation necessary before changes in therapy are instituted.[53] Technologic advances in surgical approaches, techniques, and instrumentation have rendered most tumors amenable to resection; however, for some tumor types or locations, the risk of open operation supports the choice of biopsy for obtaining diagnostic tissue. Biopsy techniques include stereotactic biopsy (with or without a stereotactic frame) using CT, MRI, or both, to choose the target. Metabolic or spectroscopic imaging can be coregistered with anatomic images to choose targets that may be of higher biologic

aggressiveness within a tumor that appears homogeneous on standard imaging. In certain settings, an approach using simple ultrasonic guidance can also be considered for obtaining diagnostic tissue.

Unless a lymphoma is being considered, patients are given corticosteroids, usually dexamethasone, immediately preoperatively and often for several days before surgery to reduce cerebral edema and thus minimize secondary brain injury from cerebral retraction. Steroid administration is then continued in the immediate postoperative period and tapered off as quickly as possible. Antibiotics are given just before making the incision to decrease the risk of wound infection.

Anesthesia and Positioning

The routine use of prophylactic anticonvulsants in the perioperative period is a common practice despite recommendations that would seem to discourage that practice.[58,59] Patients with a history of seizures need to have their anticonvulsants maintained at therapeutic dose levels. Under certain circumstances, such as for awake craniotomies with electrocorticography, the use of anticonvulsants for a short time might be warranted.

General Surgical Principles

In the past, localization of the surgical incision and craniotomy were most often performed by a neurosurgeon's understanding of cranial anatomy and an interpretation of preoperative imaging. More recently, image-guided navigation systems have been employed to more effectively localize tumor margins as they project to the cranial surface and thus allow for smaller, precisely positioned craniotomies.[59,60] For tumors not resectable because of their location or diffuseness, a biopsy can be performed stereotactically using frameless or frame-based techniques. Tumors that are limited to the cortical surface may be best sampled with an open biopsy, under direct vision, due to the risk of inadvertent injury to a cortical vessel with a more limited, needle-based approach.

Specialized technology can be used to help define the completeness of a resection. Often, preoperative mapping of functional areas and their connections with MRI-based techniques are used to delineate both cortical areas and important subcortical white matter tracts that subserve speech and motor function.[61] Image-guided navigation systems are almost always employed, but the guidance may lose accuracy over the course of an operation due to brain shift or cyst decompression. Intraoperative imaging with ultrasound, CT, or MRI may be used to determine the extent of residual tumor and to further localize areas where additional tumor may be removed safely.[62] There has been growing use of 5-aminoleuvilinic acid (5-ALA), a prodrug, which is converted by glioma cells into fluorescent porphyrins that can be visualized with an operating microscope equipped with a fluorescent imaging system. The impact of the use of 5-ALA to guide resection of glioblastoma (GBM) on completeness of surgical resection and progression-free survival (PFS) has been demonstrated in a phase III trial.[63] Its use is limited to tumors that enhance with contrast on MRI (or CT), because the conversion of prodrug in low-grade tumors does not produce a sufficient amount of fluorescent porphyrin to be visualized intraoperatively. However, this conversion can be detected with the use of specialized optical instrumentation.[57] Use of 5-ALA in this manner is approved by regulatory authorities in Europe; its use in the United States remains investigational at this time.

Intraoperative cortical-stimulation mapping facilitates the resection of tumors in or adjacent to functionally critical areas. Motor functions can be mapped even under general anesthesia; however, anesthetic agents may increase the threshold to response and hence decrease the sensitivity of mapping. Sensory and speech-associated cortex are typically mapped during an awake craniotomy. Patients are monitored in the specialized care unit overnight after surgery, and an MRI is done within 24 to 48 hours to evaluate the extent of any remaining tumor. It is important that this MRI is done before 72 hours to minimize the appearance of nonspecific contrast enhancement that is related to surgery and might be mistaken for residual tumor.[64,65]

Re-resection of recurrent cerebral astrocytomas can be modestly efficacious.[66] When the initial tumor was low grade, histologic resampling may be necessary to guide further treatment at recurrence. Reoperation offers a chance to implant polymer wafers containing carmustine (bis-chloroethylnitrosourea [BCNU]) or to administer experimental agents, such as gene therapy agents or immunotoxins. A smaller volume of disease at initiation of chemotherapy predicts longer survival; thus, reoperation may improve the efficacy of adjuvant treatment as well as relieve mass effect in some patients.[67] An increasingly important aspect of resection is the need for tumor sampling to allow for a molecular marker analysis, which might provide and aid in assessing the prognosis as well as the probability of benefit from both chemotherapeutic and targeted therapies.

RADIATION THERAPY

General Concepts

Radiation therapy plays an integral role in the treatment of most malignant and many benign primary CNS tumors. It is often employed postoperatively as adjuvant treatment to decrease local failure, to delay recurrence, and to prolong survival in gliomas; as definitive treatment in more radiosensitive diseases such as PNET and germ cell tumors; or as therapy to halt further tumor growth in schwannomas, meningiomas, pituitary tumors, and craniopharyngiomas, and as ablative therapy to abrogate hormonal overproduction in secretory pituitary adenomas. Radiation therapy is also the primary modality in palliating brain metastases, and symptomatic spinal and osseous, as well as soft-tissue skull lesions.

Radiobiologic and Toxicity Considerations

Most neoplasms can potentially be cured if the correct radiation dose can be delivered to the entire tumor and its microscopic extensions. This is not always feasible because the maximum radiation dose deliverable is limited by the tolerance of the surrounding normal tissues, and the identification of regions of microscopic extension remains vague. Radiation tolerance of the CNS depends on several factors, including total dose, fraction size, volume irradiated, underlying comorbidities (particularly hypertension and diabetes), and innate sensitivity. Adverse reactions to cranial irradiation differ in pathogenesis and temporal presentation and are not discussed in detail here.

A major radiobiologic consideration revolves around the selection of total dose and the fractionation schedule. Late or long-term toxicities are generally a function of fraction size (i.e., dose per daily fraction of treatment), and therefore, as the fraction size is increased, such as with radiosurgery, higher late toxicity rates must be anticipated, assuming that normal tissue is encompassed within the high dose field. These late toxicities from larger fraction sizes can be minimized by minimizing the volume irradiated, as is done with radiosurgery, thereby drastically reducing the volume of normal tissues exposed to high doses. Proton and charged particle therapy, such as carbon, etc., are characterized by minimal to no exit dose beyond the target (where the so-called Bragg peak [i.e., the peak region of dose deposition] is placed), thereby sharply targeting the dose, and advantageously sparing tissue distal to the target. For radiosurgery, doses in the order of 12 to 21 Gy in single fractions are often utilized. In conventional radiotherapy, fraction sizes of 2 Gy are routinely utilized and may be lowered to 1.8 Gy per fraction in proximity to the visual apparatus or may be increased

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to 3 Gy or more per fraction in patients in whom shorter palliative schedules, with lesser concern regarding long-term morbidities, exist. In general, the entire target is treated with a relatively uniform dose, but with the advent of newer delivery methods, it is possible to create dose gradients or dose inhomogeneities within the tumor to match differential radiosensitivity. However, this concept of dose painting remains investigational.

Treatment Planning and Delivery Methods

High-resolution MR fusion with CT planning images has allowed for more precise delineation of targets, although a significant margin, particularly with gliomas, is still necessary to cover microscopic extension.[68] Patient immobilization devices limit intrafraction motion and provide precision in positioning, decreasing the margin required for setup variability. Image-guided radiotherapy (IGRT), using biplanar orthogonal x-ray imaging systems, cone beam CT, megavoltage CT, surface tracking, fiducial monitoring, etc., further improves setup reproducibility and allows for decreased margins. Newer systems in development incorporate on-board MRI, but remain investigational.

IGRT can be incorporated with any radiotherapy method, such as fractionated external-beam radiotherapy and stereotactic radiosurgery (SRS), and is practically mandatory for charged-particle therapy, frameless radiosurgery, fractionated stereotactic radiotherapy (FSRT), and intensity-modulated radiotherapy (IMRT). CT-based three-dimensional conformal radiation (3D-CRT) in which noncoplanar fields with unique entrance and exit pathways can be mapped on the target has improved normal tissue sparing. This allows for avoidance of critical structures, such as the brainstem, optic apparatus, and spinal cord. In IMRT, the photon flux of a beam is modulated in multiple directions during treatment, aimed at mimicking the shape of the target from various viewpoints, thereby producing improved conformality and nonuniform dose distribution. IMRT is increasingly being utilized for CNS tumors, based primarily on dosimetric studies, which suggest superior tumor coverage and reduction in the dose to critical structures (Fig. 97.4).[69] This can be beneficial in specific instances, such as to preserve cochlear function, vision, or pituitary activity.[70]

In FSRT, the concepts of 3D-CRT or IMRT are merged with the accuracy and precision in delivery that characterizes SRS, and, typically, the radiation fraction size is considerably increased, so that the total course of therapy is reduced from the typical 20 to 30 or more fractions to 5 or fewer fractions. Various FSRT systems have been developed, with reported precision between 1 to 3 mm.[71,72] FSRT is often used for larger lesions (e.g., 4 cm or more) and for lesions located in critical regions where single-fraction

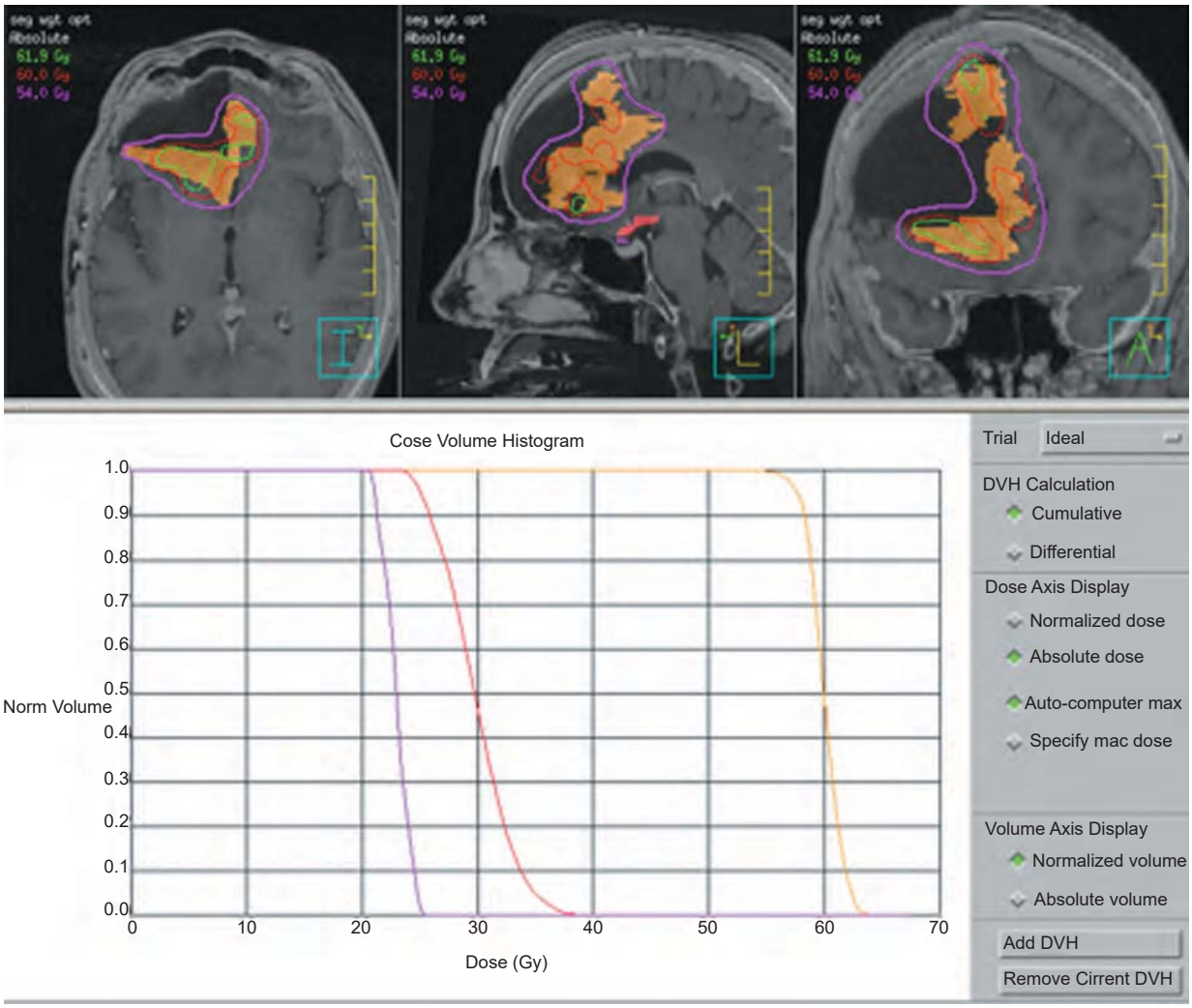

Figure 97.4 Intensity-modulated radiotherapy allows dose shaping to avoid critical structures. In this treatment plan of a right frontal oligodendroglioma (*orange*), tight target coverage and excellent conformal avoidance of the optic chiasm (*red*) and pituitary (*purple*) are achieved, as evidenced by the dose-volume histogram (DVH).

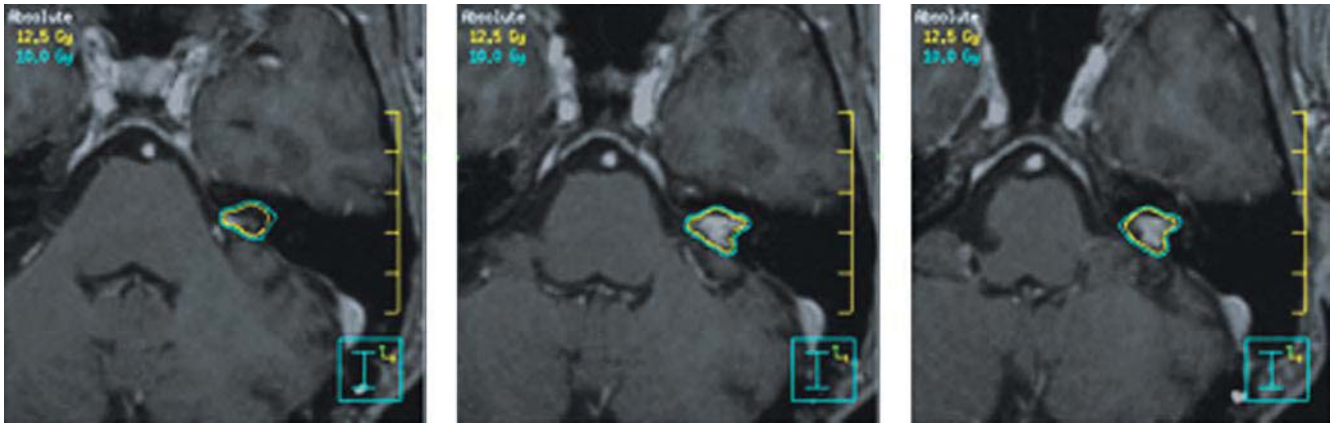

Figure 97.5 Example of radiosurgery dose distribution. This schwannoma is being treated with radiosurgery; the 12.5-Gy prescription isodose line conforms to the lesion.

SRS is disadvantageous because of a higher risk of toxicity, such as larger vestibular schwannomas or meningiomas.

SRS is used to treat a diverse group of lesions. Treatment can be carried out using either a modified or dedicated linear accelerator, cobalt-60 units, or charged particle devices. Several commercial devices have now been developed, each with slightly unique features, including robots that position the linear accelerator at various angles, collimation systems that provide prefixed circular collimators of various sizes or shaped collimated beams, and even intensity-modulated delivery from one or multiple directions, delivered serially, helically, or volumetrically.[73] Radiosurgery plays a dominant role in the treatment of oligometastases to the brain, arteriovenous malformations, schwannomas, and meningiomas and is occasionally used to treat malignant recurrences (Fig. 97.5).

Charged-particle beams, including protons (but not electrons), deposit the majority of their dose at a depth dependent on the initial energy, avoiding the exit dose of photon therapy. This localized dose is known as the *Bragg peak*. Historically, in order to cover larger volumes, proton beams have been modified by passive range modulators that disperse the Bragg peak and broaden the dose deposition, resulting in decreased proximal sparing, while still maintaining distal sparing. Charged-particle radiotherapy has been particularly utilized to treat tumors of the skull base to doses higher than can be achieved conventionally, and in reirradiation settings where conventional techniques are too unsafe. In particular, chordomas and chondrosarcomas require high radiation doses for local control. Proton beams have also been advocated for childhood tumors and tumors in young adults, because they decrease integral radiation dose, thereby decreasing the risk of second malignancies, although concern about incidental neutron production exists.[74,75] The neutron contamination issue is almost nonexistent with approaches similar to the photon technique of intensity modulation, often also referred to as intensity modulated proton therapy (IMPT) and allows for significantly superior *dose sculpting*. Increasingly, this approach is being utilized in patients with lower grade neoplasms of the CNS, where survival is anticipated to be in years, and where reduction in the volume of normal brain irradiated is likely to produce benefits in cognitive, functional, and endocrine domains.

Brachytherapy has historically been widely used, but currently has a limited role in the CNS, although it has enjoyed some resurgence and is occasionally used for recurrent gliomas. A liquid colloid of organically bound iodine-125 (^{125}I) in a spherical balloon continues to be used to treat both recurrent and newly diagnosed malignant gliomas and brain metastases in the postoperative context.[76] At least two randomized trials using seed implants have failed to demonstrate a survival advantage in malignant gliomas. The injection of radioisotopes within the cystic portion of craniopharyngiomas allows ablation of the secretory lining. A select group of patients with cystic tumors may benefit from the direct instillation of colloidal phosphorus-32 (^{32}P), yttrium-90 (^{90}Y), or gold-198 (^{198}Au).[77,78] This technique will deliver between 200 to 400 Gy to the cyst wall.

Radiolabeled therapy is in the developmental phase. The most commonly used antigenic targets for CNS malignancies are the epidermal growth factor receptor (EGFR), neural cell adhesion molecule (NCAM), tenascin, placental alkaline phosphatase (PLAP), and phosphatidylinositide. Institutions using this technique have utilized murine, chimeric, or humanized monoclonal antibodies attached to ^{131}I, ^{90}Y, rhenium-188 (^{188}Re), and astatine-211 (^{211}At). The evolution of these trials has seen the delivery route move from systemic (intra-arterial or intravenous) to local instillation of the agent into a surgically created resection cavity. Even though the blood–brain barrier is often disrupted by a rapidly growing CNS malignancy, 150 kDa antibodies would still not likely cross to a significant degree.[79] Most of the trials to date are of *dose searching pilot* or phase I design. Using ^{131}I-81C6 (antitenascin monoclonal antibody), a trend toward significant improvement in median survival was shown for patients receiving 40 to 48 Gy versus less than 40 Gy.[80] Unlike seed brachytherapy, there appears to be a very low rate of CNS toxicity with targeted isotope therapy, and a minimal need for surgical intervention for the removal of necrotic regions.

CHEMOTHERAPY AND TARGETED AGENTS

Drug therapies alone are effective for only a few types of CNS tumors (e.g., primary CNS lymphoma) but are useful as adjunctive therapy for many CNS tumors. Among the reasons for the poor efficacy of chemotherapeutic and targeted agents is the low concentration of drug penetration to the tumor because of the difficulty of agents to cross the blood–brain barrier, active transport mechanisms of drug efflux, and high plasma protein binding of agents, thereby lowering the volume of distribution of agents in the brain parenchyma.[81] Intrinsic and acquired resistance remains an important reason for the lowered efficacy of chemotherapy. Although targeted agents are in early testing, multiplicity and alternate signaling pathways limit their efficacy.

The Blood–Brain Barrier

Central to treating CNS tumors is the issue of drug delivery, due to the blood–brain barrier (BBB), a physiologic and functional barrier. The CNS microvasculature has several unique features, including the lack of fenestrations between adjacent endothelial cells and relatively fewer pinocytotic and endocytotic endothelial

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vesicles. Additionally, adjacent BBB endothelial cells are connected by a continuous extension of tight junctions, which limit passive diffusion between endothelial cells and through capillary structures. Tight junctions within the BBB are also enveloped by astrocytic foot processes, which increase the barrier to passive diffusion across the BBB.

Brain microvasculature selectively transports nutrients through 20 or more active or facilitated carrier transport systems expressed on the endothelial surface.[82] The endothelium is rich with efflux pumps, including the multidrug resistance (MDR) gene–encoded P-glycoprotein that actively removes substrate molecules that may have passed the BBB.[83] There are several methods to disrupt or circumvent the BBB, including the intra-arterial administration of mannitol,[84] which has resulted in significant toxicities and thus have limited its universal use.[85] Noninvasive delivery systems using specialized carriers such as nanosystems (colloidal carriers) with favorable pharmacokinetic and pharmacodynamic properties are being explored.[86,87] Other methods include local administration (Gliadel wafer)[88] or local drug delivery such as convection-enhanced delivery (CED). CED requires the implantation of catheters directly into the brain, followed by continuous infusion of the drug under a constant pressure gradient. Proof of principle for CED has been demonstrated in several studies, but unfortunately, phase III results have been disappointing.[89,90] Another approach is the direct administration of the agent into the CSF. With a few exceptions (e.g., methotrexate, cytarabine, thioTEPA), most compounds cause unacceptable neurologic toxicity, including death, when given into the CSF. Because of this, intrathecal chemotherapy is principally used to treat leptomeningeal metastases and for CNS prophylaxis for high-risk leukemia.

Challenges Specific for Targeted Agents

Despite the availability of targeted agents specific to aberrant signaling pathways in high-grade gliomas, the results of phase II studies of many agents have been disappointing. In addition to the difficulty of delivery of agents across the BBB, there are other challenges that limit the efficacy of these agents. These include accounting for the heterogeneity of tumors, redundancy of pathway interactions, a lack of accurate and reproducible biomarkers to select patients for specific therapies, and difficulty in assessing target modulation.[91–93] Bayesian adaptive randomized designs in clinical trials may allow for more efficient trials compared to those with balanced randomization.[94]

Other Systemic Therapy Considerations

Many antiepileptic agents, including phenytoin, carbamazepine, and phenobarbital, induce the hepatic cytochrome P-450 isoenzyme and glucuronidation drug-elimination systems. The specific isoenzymes induced by these drugs are often capable of metabolizing many agents. For example, standard paclitaxel doses commonly result in subtherapeutic serum levels in patients also using phenytoin.[95] In fact, the maximally tolerated paclitaxel dose in patients using enzyme-inducing P-450 antiepileptics is nearly threefold higher than in patients not using such agents. Similar observations have been made with regard to 9-aminocampothecin, vincristine, teniposide, irinotecan, and targeted agents.[96–99] In addition to different maximal tolerated dose (MTDs) being established depending on the use of enzyme-inducing antiepileptics, the side effect profile and dose-limiting toxicities can also differ.[99,100] Most phase I clinical trials in brain tumor patients now use separate arms for patients who are or are not taking enzyme-inducing antiepileptic drugs or limit enrollment to patients not taking enzyme-inducing antiepileptic drugs. It may be preferable to change to a non–enzyme-inducing antiepileptic agent (e.g., levetiracetam [Keppra]), although it may take days to make the switch and some time for the P-450 enzyme induction to resolve.

SPECIFIC CENTRAL NERVOUS SYSTEM NEOPLASMS

Cerebral Glioma

Pathologic Classification

The histologic subtypes of gliomas include tumors of astrocytic, oligodendroglial, ependymal, and neuroepithelial origin (Table 97.2). Based on the WHO classification,[3] noninfiltrative gliomas are classified as grade I, and infiltrating gliomas are subsequently categorized from grades II to IV. Infiltrative astrocytic tumors are divided into three categories: astrocytoma (including grade II fibrillary, gemistocytic, and protoplasmic), anaplastic astrocytoma (grade III), and glioblastoma (including grade IV giant cell glioblastoma and gliosarcoma). Oligodendrogliomas and ependymomas are either grade II or anaplastic (grade III).

WHO Grade I: Astrocytoma

Low-grade astrocytomas (WHO grade I) such as pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and subependymal giant cell astrocytoma are typically circumscribed and indolent tumors. Missense mutations of the V600E type in the v-RAF murine sarcoma viral oncogene homolog B1 (BRAF) gene were identified

TABLE 97.2

The Variety of Central Nervous System Glial Tumors (Based on 2007 World Health Organization Classification)

- **Astrocytic tumors**
  - Pilocytic astrocytoma
  - Pilomyxoid astrocytoma
  - Subependymal giant cell astrocytoma
  - Pleomorphic xanthoastrocytoma
  - Fibrillary astrocytoma
  - Gemistocytic astrocytoma
  - Protoplasmic astrocytoma
  - Glioblastoma
  - Giant cell glioblastoma
  - Gliosarcoma
  - Gliomatosis cerebri
- **Oligodendroglial tumors**
  - Oligodendroglioma
  - Anaplastic oligodendroglioma
- **Oligoastrocytic tumors**
  - Oligoastrocytoma
  - Anaplastic oligoastrocytoma
- **Ependymal tumors**
  - Subependymoma
  - Myxopapillary ependymoma
  - Ependymoma
  - Anaplastic ependymoma
- **Choroid plexus tumors**
  - Choroid plexus papilloma
  - Choroid plexus carcinoma
- **Other neuroepithelial tumors**
  - Astroblastoma
  - Anaplastic astroblastoma
  - Chordoid glioma of the third ventricle

in these noninfiltrative neoplasms.[101] The highest frequencies were found in pleomorphic xanthoastrocytomas (66%; 65% in its anaplastic variant), gangliogliomas (18%), and pilocytic astrocytomas (9%, especially in tumors with extracerebellar location).[101]

Complete surgical resection, whenever feasible, is the curative mainstay therapy for such tumors. Despite aggressive near total resection, delayed recurrence and eventual malignant transformation are, unfortunately, common. The resection of a low-grade glioma can be difficult in locations such as the optic pathway, hypothalamus, and in those involving deep midline structures. In these instances, asymptomatic patients can be observed carefully for a prolonged period of time and undergo a maximally safe resection only at the time of progression.

In patients who have a recurrent tumor that are not amenable to further resection or who have a residual tumor causing significant morbidity, adjuvant chemotherapy or radiotherapy can improve recurrence-free survival, although the role of chemotherapy in adults remains controversial. Immediate postoperative adjuvant therapies may be appropriate in some cases depending on the location of the tumor, the extent of residual disease, the impracticability of repeated surgical excision, and the availability for follow-up. Generally, radiotherapy is the primary adjuvant treatment used in older children and adults with low-grade gliomas. In young children with unresectable, progressive low-grade gliomas, there is a desire to avoid or delay radiotherapy owing to the long-term radiation-related sequelae; chemotherapy is often utilized here as the initial therapeutic option.[102–105] Some responses from chemotherapy can last for years, and nearly half of all children treated with chemotherapy ultimately require radiotherapy for tumor progression.

In terms of radiotherapy used with a curative intent, in children, the most common situation is with cerebellar and optic-pathway pilocytic astrocytoma, typically after progression on chemotherapy, whereas in adults, this tends to occur most commonly with hypothalamic pilocytic astrocytoma. The typical radiation dose used in this setting is 50.4 to 54.0 Gy, in 1.8 Gy fractions. There is evidence of improved PFS in this situation.[106] Given the young age and long expected survival of these patients, proton beam therapy is often considered for these patients, with the desire to decrease the risk of a second neoplasm, and to treat less normal brain tissue with radiation.[106]

Subependymal giant cell astrocytomas can be effectively treated with everolimus. In a prospective randomized study, 35% of patients in the everolimus group had at least a 50% reduction in the volume of their tumor versus none in the placebo group, although complete responses still remain uncommon, even with this therapy.[107]

WHO Grade II: Low-Grade Glioma

Nonpilocytic or diffusely infiltrating low-grade gliomas are classified as WHO grade II tumors. They may arise from astrocytic, oligodendrocytic, or mixed lineage. Like astrocytomas, oligodendrogliomas display various degrees of clinical aggressiveness. Three common genetic alterations, inactivation of the TP53 tumor suppressor gene, heterozygous point mutations of the isocitrate dehydrogenase-1 (IDH1), and loss of chromosome 22q are involved in the formation of WHO grade II astrocytoma. TP53, located on chromosome 17p, encodes the p53 protein that has an important role in a number of cellular processes, including cell cycle arrest, apoptosis, and response to DNA damage.[108]

Somatic mutations at codon 132 in IDH1 are present in 50% to 80% of WHO grade II and III astrocytic tumors and oligodendroglial tumors, as well as in secondary grade IV glioblastomas.[109,110] These IDH mutations promote the conversion of α-ketoglutarate into D-2-hydroxyglutarate, an oncometabolite that mediates the oncogenic activity of IDH mutations and can be measured by magnetic spectroscopy.[111] Tumors that have IDH mutations carry a better prognosis than do IDH wild-type gliomas of the same histologic grade.[112,113]

An unbalanced t(1;19)(q10;p10) translocation results in a combined loss of chromosomal arms 1p and 19q, which leads to the loss of one hybrid chromosome, and thus, a loss of heterozygosity.[114] This cytogenetic alteration is usually associated with oligodendroglial histology and is rarely found in other tumors. Patients with 1p- and 19q-codeleted tumors have a better prognosis than do histologically similar tumors of the same grade that do not harbor this codeletion.[115]

In addition to histology and molecular characteristics, several variables have been found to be of prognostic importance in low-grade gliomas. Pignatti et al.[116] performed the most comprehensive of these analyses and developed a scoring system to identify patients at varying level of risk for mortality. A multivariate analysis showed that age 40 years or older, astrocytoma histology, maximum diameter 6 cm or greater, tumor crossing the midline, and presence of neurologic deficits negatively impacted survival. Patients with up to two factors were considered low risk (median survival, 7.7 years) and patients with three or more were considered high risk (median survival, 3.2 years). Recently, 339 European Organisation for Research and Treatment of Cancer (EORTC) patients with central-pathology confirmed LGGs were used to develop a new prognostic model for PFS and overall survival (OS).[117] Data from 450 patients with centrally diagnosed LGGs recruited into two large studies conducted by North American cooperative groups were used to validate the models. Both PFS and OS were negatively influenced by the presence of baseline neurologic deficits, a shorter time since first symptoms, an astrocytic tumor type, and tumors larger than 5 cm in diameter.[117]

Surgery for Low-Grade Glioma

Retrospective analyses have suggested that the extent of resection is a significant prognostic variable. The Radiation Therapy Oncology Group (RTOG) performed a prospective evaluation of the natural history of completely resected low-grade gliomas (RTOG-9802), evaluating the recurrence risk in 111 patients with surgeon-defined gross total resections (GTR) and found that the extent of postoperative residual disease was an important variable for time to first relapse.[118] Five-year recurrence rates were 26% versus 68% for patients with less than 1-cm residual tumors versus 1- to 2-cm residual tumors.

Radiation Therapy

The role of radiotherapy—particularly the timing—remains somewhat controversial. Early intervention is indicated for patients with increasing symptoms and radiographic progression. In younger patients (less than 40 years) who have undergone complete resection, observation with imaging is an option. In RTOG-9802, median time to progression in 111 good-risk patients defined as younger than 40 years and with a gross total tumor resection was 5 years.[118] In those who have undergone a subtotal resection or those with high-risk features, postoperative radiotherapy may be recommended, typically 50.4 Gy in 1.8 Gy fractions.

Three phase III trials provide the best evidence with respect to the indications for radiotherapy as well as the dose. In a study by the EORTC (EORTC-22845), 314 patients were randomized to postoperative radiotherapy to 54 Gy (n = 157) or radiotherapy at progression (n = 157).[119] A statistically significant improvement in PFS was associated with early radiotherapy, 5.3 versus 3.4 years ($p < 0.0001$), without a difference in median survival, 7.4 versus 7.2 years.

Two other trials investigated the dose question. In EORTC-22844, 379 patients were randomized to 45 Gy versus 59.4 Gy.[120] With a median follow-up of 74 months, OS (58% versus 59%) and PFS (47% versus 50%) were similar. In an Intergroup study, 203 patients were randomized to 50.4 Gy (n = 101) or 64.8 Gy.[121] There was no significant difference in PFS or OS.

To assess the OS and cause-specific survival (CSS) impact of early adjuvant radiotherapy (EART) following the resection of supratentorial LGG in adults (16 to 65 years), 2,021 patients in

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the SEER database from 1988 to 2007 were evaluated.[122] Of the 2,021 patients, 871 (43%) received EART, and 1,150 (57%) did not. In the multivariate Cox proportional hazards model, EART was associated with worse OS and CSS. Using a propensity score and instrumental variable analyses to account for known and unknown prognostic factors demonstrated unmeasured confounding variables that may affect this finding.

Consequently, low-dose radiotherapy, 50.4 to 54.0 Gy in 1.8 Gy fractions, has become an accepted practice for selected patients with low-grade gliomas. The target volume is local, with a margin of 2 cm beyond changes demonstrated on traditional MRI sequences. FLAIR images usually show considerable abnormality beyond any enhancing or nonenhancing tumor and whether a smaller margin may be used for planning if FLAIR sequences are utilized is unknown.

Posttreatment cognition remains an important consideration. Brown et al.[123] reviewed the results of the Mini-Mental Status Examination for 203 adults irradiated for low-grade gliomas. Most patients maintained stable neurocognitive status after radiotherapy, and patients with abnormal baseline results were more likely to have improvement in cognitive abilities than to deteriorate after therapy; few patients showed cognitive decline. A more in-depth analysis of formal neurocognitive testing suggest that the tumor itself may have the most deleterious effect on cognitive function.[124] Recognition has been gaining that long-term neurocognitive functional (NCF) impairment following radiotherapy for benign or low-grade adult brain tumors could be associated with hippocampal dose. A dose to 40% of the bilateral hippocampi greater than 7.3 Gy was recently shown to be associated with long-term impairment in list-learning delayed recall.[125] Based on such data, the role of proton therapy as a potential approach to reduce cognitive deficits and other side effects is being explored.

Chemotherapy

Low-grade gliomas have historically been considered chemotherapy resistant. With the recent demonstration of the chemotherapy responsiveness of some low-grade astrocytomas and oligodendrogliomas has renewed interest in investigating chemotherapy for low-grade gliomas.[126,127] It has been demonstrated that some low-grade gliomas, especially optic pathway and hypothalamic tumors, can be responsive to chemotherapy.[31,128] In children, various single and multichemotherapeutic and biological agents are effective at controlling the growth of a low-grade glioma in a setting of a newly progressive lesion, multiply recurrent, or unresectable residual tumors.[102–105,129,130] Platinum-containing regimens result in radiographic response rates greater than 60%.[129] Vinblastine has also demonstrated substantial activity in recurrent low-grade gliomas and is a commonly used second-line agent after treatment failure with vincristine and carboplatin.[131,132] Other second- and third-line therapies for multiply recurrent tumors include thioguanine, procarbazine, lomustine, and vincristine (TPCV) and temozolomide. Irinotecan and bevacizumab are currently being investigated in a multi-institutional phase II trial for the treatment of progressive low-grade gliomas. Rapamycin, an oral immunosuppressive agent, has been effective at reducing the growth of astrocytomas associated with tuberous sclerosis.[133] Most of the chemotherapy responses seen in children with low-grade gliomas are for contrast-enhancing masses that probably represent pilocytic astrocytomas. Some of these responses can last for years, although nearly half of all children treated with chemotherapy ultimately require radiotherapy. Nonenhancing, diffusely infiltrating astrocytomas in children appear to be much less responsive to chemotherapy. Data on the use of chemotherapy for low-grade glioma in adults are sparse. In a small Southwest Oncology Group trial, adults with incompletely excised low-grade gliomas were randomly assigned to radiation therapy (RT) alone or RT and lomustine ([2-chloroethyl]-3-cyclohexyl]-1-nitrosourea [CCNU]). There was no difference in survival between the two arms.[134] The role of adjuvant procarbazine, CCNU, and vincristine (PCV) for high-risk patients (e.g., less than total resection, age older than 40 years) with low-grade gliomas was evaluated in RTOG-9802. From 1998 to 2002, 251 patients were randomly assigned to RT alone or RT followed by six cycles of PCV. An initial report of this study showed that the 5-year OS rates for RT versus RT/PCV were 7.5 years versus not reached respectively (hazard ratio [HR] = 0.72, 95% confidence interval [CI], 0.47 to 1.10; $p = 0.33$).[135] At the time of that report, however, 65% of the patients were still alive. A recent National Institute of Health press release on more mature results of this study reported significant improvement in OS in the PCV chemotherapy plus RT group (13.3 years) compared to those assigned to RT alone (7.8 years) at a median follow-up of 12 years.[136] Molecular and cytogenetic analyses (isocitrate dehydrogenase mutations and loss of heterozygosity of 1p and 19q, as well as methylation of methylguanine methyltransferase status) and clinical outcome are pending to identify predictive factors for patients with LGG.

Several studies have evaluated PCV in the recurrent setting, and, more recently, temozolomide has also been evaluated (Tables 97.3 and 97.4).[126,127,137–146] In general, approximately half of the patients treated with either temozolomide or PCV experienced imaging stability or improvement of neurologic symptoms. Although results are encouraging, the number of patients treated in these studies was small, and there are questions regarding the criteria used for radiographic response. In the first report of RTOG 0424, the primary endpoint was to compare the 3-year OS of a regimen of concurrent and adjuvant temozolomide and RT in a high-risk low-grade glioma population to the 3-year OS rate of the high-risk EORTC LGG patients reported by Pignatti et al.[116] With a median follow-up time of 4.1 years and a minimum follow-up of 3 years, MST has not yet been reached. The 3-year OS rate was 73.1% (95% CI, 65.3 to 80.8%), significantly improved in comparison to the prespecified historical control with a p value <0.0001.[147] An ongoing intergroup phase III trial is attempting to answer this issue more definitively.

Patients with low-grade oligodendroglial tumors with 1p/19q deletion or t(1p;19q) have longer PFS and OS than those without.[114] Consequently, 1p/19q determination is important in

TABLE 97.3

Procarbazine for Chemotherapy Naive Low-Grade Glioma

Author (Ref.)	Disease	N	Path	Enhancing (%)	Prior RT	RR (%)	1-Y PFS (%)
Stege et al.[140]	Recurrent	5	O, OA	0	Y	60	N/A
	Newly diagnosed	16		0	N	81	N/A
Buckner et al.[142]	Newly diagnosed	28	O, OA	46	N	52	91
Soffieti et al. [139]	Recurrent	26	O, OA	73	Y	62	80
Lebrun et al.[141]	Newly diagnosed	33	O, OA	18	N	27	N/A

CCNU (lomustine), and vincristine; O, oligodendroglioma; OA, oligoastrocytoma; RT, radiotherapy; RR, response rate; PFS, progression-free survival, N/A, not available.

TABLE 97.4

Temozolomide in Low-Grade Glioma

Author (Ref.)	N	Path	Enhancing (%)	Prior RT/ Chemo	RR (%)	1-Y PFS (%)
Quinn et al.[127]	46	A, O, AA	70	Y/Y	61	76
Pace et al.[138]	43	A, O, AA	60	Y/Y	47	39
Brada et al.[144]	30	A, O, AA	0	N/N	10	90
Hoang-Xuan et al.[126]	60	O, OA	11	N/N	31	73
Van den Bent et al.[143]	28	O, OA	100	Y/Y	25	11
Pouratian et al.[146]	28	O, OA	24	N/N	52	72
Murphy et al.[145]	13	O, OA	0	N/N	100	N/A
Van den Bent et al.[137]	39	O, OA	100	Y/N	53	40

A, astrocytoma; O, oligodendroglioma; AA, anaplastic astrocytoma; RT, radiotherapy; RR, recurrence rate; PFS, progression-free survival, N/A, not available.

patient counseling and in assessing the results of outcomes in future clinical trials. A randomized phase III EORTC trial stratified patients with low-grade glioma by 1p status prior to randomization to RT versus temozolomide.[148] In the initial results of the trial presented, PFS was not significantly different, and median OS was not reached. 1p deletion was a positive prognostic factor irrespective of treatment (PFS: 0.0003; HR = 0.59; 95% CI, 0.45 to 0.78); OS: 0.002; HR = 0.49; 95% CI, 0.32 to 0.77). First-line treatment with temozolomide compared to radiotherapy did not improve PFS in high-risk LGG patients. A molecular and genetic analysis of LGG has revealed aberrant signaling in the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) network; however, a defined role for the inhibition of this pathway in the treatment of LGG remains to be established.[149,150] Targeting this pathway is a therapeutic approach that is being investigated in clinical trials in recurrent LGG patients.

WHO Grade III: Anaplastic Astrocytoma

Prospective and/or randomized evidence indicating that a complete resection of enhancing or an MRI-visible tumor improves survival is lacking, but retrospective analyses reaffirm that this relationship is likely to be present. Nonetheless, almost all of these tumors are characterized by postoperative residual microscopic disease, and radiotherapy is used adjunctively, resulting in a 3-year survival of approximately 55%.[151]

Radiation Therapy

Partial brain fields are used for the treatment of anaplastic astrocytoma; the initial gross tumor volume (GTV) is defined as the T2 or FLAIR abnormality; the boost GTV is defined as the contrast-enhancing volume or the surgical bed; for smaller, nonenhancing tumors, the initial and the boost GTV are often equivalent.[152] The clinical tumor volume (CTV) is defined as an approximately 2-cm margin surrounding the GTV, but not expanding across natural barriers. The initial volume is typically treated to 46 Gy, with the boost volume to 60 Gy. No survival advantage for the use of bromodeoxyuridine as a radiosensitizer was demonstrated.[153] Early versus delayed radiotherapy, utilizing chemotherapy as part of the regimen, was evaluated in a German phase III trial (NOA-04), which is described in the section that follows.

Chemotherapy

The role of chemotherapy remains controversial. Most phase III trials have demonstrated no benefit compared with radiation alone. Both single-agent carmustine and PCV are associated with minimal improvement in survival. Although, for a period of time, PCV was considered the "superior" regimen, database analyses have belied this claim.[151] A meta-analysis by the Glioma Meta-Analysis Trialists' group demonstrated an approximate 6% absolute increase in 1- and 2-year survival for patients who received chemotherapy (2-year survival of 37% versus 31%).[154] A large randomized trial by the Medical Research Council found no benefit of adjuvant PCV compared with RT alone.[155] Although temozolomide is effective for the treatment of recurrent anaplastic astrocytoma, its role as an adjuvant to RT has not been rigorously assessed. Based on these results in recurrent anaplastic astrocytoma, the RTOG initiated a phase III trial (RTOG 9813) to compare radiation with BCNU or CCNU to radiation with temozolomide, and the results are pending.

A comparison of the efficacy and safety of radiotherapy versus chemotherapy with either PCV or temozolomide as initial therapy on patients with newly diagnosed anaplastic glioma showed comparable results in terms of time to treatment failure.[156] Because of the potential prognostic and predictive value of hypermethylation of the O6-methylguanine DNA-methyltransferase (MGMT) promoter and mutations of the *IDH1* gene in malignant gliomas, analyses of these was a correlative part of the study.[110,157,158] Hypermethylation of MGMT promoter and mutations of the *IDH1* gene as well as oligodendroglioma histology reduced the risk of progression. Hypermethylation of MGMT promoter was associated with prolonged PFS in the chemotherapy and RT arms.

Difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, was evaluated in a phase III trial.[159] Of 228 patients, the majority had anaplastic astrocytoma. Following RT, patients were randomized to PCV or PCV plus DFMO. There was a difference in survival during the first 2 years, but this did not continue after 2 years.

Chemotherapy for Recurrent Anaplastic Astrocytomas

Chemotherapy for anaplastic astrocytomas that recur following radiation is of benefit, and both nitrosourea-based regimens and temozolomide have efficacy. The U.S. Food and Drug Administration (FDA) granted accelerated approval for temozolomide on the basis of its activity in recurrent anaplastic astrocytoma; the response rate was 35% for patients who had not received chemotherapy and 20% for patients who had received nitrosourea-based therapy.[160] Many patients are being treated with temozolomide early in the course of their illnesses; therefore, for recurrent anaplastic astrocytoma, nontemozolomide regimens used in glioblastoma are often

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considered.[161] Several clinical trials to evaluate targeted agents in recurrent malignant glioma often include recurrent grade III histology. Based on documented activity of the antivascular endothelial growth factor antibody in recurrent glioblastoma, this agent has also been used in patients with recurrent anaplastic astrocytoma.[162] A retrospective study reported a 64% radiographic response and a 6-month PFS rate of 60% in 25 patients.[163] Prospective studies are pending.

The NOA-04 phase III trial compared efficacy and safety of RT followed by chemotherapy at progression with the reverse sequence in patients with newly diagnosed anaplastic gliomas.[156] Patients received conventional RT: procarbazine, lomustine (CCNU), and vincristine, or temozolomide at diagnosis. At occurrence of unacceptable toxicity or disease progression, patients in the RT arm were treated with PCV or temozolomide, whereas patients in chemotherapy arms received RT. Median time to failure, PFS, and OS were similar for all arms. Hypermethylation of the MGMT promoter was associated with prolonged PFS in the chemotherapy and RT arm. This study showed that IDH1 mutations are a positive prognostic factor in anaplastic gliomas, with a favorable impact stronger than that of 1p/19q codeletion or MGMT promoter methylation.[156]

WHO Grade III: Anaplastic Oligodendroglioma

Surgery

Surgery retains its role as the principal modality of treatment, as with other glial neoplasms, and the maximum safe resection is considered the standard of care. However, the consideration of risks versus benefits of an aggressive surgical resection should take into account the 1p/19q deletional status of the tumor and the potential for a more favorable natural history and response to medical therapy.

Radiation Therapy

No randomized trials that focus only on these tumors comparing radiation versus no RT have been completed. In general, patients with pure and mixed anaplastic oligodendrogliomas receive postoperative irradiation to 60 Gy in conventional daily fractions of 1.8 to 2.0 Gy using an approach similar to that used for other malignant gliomas. Recent data show a categorical and very large survival benefit for both the 1p19q codeleted and the IDH-mutated anaplastic oligodendrogliomas treated with combination chemoradiotherapy, in comparison to RT alone. Therefore, up-front RT alone should not be the preferred treatment for these good prognosis patients. Conversely, no level 1 data exist to support treating these patients with up-front chemotherapy alone, either, and although this practice is sometimes adopted in practice, it should be subjected to rigorous clinical evaluation, because of the potential for the loss of long-term survivorship in these favorable patients, if either therapy is compromised. These results are described in greater detail in the section that follows.

Chemotherapy

Retrospective series and phase II trials first suggested that oligodendrogliomas are chemosensitive.[137,164,165] In two phase III trials, RT alone was compared with RT plus PCV. In the North American trial (RTOG 9402) patients received PCV for four cycles prior to radiation or no up-front PCV. Survival in the two groups was the same. Patients with 1p and 19q deletions had significantly better outcomes, regardless of treatment.[166] An unprespecified analysis of PFS demonstrated that the benefit from PCV was most notable in patients with 1p and 19q deletions. Long-term results of this study demonstrated that patients with codeleted tumors lived longer than those with non-codeleted tumors irrespective of therapy, and the median survival of those with codeleted tumors treated with PCV plus RT was twice that of patients receiving RT (14.7 versus 7.3 years).[167] There was no difference in median survival for patients with tumors lacking 1p and 19q deletion.

In the European trial, patients received PCV or no immediate chemotherapy after radiation.[168] PFS was better in the PCV group, but OS was not different. Patients with 1p and 19q deletion had superior survival, regardless of treatment. A further molecular analysis of this cohort demonstrated that MGMT promoter methylation was of prognostic value.[169,170] Long-term follow-up showed that PFS and OS were better in the PCV group, but OS was not different between the two groups (OS in the RT/PCV arm, 42.3 months; in the RT arm, 30.6 months). In patients with a 1p/19q codeletion, there was a trend to more benefit from adjuvant PCV (OS not reached in the RT/PCV group versus 112 months in the RT group).[171] Both trials confirmed the prognostic value of 1p and 19q.

Temozolomide has produced high response rates in patients with anaplastic oligodendroglioma. In 27 newly diagnosed patients treated with temozolomide prior to radiotherapy, the objective response rate was 33% and the 6-month progression rate was 10%.[172]

Chemotherapy for Recurrent Anaplastic Oligodendroglioma

Prospective trials have demonstrated that approximately 50% to 70% of patients with anaplastic oligodendrogliomas that recur after RT respond to chemotherapy.[143] In a study of 48 patients with anaplastic oligodendroglioma/oligoastrocytoma who progressed on PCV, the objective response rate to temozolomide was 44%.[173] Although there is no evidence that the sequence of temozolomide and PCV is superior in terms of efficacy, the absence of cumulative myelosuppression with temozolomide argues for its use initially in the setting of recurrent disease.

Ongoing Clinical Trials for Newly Diagnosed Grade 3 Glioma

Two international trials are being conducted in patients with newly diagnosed grade 3 glioma stratified by 1p 19q status rather than histology. Nondeleted patients are randomized to radiation with or without temozolomide; following RT, there is a second randomization to adjuvant temozolomide or not. Codeleted patients are randomized to three arms, temozolomide alone (phase II group), RT with concomitant and adjuvant temozolomide, or RT with adjuvant PCV (phase III).

WHO Grade IV: Glioblastoma

Surgery

Gliomas are heterogeneous, and therapy is guided by the most aggressive grade in the specimen. Resection provides the best opportunity to obtain an accurate diagnosis. Studies have shown that more complete resections are more likely to provide a high-grade diagnosis and to detect an oligodendroglial component.[174] Two randomized trials of resection of malignant gliomas have been published. In a study by Vuorinen et al.,[175] survival was twice as long with resection compared to biopsy alone. Stummer et al.[63] reported that patients without residual contrast-enhancing tumor had a higher overall median survival time than did those with residual enhancing tumor (17.9 months versus 12.9 months, respectively; $p < 0.001$). Complete resection of an enhancing tumor enhances certain approved or investigational adjuvant therapies (e.g., carmustine wafers, immunotherapy). Resection also is superior to stereotactic biopsy alone for the provision of adequate tissue for the evaluation of molecular and cytogenetic classifications and certain prognostic markers (e.g., MGMT), which may be a requirement for entry into some clinical trials.

There has been extensive work in molecular subtypes of GBM in recent years that include a report of the Cancer Genome Atlas

Research Network[176] and follow-up transcriptome work of glioblastoma provided insights into the major structural and expression alterations that may drive disease pathogenesis and biology in glioblastoma.[177,178] Verhaak et al.[178] proposed a gene expression-based molecular classification of GBM into proneural, neural, classical, and mesenchymal subtypes. Aberrations and gene expression of EGFR, NF1, and platelet-derived growth factor receptor a (PDGFRA)/IDH1 were utilized to define the classical, mesenchymal, and proneural subtypes, respectively. These investigations into the genome and transcriptome reveal GBM as a heterogeneous collection of distinct diseases with multiple dependencies both within and across each particular subtype.[179]

Radiotherapy

Randomized trials have demonstrated a survival benefit with RT.[180] Localized radiation volumes are recommended based on evidence from several sources that GBMs typically recur locally, and the bulk of the infiltrative disease is within a few centimeters of the enhancing rim. However, the wide and somewhat unpredictable degree and direction of dissemination, which is not visualized well with any imaging technique, renders an RT field definition difficult. Outside of clinical trials, consensus regarding the exact field design remains difficult to obtain. In the large randomized trial RTOG 0525 for newly diagnosed GBM, which allowed both a single field treatment or a separate boost field to be utilized, no survival differences were identified, although the number of patients treated with the single field was rather small, in comparison (>80% versus <20%).[181]

Standard therapy uses a total dose of 60 Gy in 30 to 33 fractions based on dose response studies showing a survival improvement for 60 Gy compared to lower doses and without a benefit for higher doses.[182–215] For patients with poor prognostic factors and for those who are not able to tolerate conventional treatment, a shorter course may provide palliation. Older patients (older than 65 years), especially those with poor performance status, have been shown to have limited posttreatment improvement following conventional RT,[183] and several studies have not shown a significant survival difference using shorter courses.[184–218] A number of trials have evaluated the role of temozolomide versus RT in elderly patients with glioblastoma. The German phase III trial (NOA-08) randomized patients 65 years or older with anaplastic astrocytoma or glioblastoma with a minimum karnofsky performance status (KPS) of 60 to either temozolomide or radiotherapy. Of 412 patients who were randomized, 373 received at least one dose of treatment and were included in efficacy analyses. Median survival was 8.6 months in the temozolomide arm compared to 9.6 months with RT. These results met the criteria of "noninferiority" of temozolomide.[185] In an unplanned post hoc analysis, MGMT promoter methylation status was evaluated in 209 patients; promoter methylation was associated with longer OS (11.9 versus 8.2 months). Event-free survival was longer in patients with MGMT methylation who received temozolomide alone versus RT, whereas the opposite was true for patients without MGMT promoter methylation. Therefore, MGMT methylation seems to be a useful predictive biomarker and could aid decision making in elderly patients not fit to receive concurrent chemoradiation.

The Nordic three-arm phase III trial randomized elderly (>60 years) patients with glioblastoma to two different radiotherapy schedules of 60 Gy in 2 Gy per fraction (fxs) over 6 weeks or a hypofractionated schedule of 34 Gy in 3.4 Gy fxs over 2 weeks, versus temozolomide alone.[186] Median survival was significantly longer with temozolomide versus conventional RT (8.3 versus 6.0 months; HR, 0.70; $p = 0.01$), but not hypofractionated radiotherapy (7.5 months; HR, 0.85; $p = 0.24$). This trial suggests that both temozolomide alone and hypofractionated RT alone produce equivalent survival in elderly patients with glioblastoma, and both are superior to standard RT.

These trials did not address the issue of concomitant chemoradiotherapy for the elderly. An ongoing, phase III trial (EORTC 26062-22061 NCIC CTG CE6) compares the OS rates between short-course RT alone and short-course RT given together with temozolomide in newly diagnosed patients with glioblastoma who are older than 65 years of age and who are not fit for standard treatment. This study has completed accrual and results are pending.

Dose Escalation

In the pretemozolomide era, studies evaluating radiosurgery or brachytherapy boosts to conventional RT have not demonstrated a survival advantage.[222–225]

The feasibility and toxicity of dose-escalated photon radiotherapy concurrent with BCNU chemotherapy in patients with supratentorial GBM[187] was the goal of RTOG 9803. There were 209 patients who were enrolled and stratified into two groups based on the size of planned target volume (<75 mL versus ≥75 mL). Within each stratum, four RT dose levels were evaluated: 64, 72, 78, and 84 Gy; all treatments were delivered with a fraction size of 2 Gy. Acute and late grade ≥3 radiotherapy-related toxicities were no more frequent at higher RT doses or with larger tumors. No dose-limiting toxicities were observed at any dose level in either stratum, and as a result, the dose was escalated to 84 Gy in both strata. Median time to RT-related necrosis was 8.8 months (range, 5.1 to 12.5 months). This study demonstrated the feasibility and tolerability of photon dose escalation with an acceptable risk of late CNS toxicity with doses as high as 84 Gy. However, this study was conducted with concurrent BCNU chemotherapy, not the current standard approach of concurrent and adjuvant temozolomide. This chemoradiotherapy regimen has become the backbone of standard postoperative treatment for patients with GBM but has never adequately been tested in a RT dose-escalation or -intensification context. With this standard postoperative chemoradiotherapy regimen, the predominant pattern of failure remains local, highlighting the importance of investigating more intensive local therapies.

Recently, the University of Michigan published results of a clinical trial that escalated dose and dose-per-fraction from 66 Gy to 81 Gy in 30 fractions during chemoradiotherapy with temozolomide for patients with GBM.[188] The maximum tolerated dose with concurrent temozolomide was 75 Gy in 30 fractions (2.5 Gy per fraction). Median survival was 20.1 months, suggesting improved efficacy comparable to other contemporary studies. Interestingly, the probability of in-field failure decreased with increasing dose escalation, setting the stage for more definitive investigations of this approach. Small phase II studies of dose escalation using mixed photon/proton irradiation demonstrated median survival times of 20 to 22 months, and more formal comparative studies need to be performed.[244,245] Alternate particle radiation modalities used in the treatment of gliomas include neutrons, helium ions, other heavy nuclei such as carbon, negative pi-mesons, and thermal neutrons in conjunction with boronated compounds (boron neutron capture therapy). To date, most studies have been designed to determine optimal dose scheduling, efficacy, and safety.

Radiosensitizers and Radioimmunotherapy

Studies using various radiation modifiers such as hyperbaric oxygen, nitroimidazoles and tirapazamine, RSR-13, or carbogen and nicotinamide to overcome the hypoxia present in malignant gliomas have generally yielded disappointing results with no survival advantage.[228,232] Halogenated pyrimidines are incorporated into the DNA of dividing cells due to their biochemical similarity to thymidine. After being incorporated, cells are much more susceptible to single-strand breaks from radiation-induced free radicals and have impaired ability to repair DNA. Prospective clinical studies, however, have not demonstrated a survival advantage.[169] Motexafin gadolinium (MGd) is a redox-active drug that

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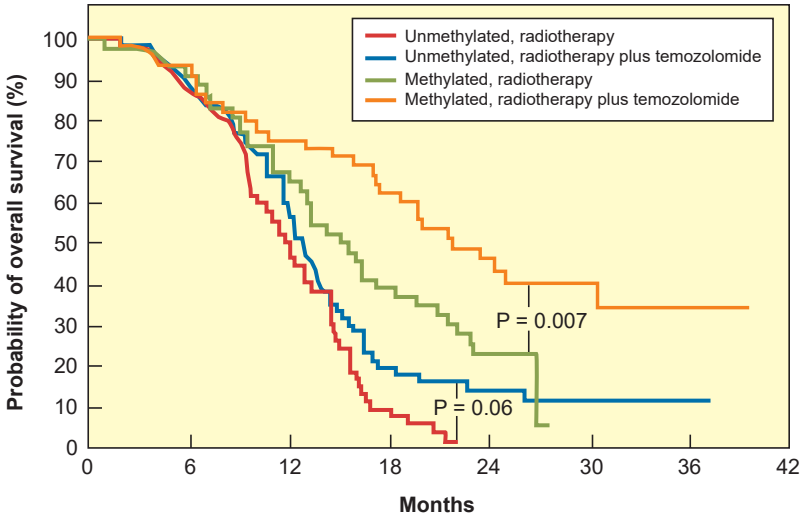

Number at risk							
Unmethylated, radiotherapy	54	47	25	5	0	0	0
Unmethylated, radiotherapy plus temozolomide	60	53	34	11	7	4	1
Methylated, radiotherapy	46	42	30	18	8	0	0
Methylated, radiotherapy plus temozolomide	46	42	34	28	16	7	1

Figure 97.6 Kaplan-Meir survival curves for the two arms of the international glioblastoma trial, demonstrating a significant survival benefit from chemoradiotherapy, compared with radiotherapy. The patients are evaluated by methylguanine DNA-methyltransferase (MGMT) gene promoter methylation status, and the maximum survival benefit is seen in the combination arm when the gene promoter is silenced. (Redrawn from Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005;352:997.)

selectively accumulates in tumor cells. It is thought to sensitize tumors through the production of reactive oxygen species that destabilize cellular metabolism. A phase II RTOG trial did not demonstrate superiority in survival.[189] Studies of radiation synergistic cytotoxics such as the camptothecins or platinum agents also did not demonstrate a survival benefit.[235] Radioimmunotherapy, using various monoclonal antibodies against EGFR or tenascin tagged with ^{125}I have been evaluated.[237,238] These were small studies and demonstrated feasibility; however, randomized controlled studies have not been performed.

Chemotherapy

In a landmark international trial, patients were randomized to RT with or without concurrent and adjuvant temozolomide. Median and 2-year survival were increased by 2.5 months and 16.1%, respectively, in patients receiving temozolomide, and long-term follow-up showed a persistent survival benefit.[190] A companion correlative study demonstrated that methylation of the promoter region of the *MGMT* gene in the tumor was associated with superior survival, regardless of treatment received, but the benefit was maximal for methylated patients.[157] MGMT removes the methyl group from the O6 position of guanine, reversing the cytotoxic effects of methylating agents (such as temozolomide), making the tumor resistant to treatment, while methylation of the promoter region of *MGMT* results in inactivation of *MGMT*. *MGMT* status was strongly associated with survival (Fig. 97.6). Recognizing that a different schedule of temozolomide may overcome chemotherapy resistance, there have been several studies of alternative dosing of temozolomide both at the time of recurrence and in the newly diagnosed setting.[191,192] A large phase III randomized international study led by the RTOG compared the standard treatment versus a 21- or 28-day adjuvant temozolomide schedule.[181]

Dose-dense temozolomide failed to result in improved efficacy regardless of tumor methylation status but was associated with more profound lymphopenia and fatigue. Strategies to increase the therapeutic ratio of existing chemotherapies, such as the inhibition of DNA repair enzymes (i.e., poly[ADP-ribose] polymerase [PARP]) are being evaluated. These agents are being combined with radiation and chemotherapy to increase the cytotoxicity of the combination approach.[193–195]

Although nitrosourea-based chemotherapy is modestly effective for patients with GBM, its use has been supplanted by temozolomide. There is evidence that carmustine-impregnated wafers implanted into the brain at the time of resection provide modest improvement in outcomes in selected patients compared with patients who received placebo wafers.[196]

Chemotherapy for Recurrent Glioblastoma

Treatment options for recurrent GBM must be tailored to the individual. Few agents have proven activity. A randomized phase II trial of temozolomide versus procarbazine in 225 patients with GBM at first relapse demonstrated that treatment with temozolomide improved median PFS (12.4 weeks versus 8.3 weeks; $p < 0.006$). Radiographic responses were disappointing (5.4% versus 5.3%). Several agents such as the platinoids, taxanes, 5-fluorouracil (5-FU), and irinotecan have been tested, most demonstrating very little activity. In a review of eight clinical trials with 225 recurrent malignant gliomas, the 6-month survival was 15% versus 31% for GBM versus anaplastic astrocytoma.[197]

Targeted Therapies

As the genetic and molecular pathogenesis of gliomas is better understood, new targets are being identified and inhibitors of associated signaling pathways are being developed. One example is EGFR as a frequently deregulated signaling molecule in GBM, prompting phase I and II trials of erlotinib and gefitinib for recurrent high-grade gliomas. Both have shown limited activity[97,198–200] Patients whose tumors demonstrate the variant 3 mutant (EGFRvIII), with resulting constitutive activation of EGFR tyrosine kinase activity, along with intact phosphatase and tensin analog (PTEN),

appear to be more responsive to EGFR inhibitors.[201] There are two reports of the combination of erlotinib with radiation and chemotherapy for newly diagnosed glioblastoma showing modest additional benefit to standard radiochemotherapy, none of which show convincing survival improvement.[202,203] Similarly, RTOG 0211, which evaluated the benefit of RT with concurrent gefitinib, showed median survival similar to that in a historical control cohort treated with radiation alone.[204] Irreversible EGFR inhibitors such as afatinib did not show efficacy when used alone or in combination with temozolomide in recurrent GBM.[205] There is an ongoing phase II study with the second-generation EGFR inhibitor, dacomitinib.

Preliminary reports using other targeted agents, including the mTOR inhibitor, temsirolimus, and the farnesyl transferase inhibitor, tipifarnib, have shown objective responses in a few high-grade gliomas.[206–210]

The most promising results have been seen for angiogenic inhibitors. The most important mediator of angiogenesis in GBM is vascular endothelial growth factor (VEGF). Antiangiogenic therapies such as the anti-VEGF monoclonal antibody bevacizumab have produced dramatic radiologic responses and prolonged PFS relative to historical controls.[211,212] Based on the results of a randomized phase II study of 167 patients who received bevacizumab with or without irinotecan, the FDA granted accelerated approval to bevacizumab for recurrent glioblastoma in 2009.[162] The PFS at 6 months was 43% for single-agent bevacizumab and 50% for the combination arm. The objective response rates were 28% and 38% for the two arms, and median survival times were 9.2 months and 8.7 months, respectively. The most common side effects associated with bevacizumab include fatigue, headache, and hypertension; proteinuria and poor wound healing are also seen. The addition of chemotherapy or targeted therapy to bevacizumab has failed to show any added benefit in recurrent GBM trials, with the exception of the BELOB study, a three-arm multicenter randomized phase II study, in which 148 patients with recurrent glioblastoma were treated with bevacizumab alone, lomustine alone, or the combination of the two. Survival at 9 months was 38%, 43%, and 59%, respectively, and the PFS at 6 months was 16%, 13%, and 41%, respectively, in the three arms.[213] The value of the combination of bevacizumab and lomustine in recurrent GBM is currently being evaluated in EORTC 26101, which was initially a randomized phase II study modified into a two-arm phase III trial to address this question.

There are several reports of small single-arm phase II studies of the combination of bevacizumab with radiation and temozolomide in the newly diagnosed setting.[214] Two large randomized trials evaluated the addition of bevacizumab to the initial combined modality therapy of RT and temozolomide. In RTOG 0825, a randomized, double-blinded, placebo-controlled trial, the addition of bevacizumab to temozolomide and radiation resulted in a longer PFS that did not reach the preset level of significance (10.7 months versus7.3 months; HR, 0.79). There was no difference in OS between two arms (16.1 versus15.7 months; HR, 1.13).[215] Another phase III, placebo-controlled randomized trial (AVAglio study) in newly diagnosed glioblastoma showed that the addition of bevacizumab to radiation and temozolomide (experimental arm) significantly prolonged PFS (HR, 0.64; $p < 0.0001$; median, 10.6 versus 6.2 months) as compared to radiation and temozolomide (control arm).[216] However, the median OS was not significantly different in both arms. One-year OS was 72% and 66%, respectively ($p = 0.049$); 2-year OS was 34% and 30%, respectively ($p = 0.24$) in the experimental and control arm. Safety was consistent with known bevacizumab side effects, serious adverse events (AEs) (grade ≥3) were 28.7% versus 15.2% grade in the two arms.

As previously discussed, patients with MGMT-nonmethylated GBM have inferior outcomes as compared to those with MGMT-methylated GBM, and temozolomide is less effective in these patients. The open-label GLARIUS trial was a randomized, multicenter study of 170 patients in which MGMT-nonmethylated GBM patients were randomized (2:1) to radiation with bevacizumab during RT followed by maintenance bevacizumab and irinotecan (experimental arm) compared to standard therapy with daily temozolomide during radiation followed by 6 cycles of temozolomide (control arm).[217] The PFS at 6 months rate of 71.1% was significantly higher in the experimental arm compared to 26.2% in the control arm ($p < 0.0001$ log-rank test).

Recognizing that tumors ultimately evade the effect of antiangiogenic agents through various mechanisms, other strategies include the evaluation of the combination of bevacizumab with chemotherapeutic and targeted agents, and the investigation of other VEGF-targeted agents. Batchelor et al.[218] reported a reduction in contrast enhancement and edema in 12 of 16 GBM patients who received cediranib (AZD2171), an orally administered pan-VEGF receptor inhibitor, with a median PFS of 3.7 months. However, a phase III randomized trial comparing the efficacy of cediranib failed to show any improvement in PFS with cediranib either as monotherapy or in combination with lomustine compared to lomustine alone in recurrent GBM.[219]

VEGF Trap (aflibercept), a recombinantly produced fusion protein that captures circulating VEGF and CT-322 (Angiocept, Adnexus Therapeutics, Waltham, MA), a pegylated recombinant peptide with a high affinity for VEGF was tested in the recurrent and in the newly diagnosed setting. The phase II study showed aflibercept had minimal evidence of single-agent activity in unselected patients with recurrent malignant glioma.[220]

Cilengitide (EMD121974), an integrin inhibitor that showed promise in the recurrent GBM,[221,222] was evaluated in two large, newly diagnosed studies. The first study, CENTRIC, a phase III trial investigated the role of cilengitide combined with the standard treatment for patients with newly diagnosed glioblastoma with *MGMT* promoter methylation.[223] The study failed to show any additional benefit with cilengitide in this patient population.[223] The other study, CORE, investigated the benefit of cilengitide in the unmethylated MGMT gene promoter in a multicenter, open-randomized phase II trial. There was suggestion of benefit of cilengitide with a median OS of 16.3 months in one of the cilengitide arms compared to the median OS of 13.4 months in the control group (HR: 0.69; $p = 0.033$). However, this drug is not being further developed.

The other antiangiogenic agents that have undergone investigation in recurrent glioblastoma include XL184, a multitargeted tyrosine kinase inhibitor that acts on the VEGFR, hepatocyte growth factor receptor (MET), and c-KIT; and enzastaurin, an inhibitor of protein kinase C-beta that targets VEGF as well as the mTOR pathway.[224] The initial results of these studies have shown similar or inferior outcomes to those reported with other agents.[224–226]

Other mechanisms of cell growth that are being targeted include epigenetic modulation through histone deacetylase inhibitors, the proteasome inhibitor bortezomib, and the glutamate receptor inhibitor talampanel.[227–229]

Gene Therapy Strategies

The efficacy and safety of a locally applied adenovirus-mediated gene therapy with a prodrug-converting enzyme (herpes simplex virus thymidine kinase; sitimagene ceradenovec) followed by intravenous ganciclovir was evaluated in 250 patients with newly diagnosed resectable glioblastoma. Temozolomide was not given in all patients. There was no evidence of a survival advantage to this approach.[230] Previous strategies have similarly been negative, and the challenges of adequate delivery of the virus and gene transduction into the tumor remain paramount.

Immunotherapies

Immunotherapeutic strategies targeting glioblastomas include recombinant immunotoxins, restoration of local and systemic immunosuppression, *one-size-fits-all*, and individualized autologous dendritic cell vaccines.[89,231–233]

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Issues in Study Designs for Novel Agents

Several key issues confront the incorporation of new agents in the up-front management of malignant gliomas. First, there is the issue of defining the appropriate end point. In recurrent malignant gliomas, PFS is frequently employed, but because of insufficient evidence linking this to survival in newly diagnosed malignant gliomas, survival remains the gold standard. However, there is considerable heterogeneity in survival outcomes based on clinical and possibly molecular prognostic variables. An adequate staging system has never been developed. The RTOG has analyzed an extensive database of prospectively treated patients (primarily with surgery, radiotherapy, and alkylating chemotherapy), and using a statistical method known as recursive portioning analysis, has developed six prognostic groups, referred to as RTOG recursive portioning analysis classes I to VI. Patients can be segmented into classes using eight variables: age, histology, Karnofsky performance score, mental status, neurologic function, symptom duration, extent of resection, and radiotherapy dose. GBM patients fall in classes III through VI, and their median survival ranges from 4.6 months to 17.9 months (Table 97.5).[234]

TABLE 97.5

Radiation Therapy Oncology Group Recursive Partioning Analysis (RPA) Classification: Survival by Class

RPA Class	Number of Patients	Median Survival (mos)	2-Year Survival (%)
I	139	58.6	76
II	34	37.4	68
III	175	17.9	35
IV	457	11.1	15
V	395	8.9	6
VI	263	4.6	4

GLIOMATOSIS CEREBRI

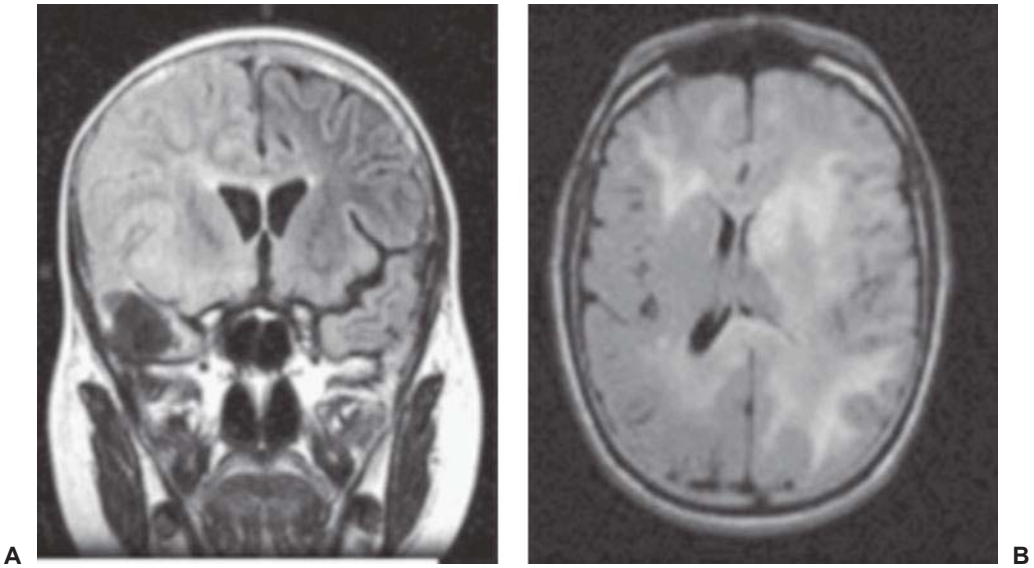
Gliomatosis cerebri is a rare condition with diffuse involvement of multiple parts of the brain (greater than two lobes). On MRI, there is typically diffuse increased signal on T2-weighted and FLAIR images and a low or absent signal in the affected areas on T1-weighted images (Fig. 97.7). Prognostic factors include age and histology as well as Karnofsky performance score. Treatment remains undefined and includes radiation and chemotherapy.[291–293]

OPTIC, CHIASMAL, AND HYPOTHALAMIC GLIOMAS

Clinical Considerations

Nearly all gliomas of the optic nerve and chiasm are discovered in patients younger than 20 and most occur in those under 10 years of age. Of patients with optic pathway glioma (OPG), 20% to 50% are affected by NF1.[235] Patients with NF1 are more likely to have lesions involving one or both optic nerves (anterior), whereas chiasmatic or hypothalamic (posterior) involvement is commonly seen among non-NF1 patients (sporadic). Lewis et al.[235] found that gliomas along the anterior visual pathway occurred in 15% of NF1 patients and were occasionally bilateral; 67% of these were neither suspected clinically nor obvious on ophthalmologic examination. In one series, 25% involved the chiasm alone, 33% the chiasm and hypothalamus, and 42% the chiasm and optic nerves or tracts.[236] Clinically, they cause loss of visual acuity (70%), strabismus and nystagmus (33%), visual field impairment (bitemporal hemianopsia, 8%), developmental delay, macrocephaly, ataxia, hemiparesis, proptosis, and precocious puberty. Funduscopic evaluation demonstrates a range of findings from normal optic discs, to venous engorgement, to disc pallor because of atrophy. Chiasmal tumors often grow to involve the hypothalamus, causing a diencephalic syndrome characterized by emaciation (especially in children between 3 months and 2 years of age), motor overactivity, and euphoria. In general, optic nerve gliomas have a better prognosis than those involving the chiasm, and tumors confined to the anterior chiasm have a better outcome than posterior chiasmal tumors.

The natural history of these tumors ranges from indolent growth or spontaneous regression (with NF1) to rapid progression or dissemination (with hypothalamic lesions).[237–239] Generally, the



A B

Figure 97.7 Two case examples of gliomatosis cerebri. Note the extensive changes visualized on fluid-attenuated inversion recovery (FLAIR) imaging, involving multiple lobes of the brain, and even an entire hemisphere.

prognosis of OPG is good with overall 5-year survival rates ranging between 70% and 90%; however, the long-term morbidity is high.[239–242] NF1 and age less than 5 years at diagnosis have better PFS.[239,243]

Pathologic Considerations

Histopathologically, a majority of these tumors are low-grade gliomas, typically pilocytic or fibrillary astrocytomas. They range from primarily piloid and stellate astrocytes (most common), with or without oligodendroglia, through the gamut of malignant astrocytomas to GBM (rarely). Typically, optic gliomas appear as fusiform expansions of any part of the nerve. They may bridge through the optic foramen and expand as dumbbell tumors. The nerve can be infiltrated by tumor originating in the chiasm, the walls of the third ventricle, or the hypothalamus. A subset of optic pathway tumors can show the more aggressive pathologic variant of *pilomyxoid astrocytoma* as compared to the better known pilocytic astrocytoma.[244]

Imaging Findings

Diagnosis is best made by MRI, which demonstrates enlargement of the affected optic pathway, often with enhancement. The T2 signal may extend posteriorly along the optic tracts as far as the visual cortex, which may represent tumor infiltration or edema. Cysts and calcification are uncommon, but the hypothalamic component can be cystic.

Treatment Decision Making

In general, children with asymptomatic lesions of the optic pathways found by MRI are not treated unless clinical or radiographic progression is documented. Tumors in children with NF1 tend to be more indolent than sporadic tumors. Only one-third to one-half of children with NF1 with asymptomatic optic pathway tumors found on screening MRIs require treatment for increasing visual symptoms.[245] Most children with sporadic tumors undergo imaging because of symptoms and should be treated. Sporadic tumors often present with advanced findings such as hydrocephalus, decreased visual acuity, and endocrinopathies.[246] Rarely, both sporadic and NF-associated optic pathway gliomas can regress spontaneously.[238]

Surgery

Surgery is only rarely indicated for optic pathway gliomas. In appropriate patients, surgery may decrease the recurrence rate and increase the time to recurrence. Patients treated with surgery, followed by radiation and chemotherapy, appear to have the highest long-term control.[247] In patients with progressive symptoms (e.g., severe visual loss and proptosis), unilateral anterior tumors that do not involve the optic chiasm may be resected. Biopsy or subtotal resection can be performed for posterior optic pathway gliomas that involve the hypothalamus and optic tract, particularly if they are symptomatic because of local compression and mass effect. Resection of the chiasm is not indicated due to resultant bilateral blindness.

If the tumor involves the chiasm and the MRI raises suspicion of another tumor type, such as an optic nerve sheath meningioma or another parasellar mass, a confirmatory biopsy can be performed. This is rarely needed in patients with NF1, in whom there is a high index of suspicion for an optic nerve glioma. A subtotal resection is indicated if mass effect produces dysfunction of adjacent structures such as the hypothalamus or the nerve itself. Hydrocephalus can be produced by more posteriorly situated tumors and may be alleviated by debulking. If hydrocephalus persists after debulking, CSF shunting (which may need to be biventricular or require fenestration of the septum) becomes necessary. For unknown reasons, hydrocephalus is often persistent after the CSF pathways have been cleared, requiring the insertion of a CSF diversion device even after tumor removal.

Radiation Therapy

Untreated optic gliomas, especially those involving the chiasm or extending into the hypothalamus or optic tracts, progress locally or are fatal in 75% of patients. Tenny et al.[248] found that only 21% of patients who were followed after biopsy or exploration survived compared with 64% of those who received RT.

Routine postoperative irradiation is not indicated for gliomas confined to the optic nerve, which can be completely resected.[249] RT can prevent tumor progression, improve disease-free survival, and stabilize or improve vision in patients with chiasmal lesions, for whom postoperative residual is the rule. Wong et al.[250] reported that 86% of chiasmal gliomas not treated with RT progressed locally, whereas treatment failure occurred in 45% that underwent RT. Furthermore, control was achieved in 87% of the irradiated patients who received a dose of 50 to 55 Gy compared to 55% of those who received 46 Gy or less.

The prognosis for patients with optic nerve tumors may be better than for those with chiasmal-hypothalamic lesions. In a literature review, local control was found to be achieved for 154 of 189 irradiated anterior chiasmal tumors (81%), whereas 92 of 142 posterior tumors (65%) were controlled. Vision improved in 61 of 210 evaluable patients (29%) and remained stable in 118 of 210 patients (56%).[251] For chiasmal-hypothalamic tumors, RT produced radiographic shrinkage in 11 of 24 (46%) with a median PFS of 70 months compared with 30 months for patients who did not receive RT.[252] Age and tumor location were important prognostic factors, with younger children (less than 3 years), and children with lesions posterior to the chiasm faring less well after radiotherapy.

Three-dimensional conformal radiotherapy, IMRT, and stereotactic techniques are used to minimize the dose to adjacent structures. A report by Debus et al.[253] summarized results in patients treated with fractionated stereotactic radiation therapy (FSRT) (52.2 Gy median dose at 1.8 Gy per day).[253] All patients remained disease free, and no significant complications or marginal failures were seen despite highly conformal radiation fields. Because these tumors are often focal, techniques like FSRT can offer both excellent local control and decreased late effects.

Chemotherapy

In recent years, chemotherapy has played a pivotal role in the management of OPG in young children in order to spare the developing brain from the adverse effects of irradiation.[241,254–257] This is especially important in patients with NF1 who are at significant risk of developing vasculopathy such as moyamoya syndrome and secondary malignancy after receiving radiotherapy.[256,258] Retrospective series suggest that cognitive function is preserved better in children who receive initial chemotherapy compared with RT.[241,259] Although the appropriate agents are still evolving, vincristine plus carboplatin remains the most common first-line regimen.[128] Gnekow et al.[260] reported a 5-year PFS of 73% in 55 patients who were treated with this regimen. The randomized Children's Oncology Group A9952 study showed a 5-year PFS of 35% using carboplatin with vincristine and 48% using thioguanine, procarbazine, lomustine, and vincristine regimen in children with newly diagnosed progressive low-grade glioma.[261] Cisplatin-based regimens have shown responses between 50% and 60% and 5-year PFS of 50%.[262–264] Other studies have shown temozolomide to be effective.[265–267] Vinblastine has also been active in these tumors and is generally a second-line agent.[131,132] Collectively, these data suggest that chemotherapy is helpful in delaying tumor progression in a significant portion of children.

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Whether chemotherapy alone can improve vision is controversial. Most studies in the literature lack objective data on visual outcome prior to and after chemotherapy. Moreno et al.[268] conducted a systematic review of eight reports and found only 14.4% of the children treated with chemotherapy had improvement in their vision. Due to the risk of second malignancy, alkylator-based chemotherapies are generally avoided in patients with NF1.

BRAINSTEM GLIOMAS

Clinical and Pathologic Considerations

Brainstem gliomas account for 15% of all pediatric brain tumors but are rare in adults. They can be divided into several distinct types. The diffuse intrinsic pontine gliomas (DIPG) tumors are generally high-grade astrocytomas, either anaplastic astrocytomas or GBM. Completely separate, and clinically distinct are the focal, dorsally exophytic or cervicomedullary lesions that are usually low grade with a better prognosis. Although rare, ependymomas, PNETs, and atypical teratoid-rhabdoid tumors also occur in the brainstem. Nonneoplastic processes that may be confused with a brainstem tumor include neurofibromatosis, demyelinating diseases, arteriovenous malformations, abscess, and encephalitis.

The diagnosis of a DIPG is usually based on a short history of rapidly developing neurologic findings of multiple cranial nerve palsies (most commonly VI and VII), hemiparesis, and ataxia. The initial manifestations of a brainstem glioma are unilateral palsies of cranial nerves VI and VII in approximately 90% of patients. The classic MRI finding is diffuse enlargement of the pons with poorly marginated T2 signal involving 50% or greater of the pons (Fig. 97.8).[269] Most are nonenhancing; in children, enhancing lesions could have either a pilocytic or malignant component; in adults, enhancement is worrisome for a malignant glioma.[270] Cervicomedullary tumors are nonenhancing, well-circumscribed lesions with an exophytic component. Tectal gliomas are nonenhancing and enlarge the tectal plate, often expanding it into the supracerebellar cistern with associated hydrocephalus. Overall, the prognosis is poor for patients with DIPG, with few patients surviving longer than 1 year.[271]

Surgery

Complete resection is almost never possible for the majority if brainstem tumors, and even a biopsy is restricted because of substantial morbidity and mortality.[272,273] In most centers, biopsy of DIPG is not undertaken for very typical cases. Stereotactic needle biopsy can be performed if atypical imaging findings or clinical characteristics suggest another diffuse brainstem disorder. Resection has no place in the treatment of diffuse pontine gliomas in children or adults. For the rare focal astrocytic lesions of the adult or pediatric brainstem, surgery may play a larger role. Tectal gliomas have a typical imaging appearance, and biopsy is neither necessary nor safe. However, the accompanying noncommunicating hydrocephalus (from compression of the aqueduct of Sylvius) can be treated with CSF diversion, either by third ventriculocisternostomy or by ventriculoperitoneal shunting.[274] Dorsally exophytic astrocytomas within the fourth ventricle or at the cervicomedullary junction are often partially resectable with low morbidity and excellent long-term results.[275] These dorsally exophytic brainstem tumors arise from the substance of the pons and are very dangerous to resect in totality. Because many of them will remain indolent after partial resection, complete removal at the first surgery is likely not warranted and associated with severe neurologic deficits. Intrinsic astrocytomas or ependymomas at the cervicomedullary junction can often be completely removed through a posterior midline approach.[276] In a retrospective review of 28 patients with juvenile pilocytic astrocytoma of the brainstem treated with resection in 25 cases and biopsy in 3,[277] the 5- and 10-year PFS rates were 74% and 62%, respectively, after gross total resection or resection with linear enhancement and 19% and 19%, respectively, when solid residual tumor was present, suggesting that long-term survival after resection of these tumors may relate to the extent of initial excision.

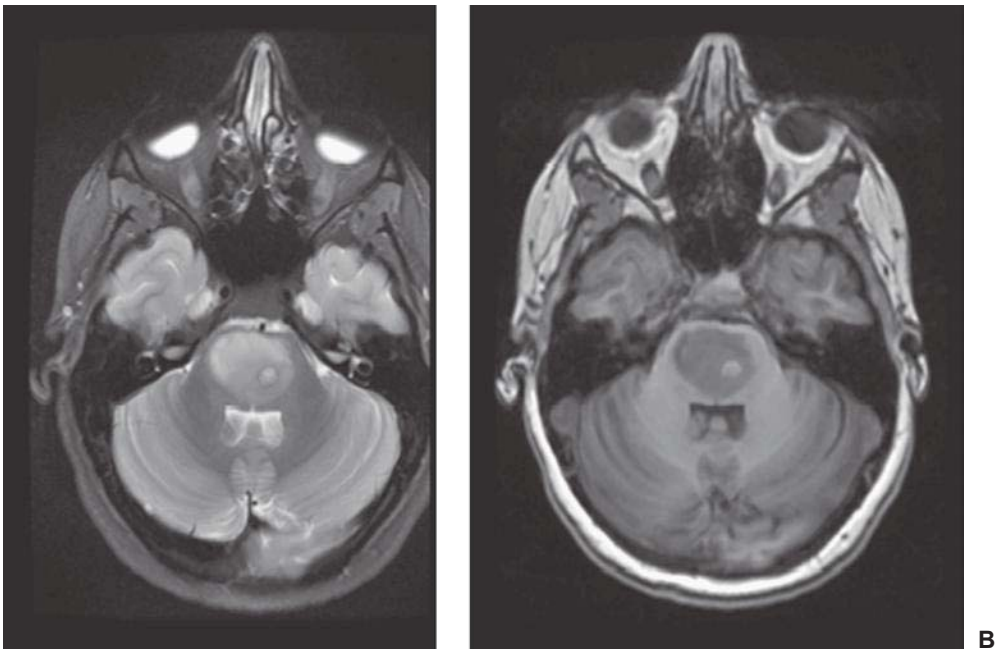

Figure 97.8 Typical magnetic resonance appearance of a diffuse pontine glioma. Diffuse enlargement of the pons is visualized on the T2-weighted image **(A)**; a small amount of hemorrhage is visualized on the noncontrast T1-weighted image **(B)**.

Radiation Therapy

RT, the primary treatment for brainstem tumors, improves survival and can stabilize or reverse neurologic dysfunction in 75% to 90% of patients. The GTV is usually best defined using T2-weighted or FLAIR MRI. A margin of 1.0 to 1.5 cm is added to create a CTV. These lesions should be treated to 54 to 60 Gy using daily fractions of 1.8 to 2.0 Gy. In a multi-institutional survey by Freeman and Suissa,[278] the 1-, 2-, and 5-year survival rates of children treated with conventional RT techniques were 50%, 29%, and 23%, respectively. Hyperfractionation, designed to deliver higher tumor doses, has been evaluated, without a significant survival advantage (median survival, 8.5 months versus 8.0 months for conventional versus hyperfractionated regimens).[279] Several drugs, such as topotecan, and motexafin-gadolinium have been investigated as radiosensitizers, without clear evidence of benefit, and therefore, the role of sensitizers remains investigational.

Fewer data exist with respect to brainstem glioma in adults, but there is some evidence that these tumors may be less aggressive in adults, with OS that ranges from 45% to 66% at 2 to 5 years, perhaps because of a greater frequency of more favorable tumor types.[280] In the series from ANOCEF, 48 adult patients with brainstem gliomas were grouped on the basis of their clinical, radiologic, and histologic features.[270,281] Nearly half had nonenhancing, diffusely infiltrative tumors and had symptoms that were present for longer than 3 months. Eleven of these 22 patients underwent biopsy, and 9 had low-grade histology. Nearly all underwent radiotherapy and had a median survival of 7.3 years. A second group of 15 patients who had presented with rapid progression of symptoms and had contrast enhancement on MRI were described. Fourteen of these patients underwent biopsy, and anaplasia was identified in all 14 specimens. Despite radiotherapy, the median survival in this group was 11.2 months, which approximates the survival in pediatric series.

Chemotherapy

Despite numerous clinical trials, there is no clear evidence to show increased survival for patients with DIPG who receive radiation and chemotherapy as compared to radiation alone. The recent discovery that the majority of DIPGs harbor mutations of lysine 27 (K27) in the histone 3.3 gene (so called K27M mutations) may allow for the development of targeted therapies.[282,283] Similarly, a subset of DIPGs have amplifications of receptor tyrosine kinase family members (i.e., PDGFR-α) suggesting other avenues to targeted therapy.[284] Clinical trials using temozolomide during and after RT have not shown improvement in the outcome.[285–287] Thus, no agent used either during or after radiation treatment has been shown to have benefit over radiation alone.

CEREBELLAR ASTROCYTOMAS

Clinical and Pathologic Considerations

Cerebellar astrocytomas, which occur most often during the first 2 decades of life, arise in the vermis or more laterally in a cerebellar hemisphere. They are usually well circumscribed and can be cystic, solid, or some combination of both. It is not uncommon to have a small tumor (mural nodule) associated with a large cystic cavity.

Histologically, most are low-grade pilocytic astrocytomas that lack anaplastic features. In a series of 451 children, cerebellar astrocytomas accounted for 25% of all posterior fossa tumors, and 89% of the 111 cerebellar astrocytomas were low grade.[288] Approximately 75% of these tumors are located only in the cerebellum, with the remainder involving the brainstem as well. Because these tumors usually arise in the vermis or median cerebellar hemisphere, the clinical presentation is similar to that of medulloblastoma, with truncal ataxia, headache, nausea, and vomiting. In infants, head enlargement from hydrocephalus is seen. The majority of cerebellar pilocytic astrocytomas have an oncogenic fusion gene (KIAA1549-BRAF) that results in the activation of the BRAF oncogene, and which might be a rational target for future therapy.[289,290]

Surgery

Gross total resection is tantamount to a cure for these lesions.[291,292] In most cases, incomplete removal should be managed by conservative monitoring because the majority of remnants will not grow, will remain low grade, and are easy to remove if they progress in the future.

Radiation Therapy

Nearly all completely resected cerebellar astrocytomas do not require RT. Even when they progress, repeat resection is reasonable if a majority of the tumor can be removed. Only in the most exceptional circumstances would RT be necessary for the treatment of child with a true cerebellar pilocytic astrocytoma.

Chemotherapy

In general, chemotherapy is not indicated. Based on the experience with optic pathway gliomas, several of which have pilocytic features, carboplatin has been used for recurrent tumors.[293,294] There is limited experience with the use of temozolomide in this setting. High-grade gliomas that arise in the cerebellum are treated with regimens identical to their supratentorial counterparts.

GANGLIOGLIOMAS

Clinical and Pathologic Considerations

Gangliogliomas, along with pilocytic astrocytomas, pleomorphic xanthoastrocytomas, and subependymal giant cell astrocytomas, are considered *astroglial variant* forms of low-grade gliomas.[295] They are more circumscribed than diffuse low-grade gliomas, are classified as grade 1 or 2, and do not typically invade the normal brain. Because they less frequently progress to higher grade lesions, surgery alone is often curative. Gangliogliomas are more common in children than adults. They are the most common neoplasms to cause chronic focal epileptic disorders, and they typically arise in the temporal lobe but may also occur in the brainstem, spinal cord, and diencephalon.[296] They may include a cystic component, and the solid portion is free of normal brain parenchyma. Unlike diffuse low-grade gliomas, gangliogliomas enhance on MRI scans. They contain both glial and neuronal elements. The glial elements, which stain for glial fibrillary acidic protein, are almost always astrocytic and often pilocytic, but fibrillary astrocytes are also common. The glial elements dictate whether the lesion is grade 1 or 2. The neurons in the tumor are neoplastic and are characteristically large and relatively mature (i.e., they contain ganglion cells). The presence of neoplastic neurons may be confirmed by immunostaining for neuron-specific enolase and synaptophysin. Grade 2 lesions have rarely been observed to progress to a higher grade.[297,298]

Surgery

Surgical resection is directed at removal of the contrast-enhancing portion of the tumor. Nevertheless, although lesions located within eloquent brain regions are resectable, they may present significant surgical challenges because the boundary between tumor and functional brain may be difficult to define, even with the aid of

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modern surgical adjuncts (e.g., operating microscope, computer-assisted navigation, functional brain mapping). Although no phase 3 prospective studies have documented the superiority of surgery over other approaches (e.g., radiotherapy), retrospective studies have indicated that complete resection is associated with a very favorable long-term survival.[297–299] Resection of gangliogliomas also can result in seizure control.[300] Grade 2 gangliogliomas may recur, and some patients do poorly. The degree of anaplasia determines the prognosis.

Radiation Therapy

Because resection has the potential to cure most of these lesions, radiotherapy is generally reserved for subtotally resected cases or for recurrences.[301] It is also used for lesions in complex locations where further resection may result in significant morbidity. To determine the optimal strategy for gangliogliomas, Rades et al.[302] conducted a literature-based retrospective study of more than 400 patients treated for ganglioglioma. They examined four different treatment strategies (GTR or subtotal resection [STR] with or without radiotherapy) in 402 patients identified from reports published between 1978 and 2007. Surgery was found to be the mainstay of therapy, with 209 patients undergoing GTR and 193 undergoing STR. Adjuvant radiotherapy was used in 101 patients (20 following GTR and 81 following STR). Patients who underwent GTR had higher rates of OS and PFS than individuals who underwent STR. For patients undergoing GTR, the 10-year rates of local control and OS were 89% and 95%, respectively, better than the 52% and 62% observed for patients undergoing STR. This indirectly indicates that GTR is the most effective treatment strategy for gangliogliomas. For patients undergoing STR followed by postoperative radiotherapy, the 10-year rate of local control was 62%, better than the 52% for patients undergoing STR without postoperative radiotherapy; although the 10-year survival also improved from 65% to 74% with the use of postoperative radiotherapy in patients with subtotally resected tumors, this did not reach statistical significance. For the 40 patients undergoing STR for whom radiotherapy details were known, local recurrence was observed in 6 of 22 (27%) receiving 54 Gy, compared to 7 of 18 (39%) receiving greater than 54 Gy, implying no specific dose–response relationship.

Chemotherapy

Chemotherapy for gangliogliomas is generally reserved for young children who have undergone subtotal resection and who demonstrate disease progression. In older patients, it is typically used as salvage therapy to treat recurrent tumors after the failure of surgery and RT. In general, for astroglial variants such as gangliogliomas, no optimal chemotherapeutic regimens have been defined, and most researchers consider disease stabilization (rather than a complete tumor response) to be a successful outcome.

EPENDYMOMA

Clinical and Pathologic Features

Ependymomas were originally thought to arise from the ependymal cells lining the cerebral ventricles and the vestigial central canal of the spinal cord as they resemble this tissue under the microscope, although more recently it has been accepted that they arise from radial glial cells, a type of CNS stem cell.[303,304] Ependymomas can arise throughout the nervous system, and are usually divided into those from the supratentorial, infratentorial (posterior fossa), and spinal regions. Those in the spinal region are broken down into the intramedullary lesions and the myxopapillary ependymomas of the conus medullaris and cauda equine. Although these tumors look very similar under the microscope (histology), they are demographically, clinically, transcriptionally, and genetically distinct and should not be regarded as the same entity.[304,305] More recently, it has been shown that there are two clear groups of posterior fossa ependymoma, the posterior fossa type A (PFA) tumors and the posterior fossa type B (PFB) tumors.[306,307] PFA tumors occur in young children (infants), are more likely to be lateral (CP angle), and have a terrible prognosis. PFB tumors are diagnosed in older children, found in the midline, and have a much better prognosis than PFA tumors. Figure 97.9 shows the typical magnetic resonance appearance of a midline, the posterior fossa ependymoma. In the past, much was made of the pattern of anaplasia in ependymoma histology, with the diagnosis of anaplasia taking an ependymoma from WHO grade II to WHO grade III. More recently, a number of luminaries in the field of neuro-oncology have shown that the intra- and interobserver reliability in the diagnosis of anaplasia in ependymoma is very high and its clinical utility is, therefore, very limited.[308]

Clinical presentation depends on location. Tumors with ventricular involvement often cause increased ICP and hydrocephalus by obstruction of CSF pathways. Headaches, nausea and vomiting, papilledema, ataxia, and vertigo are frequent. Focal neurologic signs and symptoms are seen with supratentorial ependymomas that involve the parenchyma. The presence of calcification in a fourth ventricular tumor on CT is very suggestive of an ependymoma. Supratentorial parenchymal tumors cannot be readily distinguished from other gliomas by imaging. Posterior fossa tumors in infants that protrude below the foramen magnum are more likely to be ependymomas, as are posterior fossa tumors that cause a head tilt (due to compression of the XIth cranial nerve as it crosses the foramen magnum).

Metastatic dissemination of ependymomas occurs in the leptomeningeal space in a similar pattern to that seen in medulloblastomas, albeit at a much lower rate (<5% of patients at presentation).

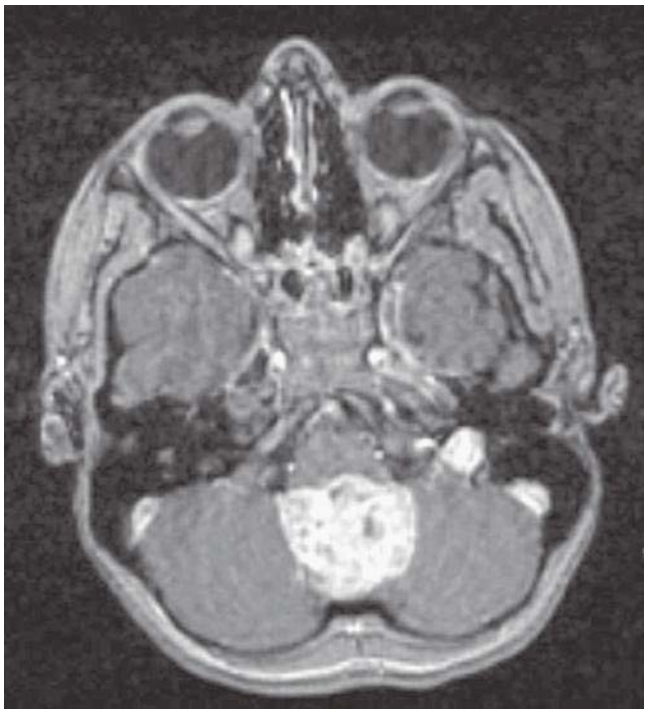

Figure 97.9 Typical magnetic resonance appearance of a posterior fossa ependymoma. The tumor arises from the floor of the fourth ventricle and rapidly expands to occupy it, and compresses the pons/medulla ventrally and the vermis of the cerebellum dorsally. The enhancement is typically heterogenous.

This low rate of observable dissemination at diagnosis has lead to the almost universal use of local, rather than craniospinal radiotherapy at diagnosis for patients with ependymoma.

Subependymomas are benign tumors with an admixture of fibrillary subependymal astrocytes. They are distinct from subependymal giant cell astrocytomas, which occur in the lateral ventricles in tuberous sclerosis. Subependymomas occur most often in the floor or walls of the fourth ventricle in older men. Most are asymptomatic and slow growing, and treatment is rarely needed except for hydrocephalus or demonstrated growth. They are often incidentally found at autopsy.

Surgery

Several retrospective studies support the relationship between postsurgical residual ependymoma and a poorer outcome, and therefore, maximal safe resection is the goal.[309–311] These tumors may also extend through the foramen of Luschka, entangling the cranial nerves in the basal cisterns, which also precludes a complete resection. The less common supratentorial tumors are removed as with any glioma. Avoidance of bleeding into the ventricular system is important to prevent postoperative hydrocephalus.

Radiation Therapy

Postoperative irradiation improves the recurrence-free survival of patients with intracranial ependymomas, and 5-year survival rates with doses of 45 Gy or more range from 40% to 87%.[152] Therapeutic utility of local radiation is established for ependymoma patients, even in infants.[312] Because local failure usually dominates the recurrence patterns, low-grade supratentorial ependymomas are typically treated using partial brain fields with a dose of approximately 54 Gy. Low-grade infratentorial ependymomas are also treated using limited fields. The best survival results in retrospective series have been shown for patients who undergo gross total resection followed by radiotherapy.[374,375] For most patients, a more usual volume consists of the tumor bed and any residual disease plus an anatomically defined margin of 1 to 1.5 cm to create a CTV. Larger margins may be required in areas of infiltration, and special attention must be paid to areas of spread along the cervical spine because 10% to 30% of fourth ventricular tumors extend down through the foramen magnum to the upper cervical spine.[313,314] Patients with neuraxis spread (positive MRI or positive CSF cytology) should receive craniospinal irradiation (40 to 45 Gy) with boosts to the areas of gross disease and to the primary tumor to total doses of 50 to 54 Gy.

Chemotherapy

There is no evidence that any type of chemotherapy improves survival in children with ependymomas.[315] Single-agent carboplatin, cisplatin, and etoposide, as well as multiagent chemotherapy, have been evaluated in small series and, to date, few if any drugs have shown even modest consistent activity in ependymomas.[377–380]

Although a complete removal of the ependymoma has a positive impact on the outcome, a complete resection is achieved in only 40% to 60% of cases.[316,317] Therefore, responsiveness to preirradiation chemotherapy was investigated in a Children's Oncology Group (COG) study where an objective response rate of 58% to preirradiation chemotherapy, consisting of cisplatin, etoposide, cyclophosphamide, and vincristine, in children with incompletely resected ependymoma was seen.[383] The 3-year event-free survival in patients assigned to preirradiation chemotherapy because of incomplete resection was 58% and was comparable to those who had a complete resection and were assigned to irradiation alone. However, 15% of the children who received preirradiation chemotherapy experienced progression prior to RT. Therefore, a subsequent COG study was carried out that aimed to decrease the progression rate prior to radiotherapy by employing a strategy of *second-look* surgery following the preirradiation chemotherapy in children with residual disease. In this study, patients who had a complete resection of a differentiated supratentorial ependymoma were observed without any further therapy. The results of this study are pending. The recently opened randomized COG study is exploring whether maintenance chemotherapy following radiation will improve event-free survival and OS.

The primary application of chemotherapy, therefore, is investigational, and it is within the realm of neoadjuvant therapy to improve resectability, as primary adjuvant therapy in young children to delay radiotherapy and as possible salvage. In the Baby Pediatric Oncology Group study, a 48% response rate was reported to two cycles of vincristine and cyclophosphamide in 25 children younger than 3 years of age with ependymoma, allowing a delay in radiotherapy by 1 year without impacting the outcome.[318] However, the use of chemotherapy to delay radiotherapy has to be approached cautiously. In a trial of 34 patients with anaplastic ependymoma, 25 patients relapsed relatively rapidly and only 3 patients who did not receive radiotherapy survived.[319]

Despite multimodal therapy, 50% of the patients with ependymoma will experience a relapse. The majority of the recurrences are local, and prognosis is poor after relapse.[320] Resection, reirradiation, and chemotherapy are the common treatment modalities for relapsed ependymoma. Various antineoplastic agents such as etoposide, cyclophosphamide, temozolomide, cisplatin, and irinotecan have failed to improve survival in these patients.[287,321,322] Novel therapies to target molecular pathways are currently under investigation.

MENINGIOMAS

Clinical and Pathologic Considerations

Meningiomas are believed to arise from epithelioid cells on the outer surface of arachnoid villi in the meninges, also known as *arachnoidal cap cells*. The most frequent locations are along the sagittal sinus and over the cerebral convexity (Fig. 97.10). Meningiomas are extra-axial, intracranial, and sometimes intradural—extramedullary spinal tumors that produce symptoms and signs through the compression of adjacent brain tissue and cranial nerves. They often also produce hyperostosis; bony invasion does not indicate malignancy. They rarely metastasize except after multiple resections when they may spread to the lung, where growth is typically slow.

The WHO categorizes this tumor into three grades.[4] Benign (WHO grade I) meningiomas comprise about 70% to 85% of intracranial primaries. With appropriate treatment, approximately 80% of WHO grade I meningiomas remain progression free at 10 or more years.[323] Atypical (WHO grade II) meningiomas account for 15% to 25% of patients. These have greater proliferative capacity, and a seven- to eightfold increased recurrence risk within 5 years.[324] Only about 35% patients with WHO grade II meningiomas remain disease free at 10 years. About 1% to 3% of intracranial meningiomas are anaplastic (WHO grade III). These aggressive malignant tumors have a median OS of less than 2 years.[325]

Surgery

The goal is total resection, including a dural margin, because this is often curative for WHO grade I tumors. The risks of resection must be balanced against the advantages of less-aggressive removal because these tumors are typically slow growing, and the patients are sometimes elderly. Observation is appropriate for some, especially small tumors that are incidentally discovered. In a series of 603 patients who had asymptomatic meningiomas that were

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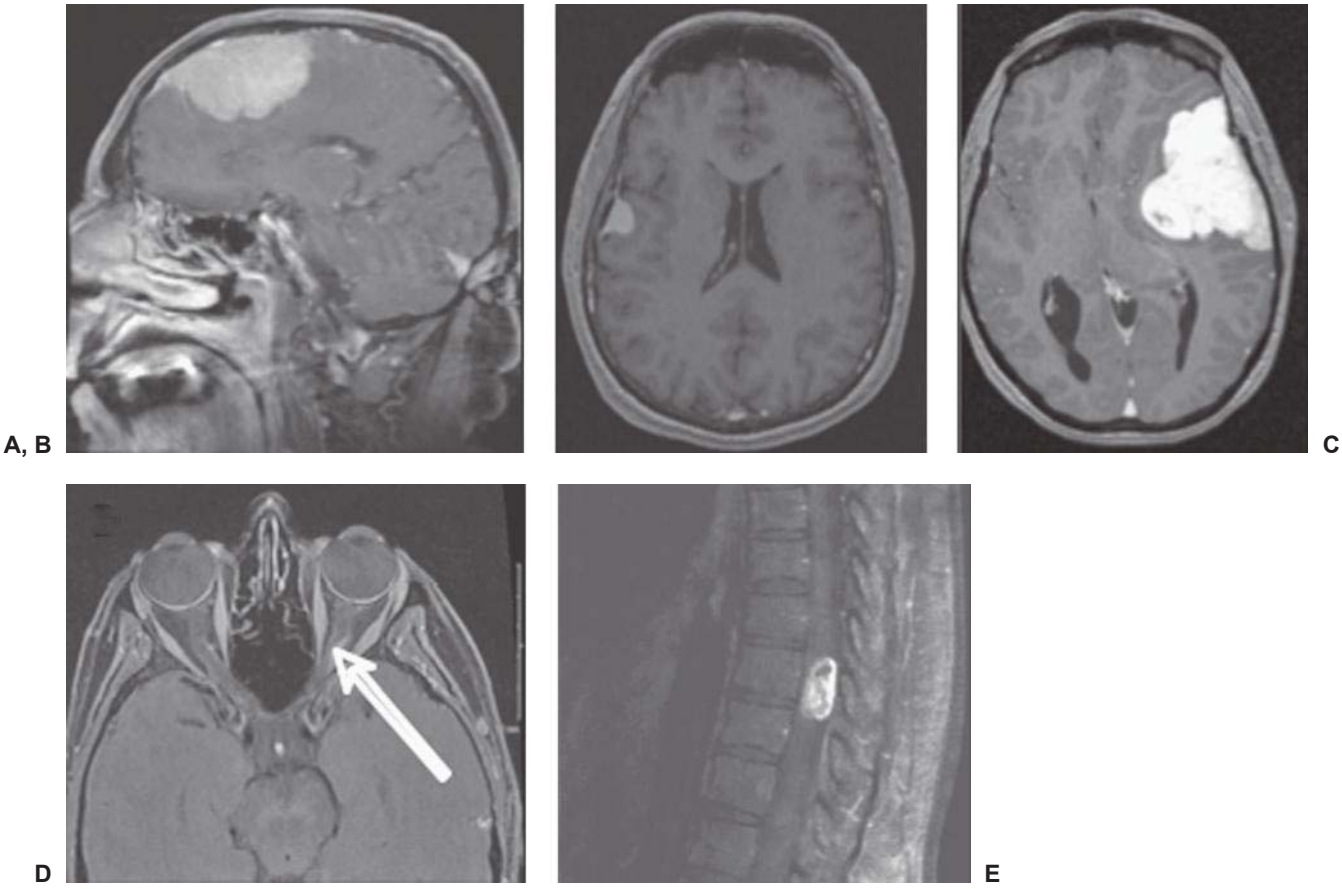

Figure 97.10 These five images show various appearances of meningioma. The most common location is parasagittal **(A)**. Some meningiomas remain small **(B)**, whereas others achieve a massive size with midline shift **(C)**. An optic nerve meningioma (*arrow*) is illustrated in **(D)**, whereas spinal locations are also possible **(E)**.

treated conservatively, Yano and Kuratsu[325] found that approximately 63% exhibited no growth, and only 6% ultimately experienced symptoms. However, a subsequent study of 244 patients with 273 meningiomas indicated that T2 hyperintensity, lack of calcification, size greater than 25 mm, and edema are associated with a shorter time to progression, and those tumors should be followed more closely.[326]

Preoperative Planning

Meningioma surgery requires a detailed knowledge of surgical anatomy. A preoperative angiogram to assess vascularity and to identify or embolize surgically inaccessible feeding arteries is sometimes indicated. Typically, embolization is done within 24 to 96 hours of surgery so that collateral vascular supply to the tumor does not develop. Normally, only the vascular supply from the external carotid artery can be embolized safely. In meningiomas that receive more than 50% of their blood supply from this artery, Kai et al.[327] found the optimum interval between embolization and surgery to be 7 to 9 days, which allowed the greatest degree of tumor softening. For convexity and parafalcine tumors, preoperative imaging may be performed to allow for the use of a neuronavigation system to aid in planning the scalp incision and bony opening.

Simpson Grades of Resection

The completeness of surgical removal is a crucial prognostic factor, and historically, the definitions provided by Donald Simpson have served as a useful guideline.[328] By following 470 patients during a 26-year span, he described five *grades of resection* based on recurrence. A grade 5 resection refers to a biopsy only and is associated with near-universal progression. A partial tumor resection is labeled Simpson grade 4 and is associated with a recurrence rate of 44%. A Simpson grade 3 resection refers to gross total resection of the tumor, without addressing hyperostotic bone or dural attachments, and is associated with a 29% rate of relapse. A Simpson grade 2 resection includes gross tumor removal, and the dural attachments are either removed or coagulated and the relapse rate drops to 19%; and finally, when hyperostotic bone is also removed for a Simpson grade 1 resection, the relapse rate is 9%.

This definition has subsequently been expanded to include a category referred to as grade 0 resection. Kinjo et al.[329] reported on 37 convexity meningioma patients who underwent gross total resection of the tumor, any hyperostotic bone, and all involved dura with a 2-cm dural margin, and observed no local recurrences, with over half of the patients followed beyond 5 years; this is now widely termed the grade 0 resection. However, apart from convexity primaries, resection to this extent is usually not feasible in other locations.

The likelihood of gross total resection varies considerably among primary sites, with convexity lesions most amenable to complete resection and skull-base lesions least likely to be completely resected. In most surgical series, at least a third of meningiomas reported are not fully resectable.[330]

Recurrence Following Resection

Gross total resection for benign meningiomas remains the preferred treatment and is generally considered definitive. Three large series with extended follow-up are available (Table 97.6). These have remarkably similar rates of local recurrence after gross-total

TABLE 97.6
Recurrence After Gross Total Resection Alone of Meningioma

		Local Recurrence Rate (%)		
Study (Ref.)	**Number of Patients**	**5-Y**	**10-Y**	**15-Y**
Mirimanoff et al.[330]	145	7	20	32
Stafford et al.[333]	465	12	25	—
Condra et al.[331]	175	7	20	24
Total	785	7–12	20–25	24–32

resection: 7% to 12% at 5 years, 20% to 25% at 10 years, and 24% to 32% at 15 years.[330–333]

As expected, recurrence following subtotal resection is more frequent. Outcomes following subtotal resection alone, from four single institutions with up to 20 years of follow-up, are available. Collectively, the rates of progression following subtotal resection at 5, 10, and 15 years are 37% to 47%, 55% to 63%, and 74%, respectively.[330–333]

Radiation Therapy

Given the long natural history of meningiomas and the relatively late recurrences, radiotherapy has not been routinely adopted in the adjuvant context. Further, there is a paucity of clinical trials on which to base recommendations. However, in almost every retrospective series, cohort comparisons suggest that radiotherapy leads to a decrease in recurrence, and some suggest possible survival improvement.

The need for adjunctive RT is determined by the extent of resection, tumor grade, patient age, and performance status. The risk of recurrence following resection has been outlined previously. In general, it is common practice to not use adjunctive radiotherapy after Simpson grade 0, 1, 2, and sometimes 3 resection for grade 1 meningioma.

The risk of relapse after subtotal resection is high.[330–333] Several reports, now numbering over 60, suggest that postoperative irradiation prolongs the time to recurrence. As an illustrative series, Goldsmith et al.[334] reported the results for 140 patients (117 with benign and 23 with malignant tumors) treated with subtotal resection and postoperative irradiation. For patients with benign meningiomas, the 5- and 10-year PFS rates were 89% and 77%, respectively. Patients who received at least 52 Gy had a 20-year PFS rate of greater than 90%. The 5-year PFS of patients treated after 1980 was 98%, compared to 77% for those treated prior to 1980. This improvement was attributed to the availability of cross-sectional imaging for tumor localization and 3-D treatment planning. The size of the residual tumor as well as grade can affect the outcome after radiotherapy. Connell et al.[335] showed that for tumors 5 cm or larger, the 5-year PFS rate was 40%, significantly lower than the 93% observed for smaller tumors. Among patients irradiated for unresectable tumors and in those with residual disease, the volume of visible tumor on imaging studies rarely decreases by more than 15% and often only after many years.

Radiation Therapy for Anaplastic and Malignant Meningioma

Atypical and malignant meningiomas behave more aggressively. Goldsmith et al.[334] reported a 5-year PFS of 48% for 23 patients treated by subtotal resection and irradiation. The recurrence rate among 53 patients with malignant meningiomas collected from six series in the literature was 49%. The recurrence rates were 33% for patients treated with complete resection alone, 12% for those undergoing complete resection and RT, 55% for patients treated by subtotal resection and irradiation, and 100% for those treated by subtotal resection alone.[336] However, other studies in the literature do not categorically support a reduction in local failure with the use of adjuvant radiotherapy for gross totally resected G2 meningioma, and given that the patients referred for this therapy are often selected because of a variety of one or more negative prognostic factors, compared to the cohort of patients that is observed, conclusions regarding the precise value of postoperative radiotherapy for G2 totally resected meningioma remain nonuniform. However, most data suggest that all patients with malignant G3 meningiomas, regardless of the extent of resection, and those with subtotally resected G2 meningioma should be offered postoperative irradiation.[337,338]

Primary Radiotherapy

RT has been used as primary treatment following biopsy or on the basis of imaging findings alone in several small series. An early report from the Royal Marsden Hospital found 47% disease-free survivorship at 15 years in 32 patients.[339] In a recent series, Debus et al.[340] noted no recurrences in patients treated by radiotherapy alone (n = 59).

Optic nerve sheath meningiomas are rare tumors, generally not resected, but treated with radiotherapy as primary management. Narayan et al.[341] found no radiographic progression in any of 14 optic nerve sheath meningioma patients treated with conformal radiotherapy, with more than 5 years of median follow-up. In a study by Turbin et al.,[342] RT alone provided more favorable outcome than observation or surgery alone.

Radiation Dose and Volume Considerations

For benign meningiomas, the planning target volume consists of the residual tumor with a modest margin of normal tissue, defined by MRI and modified by the neurosurgeon's description of the site of residual disease. Extensive tumors of the base of the skull and malignant meningiomas require more generous margins, with special attention to dural extensions toward and through the skull foramina. The preoperative tumor volume is used for planning for completely resected malignant lesions. A dose of 54 Gy in daily fractions of 1.8 Gy is recommended for benign meningiomas, and 60 Gy or higher for atypical and malignant tumors. Complex 3D-CRT treatment planning and delivery techniques and IMRT are used to restrict the dose to normal tissues.

Radiosurgery

Numerous retrospective reports describe the use of radiosurgery for small meningiomas, either residual or progressive after resection, or untreated, skull-base lesions. Local control rates range from 75% to 100% at 5 to 10 years. Complications such as cranial neuropathies, transient neurologic deficits, radiation necrosis, and significant edema have been reported in 6% to 42% of patients treated with radiosurgery.[343,344] Complications are more frequent in patients with large or deep-seated tumors and in those treated with high single doses.[345,346] Fractionated radiotherapy may be preferable for larger tumors.

Chemotherapy

There is currently no defined role for chemotherapy for newly diagnosed or nonirradiated meningiomas. Chemotherapy is generally reserved for recurrent meningioma not amenable to further surgery or radiotherapy. Responses are anecdotal, with no drug or a combination yielding consistent responses. Because many

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meningiomas express estrogen and progesterone receptors, there have been unsuccessful attempts to use agents such as tamoxifen or antiprogesterones.[347,348] Preliminary data suggest that hydroxyurea[349,350] or interferon-alpha-2B (IFNα-2B)[351] may have activity; however, assessment of efficacy is limited by small numbers of patients treated. Targeted agents such as STI-571 (imatinib), angiogenesis inhibitors such as sunitinib, vatalanib, and EGFR inhibitors have been evaluated, without clear efficacy.[352–355]

PRIMITIVE NEUROECTODERMAL OR EMBRYONAL CENTRAL NERVOUS SYSTEM NEOPLASMS

These tumors of putative embryonal origin predominantly arise in children and include supratentorial PNETs, pineoblastomas, medulloblastomas, ependymoblastomas, and atypical teratoid or rhabdoid tumors. They are characterized by sheets of small, round, blue cells with scant cytoplasm. Historically, small round cell tumors arising in the posterior fossa were called *medulloblastomas*. Given the cytologic similarity between all these tumors regardless of location, it was suggested in the 1980s that they all be designated as PNETs. Although still controversial, the current WHO classification retains medulloblastoma as a distinct type of PNET within the larger group of *embryonal* tumors that includes medulloepitheliomas, neuroblastomas, and ependymoblastomas. Pineoblastomas also retain a separate position within the category of pineal parenchymal tumors. Regardless of formal classification, these tumors are viewed as developmentally aberrant early neural (glial or neuronal or both) progenitor or stem cell neoplasms.

Embryonal tumors with abundant neuropil and true rosettes (ETANTR) are a recently identified variant of PNET and, histologically, have features of ependymoblastomas and neuroblastomas, demonstrating areas of fine fibrillary neuropils intermingled with ependymoblastic rosettes and zones of undifferentiated neuroepithelial cells. ETANTRs are distinguished pathologically from other embryonal tumors by the striking abundance of neuropils.[356]

Recent data have shown quite clearly that medulloblastomas, supratentorial PNETs, atypical teratoid rhabdoid tumor (ATRTs), ETANTRs, and pineoblastomas probably all represent very distinct entities, each with their own biologies and indeed, in many cases, their own biologically important subgroups.[357–363]

Medulloblastoma

Epidemiology

Medulloblastomas comprise 15% to 30% of CNS tumors in children and an estimated 350 to 400 cases are diagnosed in the United States annually.[4] There is a 1.5:1 male-to-female predominance, and 70% are diagnosed by age 20 years. Medulloblastomas become progressively more rare with increasing age, with few cases found in those older than 50 years.[364,365] Gorlin and Turcot syndromes have increased rates of medulloblastoma, but account for only 1% to 2% of medulloblastomas.[29,366]

Pathology

Classically, medulloblastomas have Homer-Wright (neuroblastic) rosettes, although these are found in less than 40% of the cases.[367] Mitoses are frequent, representing a high proliferative index. Immunohistochemical analyses are positive for synaptophysin, which is most prominent in nodules and within the centers of the Homer-Wright rosettes, correlating with a presumed neuronal progenitor origin.[368] According to the last round of WHO classification, medulloblastomas are histologically grade IV and classified into five variants: classical, desmoplastic/nodular, medulloblastoma with extensive nodularity, anaplastic, and large cell.[4] The desmoplastic subtype has collagen bundles interspersed with the densely packed undifferentiated cells of the classic subtype as well as nodular, reticulin-free "pale islands," or follicles.[369] Medulloblastomas with extensive nodularity are similar to the desmoplastic variant except that the reticulin-free zones are large and rich in neuropil-like tissue. Anaplastic medulloblastomas are relatively rare, accounting for approximately 4% of cases, and have marked nuclear pleomorphism, nuclear moulding, cell-cell wrapping, and high mitotic activity, with a high degree of atypia.

More recently, it has become agreed upon and apparent that, in fact, medulloblastomas are comprised of at least four different molecular subgroups (Wnt, Shh, Group 3, and Group 4), each with their own demographic, clinical, epidemiologic, transcriptional, genetic, and epigenetic features.[359–361,363,370] Because targeted therapies are likely to only be effective within a single subgroup, the next group of clinical trials will likely be subgroup specific.

Radiographic and Clinical Features

Childhood medulloblastomas typically arise within the vermis, expanding into the fourth ventricle. In older patients, tumors in the lateral cerebellar hemispheres are more common (greater than 50% in adults compared with 10% in children).[364]

Clinical signs and symptoms depend on both age, with infants having less specific symptoms, and the anatomic location within posterior fossa. Midline tumors usually present with symptoms of increased intracranial pressure, including nocturnal or morning headaches, nausea and vomiting, irritability, and lethargy—manifestations of progressive hydrocephalus from fourth ventricle compression. Truncal ataxia may be present because of involvement of the vermis, and sixth nerve palsies are the most common nerve deficit. In younger children, a bulging of open fontanelles may occur. Tumors of a lateral origin more frequently have ataxia and unilateral dysmetria. On CT, medulloblastomas are classically discrete vermian masses that are hyperattenuated compared with the adjacent brain and enhance avidly. Imaging variance is common, with frequent cyst formation and calcification (59% and 22% of cases, respectively).[371] MRI is the gold standard. Medulloblastomas are typically iso- to hypointense on T1-weighted images, of variable signal intensity on T2-weighted images, and enhance heterogeneously.[372] MRI provides improved evaluation of foraminal extent beyond the fourth ventricle, invasion of the brainstem, and subarachnoid metastases. Diffusion-weighted images exhibit restriction, allowing PNETs to be distinguished from ependymomas.[373,374]

Staging and Risk Groups

A modified version of the Chang staging system is currently used.[375] T stage has been made less relevant than the extent of residual disease due to advances in neurosurgical techniques. M stage remains crucial. M0 represents no tumor dissemination, whereas M1 represents tumor cells in the CSF. M2 represents presence of gross tumor nodules in the intracranial, subarachnoid, or ventricular space, and M3 represents gross tumor nodules in the spinal subarachnoid space. M4 represents systemic metastasis.

Clinical staging requires the assessment of tumor dissemination and includes CSF cytologic examination. This is frequently not performed prior to surgery because of concern for cerebellar herniation from increased pressure within the posterior fossa. Ventricular fluid is not as sensitive as lumbar fluid in detecting dissemination within the neuraxis.[376] Negative CSF cytology does not preclude more advanced leptomeningeal disease.[377] An MRI examination of the spine has supplanted conventional myelography. CSF dissemination is identified on MRI scans as diffuse enhancement of the thecal sac, nodular enhancement of the spinal cord or nerve roots, or nerve root clumping, predominantly seen along the posterior aspect of the spinal cord based on CSF circulatory patterns.[372] Spine MRI is ideally performed prior to surgery if a medulloblastoma is suspected and the patient is stable; otherwise, 10 to 14 days should elapse after surgery to avoid a potential false

positive interpretation from surgical cellular debris and blood products.[378] Metastases outside the CNS are less common and occur in less than 5% of patients and correlate with advanced disease within the neuraxis. Eighty percent of systemic metastases are osseous. A bone scan, chest x-ray, and bilateral marrow biopsies should be routinely performed for M2 and M3 stages.

Patients with medulloblastomas are currently classified as *average* or *high risk* based on age, M stage, extent of residual disease, and pathology. Average-risk patients have M0 stage arising within the posterior fossa, are more than 3 years old, and have less than 1.5-cm tumor residual. Due to the poor prognosis, all patients with anaplastic medulloblastoma are classified as high risk.[379,380] Patients less than 3 years old have particularly poor prognoses. This may represent the presence of more primitive, aggressive tumors, but could also be due to the higher likelihood metastatic disease, subtotal resection, and reduced dose or withholding of radiotherapy. Between 20% and 30% of patients present with neuraxial dissemination, most commonly along the spinal cord. The presence of metastatic disease is prognostically significant, with 5-year PFS rates of 70% for M0 disease, to 57% for M1, and to 40% for M2 or higher in CCG-921.[381] The disease-free survival of high-risk patients treated with craniospinal irradiation (CSI) with or without chemotherapy is 25% to 30%.[401] Historically, average-risk patients have had a 5-year disease-free survival of 66% to 70%, which has increased to 70% to 80% in recent reports.[381,382]

Surgery

In one study, 3-year survival was reduced by 60% in patients who had an incomplete resection of their primary tumor.[383] Although hydrocephalus associated with medulloblastoma obstructing the fourth ventricle can be relieved with a ventriculostomy, ICP may be controlled with corticosteroids, and in most patients, aggressive tumor resection is sufficient to relieve hydrocephalus. Following surgery, gradual weaning of the ventriculostomy is attempted, with internalization 7 or more days after surgery if clamping is untenable. Postoperative shunting for hydrocephalus is necessary in approximately 35% to 40% of patients because of scarring and decreased capacity to resorb CSF.[384] Patients who require long-term shunting are younger and have larger ventricles and a more extensive tumor at presentation.[385] Concern has existed that a ventriculoperitoneal shunting (VP) shunt may cause peritoneal seeding, but this has not been upheld.[386]

With advances in neurosurgical technique, the number of patients not undergoing a gross total or near-total resection is dwindling. MRI should be performed to evaluate the extent of residual disease within 48 to 72 hours following surgery to prevent postsurgical changes from influencing interpretation. Patients with either gross total resection or subtotal resection have better 5-year OS and posterior fossa local control rates than patients who undergo biopsy alone. Although retrospective data infer that a total resection is prognostically favorable, the majority of trials have found that patients who undergo substantial subtotal resection with minimal residual disease treated with both chemotherapy and radiation do just as well as those who undergo total resection.[387] This justifies opting for a near-total resection, particularly when there is invasion of the floor of the fourth ventricle or envelopment of cranial nerves or the posterior inferior cerebellar artery. It is clear, however, that the extent of resection does not impact survival in patients with disseminated disease.[387]

The value of an aggressive resection must be balanced against surgical complications, interchangeably referred to as *posterior fossa syndrome* or *cerebellar mutism syndrome*. These conditions consist of diminished speech and can include emotional lability, hypotonia, long-tract signs, bulbar dysfunction, decreased respiratory drive, urinary retention, and ataxia. These changes can be seen in up to 25% of patients who have undergone a resection of a midline posterior fossa tumor.[388] Although thought to be a temporary, a significant number have persistent deficits.

Radiation Therapy

The aims of radiotherapy are to treat residual posterior fossa disease (or gross deposits of disease anywhere in the craniospinal axis) and also to treat microscopic disease in the craniospinal axis. Historically, CSI has been delivered to 36 Gy with a posterior fossa boost of 54 Gy using conventional fractionation of 1.8 Gy per day.[389] Radiation is typically initially withheld in patients younger than 3 years of age because of the higher risk of neurocognitive damage. Supratentorial PNETs and other embryonal tumors have been treated with the same CSI regimen, with a boost to the tumor bed and residual disease. Supratentorial PNETs treated with an appropriate dose and volume of radiotherapy were found to have a 49% PFS at 3 years compared with 7% with major violations of radiotherapy.[390]

Various alterations to the radiotherapy regimen have been made endeavoring to limit late toxicities. Hyperfractionation has been examined, with one study showing no improvement in survival and an excess of failures outside the primary site, although this was likely attributable to a reduced craniospinal dose of 30 Gy.[391] A recent trial showed that with a reduction in craniospinal dose, adequate disease-free survival with possible preservation of intellectual function is possible.[392] IMRT has been used to provide radiation to the posterior fossa, with a 32% redu. A recent trial showed that with a reduction in craniospinal dose, adequate disease-free survival with possible preservation of intellectual function is possible. p ction in dose to the cochlear apparatus, reducing the risk of grade 3 or 4 hearing loss from 64% to 13%.[70] Improved imaging methods have allowed for more precise delineation of the tumor within the posterior fossa, providing the possibility of avoiding treatment to the entire posterior fossa with the boost dose. Although standard practice has been to boost the entire posterior fossa, retrospective data have shown isolated recurrences outside the tumor bed to be rare.[393,394] Encompassing the tumor bed and a 2-cm margin only for the boost led to less than 5% isolated posterior fossa recurrences.[395]

A combined CCG/Pediatric Oncology Group trial compared standard and reduced dose CSI (36 versus 23.4 Gy) with a posterior fossa boost to 54 Gy in average-risk patients. All patients received concurrent vincristine during radiation with no adjuvant chemotherapy. Patients who received the lower dose had a higher rate of early relapse, lower 5-year event-free survival (67% versus 52%), and lower OS.[396] A comparison of CSI doses of 35 versus 25 Gy in the International Society of Paediatric Oncology (SIOP) II yielded similar results.[397] Further dose reduction is being evaluated in ongoing prospective trials.

Strong advocates for proton therapy have emerged as a result of the sharply diminished exit dose from spinal irradiation and the more conformal treatment of the posterior fossa. A dosimetric analysis that compared photons to protons has demonstrated a decrease in the dose to 50% of the heart volume from 72.2% to 0.5%, and the dose to the cochlea was reduced from 101.2% of the prescribed posterior fossa boost dose to 2.4%.[398] Proton-based radiotherapy also demonstrated a decreased radiation dose to normal tissues compared with IMRT. Overall, a reduction in second malignancies is also anticipated and modeled based on available data, although one controversial report contends that the older generation of proton-beam machines might pose a greater risk of second malignancies because of a higher rate of neutron production and contamination, which is more carcinogenic.[74]

Chemotherapy

Chemotherapy has been used in medulloblastomas with the dual goals of reducing the radiation dose while maintaining optimal disease-free survival rates in average-risk patients and improving disease-free survival in high-risk patients. Tait et al.[399] in SIOP I compared radiotherapy alone versus radiotherapy with concurrent vincristine followed by maintenance vincristine and CCNU. Overall, there was no survival benefit from chemotherapy, but an

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unprespecified post hoc subgroup analysis identified subgroups that appeared to benefit from chemotherapy, including T3 or T4 disease, and subtotal resection. Similar results were seen in a CCG study.[382] The 5-year disease-free survival rates in the CCG and SIOP studies were 59% and 55%, respectively, for RT plus chemotherapy, and 50% and 43% for RT alone. Based on these results, the routine use of chemotherapy for "high"-risk medulloblastomas has become standard.

For "standard"-risk patients, chemotherapy has been postulated to lead to a reduction in the CSI dose necessary to control microscopic disease. A phase II trial of CCNU, vincristine, and cisplatin for eight cycles following the reduced CSI prescription of 23.4 Gy had a PFS rate of 86% and 79% at 3 and 5 years, respectively.[396] This was superior to historical controls, and CSI to 23.4 Gy with chemotherapy was adopted as the standard of care and reference dose for further trials.

The most recent COG trial for average-risk patients compared cisplatin and vincristine with either CCNU or cyclophosphamide and 23.4 Gy CSI. No differences in outcome were noted, with a 5-year event-free survival and OS rates of 81% and 86%, respectively. The overall outcomes indirectly validated the use of reduced-dose CSI in conjunction with chemotherapy. The ongoing COG trial for average-risk patients is investigating a CSI dose of 18 Gy in patients between 3 to 7 years of age. The 2 × 2 randomization also compares boosting the entire posterior fossa versus a local boost.

Current approaches for high-risk medulloblastomas focus on chemotherapy dose intensification. Vincristine, CCNU, and prednisone had a 63% 5-year PFS rate, better than an 8-in-1 chemotherapy regimen. High-dose cyclophosphamide with autologous stem cell rescue is feasible and provided a 5-year event-free survival of 70% in patients with high-risk disease.[400] In a pilot study involving 57 children, the COG incorporated carboplatin as a radiosensitizer with CSI to 36 Gy and a posterior fossa boost followed by six cycles of maintenance cyclophosphamide, vincristine, and cisplatin. Four-year OS and PFS rates were 81% and 66%, respectively, with an inferior outcome in patients with anaplastic medulloblastoma.

Because the risk of cognitive deficits increases with decreasing patient age, extensive effort has been made to develop regimens that can delay or potentially eliminate the need for radiation in patients younger than 3 years of age. The avoidance of radiation has proved to be more feasible for patients with M0 disease.[401] The addition of intraventricular methotrexate following surgery in a five-drug chemotherapy regimen provided 5-year PFS and OS rates of 58% and 66%, respectively.[383] Although asymptomatic leukoencephalopathy was detected by MRI and mean intelligence quotient (IQ) scores were lower than healthy controls, the mean IQ scores were significantly higher than previous cohorts who had received radiation. A prospective randomized trial of supratentorial PNETs in children younger than 3 years old treated with chemotherapy and omitted or delayed radiation yielded less promising results, with a PFS and OS rates at 3 years of 15% and 17%, respectively. The administration of radiation was the only positive prognostic variable for PFS and OS.[402] The Head Start I trial for young children with localized medulloblastoma consisted of five cycles of cisplatin, vincristine, etoposide, and cyclophosphamide followed by a single high-dose myeloablative chemotherapy regimen of thioTEPA, carboplatin, and etoposide.[403] The 5-year survival was 79%. With the addition of methotrexate, children with disseminated disease had a 5-year PFS of 45% and an OS of 54%.[404]

The addition of conformal RT limited to the posterior fossa and primary site to chemotherapy (cyclophosphamide, vincristine, cisplatin, etoposide) in children between 8 months and 3 years of age with nonmetastatic medulloblastoma increased event-free survival compared with the use of postoperative chemotherapy alone (Children's Oncology Group trial P9934). Neurodevelopmental assessments did not show a decline in cognitive or motor function.[405]

Recurrent medulloblastomas are essentially an incurable and lethal disease. Although it is responsive to a variety of neoplastic agents, including vincristine, nitrosoureas, procarbazine, cyclophosphamide, etoposide, and cisplatin, with several regimens yielding relatively high response rates, durability is limited. A CCG trial to evaluate carboplatin, thioTEPA, and etoposide with peripheral stem cell rescue showed a 3-year event-free survival and OS of 34% and 46%, respectively.

Long-term effects from treatment can be categorized as neurocognitive, neuropsychiatric, neuroendocrine, and growth retardation. Hypothalamic and pituitary endocrinopathies such as delayed hypothyroidism and decreased growth hormone secretion may occur. Growth retardation can also be secondary to delayed or reduced bone growth, leading to a reduction in sitting height. Neurocognitive deficits have long been recognized secondary to surgery, radiotherapy, and chemotherapy. In one study, 58% of children showed an IQ above 80 at 5 years after treatment, but by 10 years after treatment, only 15% of the patients had an IQ that remained above 80.[406] A prospective study of cognitive function showed an average decline of 14 points in mean IQ, with an average decline of 25 points in patients younger than 7 years of age.[407] Even with risk-adapted RT, patients had a significant yearly decrease in mean IQ, reading, spelling, and math.[408] Psychologic secondary effects are partially attributable to the diminished cognitive function as well as the social challenges caused by the physical manifestations of CSI (e.g., hearing loss, decreased truncal stature, and thin hair) and potential ataxia and abnormal speech patterns. The risk for secondary malignancies also exists. A population-based study tabulated a 5.4-fold increased rate of malignancy when compared with the general population, although this only affected 20 of 1,262 patients at risk.[409]

PINEAL REGION TUMORS AND GERM CELL TUMORS

Clinical and Pathologic Considerations

Pineal and germ cell tumors account for less than 1% of intracranial tumors in adults and 3% to 8% of brain tumors in children.[410] Germinomas are the most common type, accounting for 33% to 50% of pineal tumors. The peak incidence of germ cell tumors is in the 2nd decade, and few present after the 3rd decade. Gliomas are the next most common pineal region tumor (approximately 25%). Pineal parenchymal tumors are nearly as common as glial tumors and are called *pineocytomas* if benign and *pineoblastomas* (a variant of PNET) if malignant; a rare intermediate form also exists.

Germ cell tumors commonly involve the two midline sites, suprasellar and pineal regions, and occasionally are found in other areas such as the basal ganglia, ventricles, cerebral hemispheres, and the spinal cord. Germinomas can occur bifocally or, rarely, even multifocally; the most common bifocal presentation is synchronous involvement of the suprasellar region and the pineal gland.[411] Based on histology and the presence of tumor markers in the serum or CSF, the WHO classification system divides intracranial germ cell tumors into germinomas and nongerminomatous germ cell tumors.[4] Nongerminomatous germ cell tumors are further divided into embryonal carcinomas, yolk sac tumors, choriocarcinomas, and teratomas (mature, immature, or teratoma with malignant transformation). A quarter of the intracranial germ cell tumors have more than one histologic component and are known as mixed germ cell tumors. Alpha-fetoprotein (elevated in yolk sac tumors) and β-human chorionic gonadotropin (elevated in choriocarcinoma, and to a modest extent in germinoma) are generally secreted by these tumors. Mature teratomas do not have elevated tumor markers.

Neurologic signs and symptoms are caused by obstructive hydrocephalus and involvement of ocular pathways. Major symptoms are headache, nausea and vomiting, lethargy, and diplopia. Signs are primarily ocular, but can include ataxia and hemiparesis. The major ocular manifestation is paralysis of conjugate upward

gaze (Parinaud syndrome), but pupillary and convergence abnormalities are seen, as are skew deviation and papilledema. Some patients with a pineal germ cell tumor can present with symptoms of diabetes insipidus (DI) without any radiologic evidence of overt suprasellar disease.[412,413]

On CT, these lesions are hyperdense. On MRI, the mass is hypointense on T2-weighted sequences (due to the high cellularity of the mass) and shows enhancement with gadolinium. Calcification and fat may be seen in teratomas or mixed malignant germ cell tumors. Germinomas tend to surround a calcified pineal gland, whereas pineal parenchymal tumors tend to disperse the calcification into multiple small foci. The potential for leptomeningeal dissemination requires imaging of the neuraxis before surgery. Determination of histology, tumor markers, and extent of disease is critical for the optimal management of pineal region tumors. The prognosis varies depending on the histologic type, the size of the tumor, and the extent of disease at presentation.

Surgery

Because pineal tumors are often near the center of the brain, they are among the most difficult brain tumors to remove. Having said that, there is no role for cytoreductive surgery in the treatment of germinoma, which requires only a biopsy from the neurosurgeon followed by radiation, chemotherapy, or both. The application of modern surgical technology with superb illumination, magnification, surgical guidance, and neuroanesthesia has made this region much more accessible. Surgeons can choose from several approaches depending on preference and the tumor's position and extent.[414] The current recommendation is to obtain tissue when a diagnosis cannot be made from serum tumor markers, CSF tumor markers or cytologic examination. Whenever possible, the tumor is completely excised, except when a germinoma is found at open surgery; a biopsy suffices in this situation because germinomas respond well to radiation.[415] Resection is important when tumors are radioresistant or when an excision may be curative (e.g., as in teratomas, arachnoid cysts, and pineal parenchymal tumors).

The place of stereotactic biopsy in the diagnosis of pineal region tumors is unclear. Although biopsies have been described as safe, particularly for large tumors, some avoid it because of the risk of damaging large veins that flank the pineal gland.[416] In addition, there is a risk that tissue sampling of these heterogeneous tumors may not depict the correct histologic nature of all parts of the tumor. Without an accurate diagnosis, treatment planning may be erroneous or inadequate. In favor of biopsy are the advantages of a rapid tissue diagnosis and shortened hospital stay. A transventricular endoscopic biopsy is a much more common clinical scenario currently because it can be performed under direct vision with little risk of damaging deep venous structures. The downside of an endoscopic biopsy is the small size of the biopsy, which can be problematic in the face of a polyphenotypic germ cell tumor.

In patients with a pineal mass and obstructive hydrocephalus from blockage of the aqueduct of Sylvius, endoscopic surgery can play a special role. An endoscopic third ventriculostomy is performed by making a fenestration in the floor of the third ventricle, which relieves hydrocephalus, and the mass in the posterior third ventricle can be viewed and biopsied through a flexible endoscope. A rigid endoscope can also be safely used by placing a second burr hole for the biopsy. CSF for cytology and marker studies can also be obtained, and the walls of the third ventricle inspected for tumor studding. There is a small risk of intraventricular hemorrhage.[417]

Radiation Therapy

With certain exceptions, such as benign teratomas, RT has an established role in the curative treatment of pineal germ cell and parenchymal tumors. The location and infiltrative nature of these lesions often does not allow for a complete resection. In the past, the risk of biopsy or attempted resection often led to the use of RT without histologic confirmation. In such instances, response to low-dose RT, the measurement of α-fetoprotein and human chorionic gonadotropin-β, and CSF cytology were used to provide diagnostic information. There is a tendency to increase the use of biopsy and resection, and treatment without histology is less common.

Five-year survival rates with RT range from 44% to 78% and vary with histology, extent of disease, age, radiation volume, and dose to the primary tumor.[152] In a multi-institutional survey by Wara and Evans,[418] the survival of patients with pineal parenchymal cell tumors or malignant teratomas was 21% (3 of 14) compared with 72% (26 of 36) for those with germinomas. Wolden et al.[419] reported 5-year disease-free survival rates of 91% for germinomas, 63% for unbiopsied tumors, and 60% for nongerminomatous germ cell tumors irradiated to 50 to 54 Gy to the local site with or without treatment to the whole brain or ventricular system. Patients younger than 25 to 30 years old have survival rates of 65% to 80% compared with 35% to 40% for older patients. This may reflect the increased incidence of germinomas in younger patients.

Germinomas are infiltrative tumors that tend to spread along the ventricular walls or throughout the leptomeninges. The incidence of CSF seeding ranges from 7% to 12%. For this reason, fields encompassing the entire ventricular system, the whole brain, and even the entire craniospinal axis have been recommended. The appropriate treatment volume for pineal germinomas was evaluated by Haas-Kogan et al.[420] in 93 patients treated at the University of California–San Francisco (UCSF) or at Stanford. The UCSF group favored whole ventricular irradiation; the Stanford group included CSI. Five-year survival for the combined cohort was 93%, with no difference in survival or distant failure regardless of whether CSI or whole ventricular radiation was given. In some institutions, 25.5 Gy (1.5 Gy per day) whole-brain or whole-ventricular radiation is followed by a boost to the primary site to 45 to 50 Gy. CSI is reserved for patients with disseminated disease at presentation.

Neoadjuvant chemotherapy and low-dose (30 to 40 Gy) focal irradiation is employed by some.[421,422] Chemotherapy might be useful in the young child to defer irradiation. For disseminated or multiple midline germinomas, systemic chemotherapy or CSI is given. CSI doses of 20 to 35 Gy have been used when CSF cytology results are positive. When response to primary chemotherapy is incomplete or the tumor recurs, salvage radiotherapy yields good results.[423]

Nongerminomatous malignant germ cell tumors, whether localized or disseminated, are treated with chemotherapy followed by restaging. After restaging, localized tumors receive focal RT to 54 to 60 Gy, and disseminated tumors receive CSI (54 to 60 Gy to the primary, 45 Gy to the ventricular system [controversial], 35 Gy to the spinal cord, and 45 Gy to localized cord lesions).[419] In a German study, 63 supratentorial PNETs were treated with chemotherapy before or after radiation (35 Gy CSI with a boost to the primary of 54 Gy).[390] The 3-year survival was 49.3% in those for whom treatment was delivered as prescribed, but only 6.7% in those with major protocol violations. This indicates the importance of CSI in pineoblastoma, analogous to the situation with medulloblastoma.

Tumors that tend not to metastasize to the cord, such as teratomas, pineocytomas, and low-grade gliomas, are treated by resection, with localized radiotherapy reserved for patients with residual disease.[424] For selected patients with small residual disease, radiosurgery has been shown to be effective in terms of local control.

Chemotherapy

Chemotherapy for pineal glial neoplasms is similar to that for gliomas elsewhere. Germinomas are chemosensitive and responsive to cisplatin, carboplatin, ifosfamide, cyclophosphamide, bleomycin,

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and etoposide. Adjuvant multidrug therapy with radiotherapy has produced encouraging disease-free survival and OS. Newly diagnosed germinomas treated with two courses of high-dose cyclophosphamide showed a complete response rate of 91%.[425] Building on this, several studies have reported excellent outcomes with reduced dose radiation combined with chemotherapy.[298,495,499–502]

Given the poor outcome of CNS nongerminomatous germ cell tumors after radiotherapy alone, there is significant interest in the use of chemotherapy. Balmaceda et al.[426] reported the results from using four cycles of carboplatin, etoposide, and bleomycin without radiation. Of 71 patients (45 germinoma and 26 nongerminomatous), 68 were assessable for response; after four cycles, the complete response rate was 57%. The 29 patients with less than a complete response received dose-intensified chemotherapy or surgery, and a further 16 achieved a complete response, for an overall complete response rate of 78%. Despite these high response rates, only 28 of 71 (39%) patients were alive and progression free within 31 months. Subsequently, they treated 20 patients with two cycles of cisplatin, etoposide, cyclophosphamide, and bleomycin, and the 16 patients achieving a complete response received two additional cycles of carboplatin, etoposide, and bleomycin.[427] Nine of the 14 survivors received RT. The chemotherapy response rate was 94%, 5-year OS was 75%, and 36% of patients were event free. Although the complete response rate was high, approximately half the patients developed recurrent disease, suggesting that a multimodal therapeutic approach of surgery, chemotherapy, and radiotherapy is necessary to improve the overall outcome of these tumors. Matsutani et al.[428] analyzed 153 germ cell tumors treated with surgery and RT with or without chemotherapy. The 10-year survival rates for mature and malignant teratomas were 92.9% and 70.7%, respectively. Patients with pure malignant germ cell tumors (embryonal carcinoma, yolk sac tumor, or choriocarcinoma) had a 3-year survival rate of 27.3%. The mixed tumors were divided into three subgroups: (1) mixed germinoma and teratoma; (2) mixed tumors whose predominant characteristics were germinoma or teratoma combined with some elements of pure malignant tumors; and (3) mixed tumors with predominantly pure malignant elements. The 3-year survival rates were 94.1%, 70.0%, and 9.3%, respectively, for the three groups.

High-dose chemotherapy with autologous stem cell rescue has been used for pineoblastoma.[429] Twelve patients were treated with induction chemotherapy followed by CSI with a pineal boost (36 Gy CSI, 59.4 Gy to primary), then with high-dose chemotherapy with stem cell support. Nine of the 12 patients remained disease free, including two infants who never received radiation. The actuarial 4-year PFS and OS were 69% and 71%, respectively. Although still considered investigational, the survival results are impressive. The use of high-dose chemotherapy and autologous bone marrow support has not been as promising for patients with recurrent tumors, although reported data are few.[429]

PITUITARY ADENOMAS

Clinical and Pathologic Considerations

Pituitary tumors are identified incidentally or present through symptoms of local mass effect or as a result of endocrine effects. Pituitary adenomas almost always arise from the anterior pituitary, the adenohypophysis. The tumor initially compresses the gland and, subsequently, the optic chiasm and nerves. Tumors less than 10 mm—microadenomas—rarely compress the optic apparatus. Larger macroadenomas can involve the cavernous sinus bilaterally, the third ventricle (sometimes producing hydrocephalus), and, less commonly, the middle, anterior, or even the posterior fossae. The classic ophthalmologic finding is visual loss, typically starting with bitemporal hemianopsia and loss of color discrimination. Automated visual field testing is more sensitive than simple confrontation. Occasionally, extraocular palsies can result from the compression or invasion of the nerves in the cavernous sinus. Tumors that present with mass effect are often nonsecreting, but prolactin, growth hormone, thyrotropin, and gonadotropin-producing tumors may also present in this way.

Neuroendocrine abnormalities are usually from tumors that oversecrete hormones but can also result from compression of the pituitary gland and the stalk. The most commonly secreted hormones are prolactin, adrenocorticotropic hormone, or growth hormone. The incidence of the various types of adenoma is variable. In 800 patients operated on at UCSF between 1970 and 1981, 79% were endocrinologically active. Of these, 52% were prolactin secreting, 27% were growth hormone secreting, 20% were corticotropin secreting, and only 0.3% were thyroid-stimulating hormone secreting.[430] Sexual impotence in men and amenorrhea and galactorrhea in women are hallmarks of a prolactin-secreting tumor. Hypogonadism, infertility, and osteopenia are also common.[431] Growth hormone hypersecretion causes acromegaly or, in the rare patient with a tumor occurring before epiphyseal closure, gigantism. The secondary production of insulin-like growth factor-1 (IGF-1; primarily from the liver) or somatomedin C produces skeletal overgrowth changes (e.g., increased hand and foot size, macroglossia, frontal bossing). Soft tissue swelling, peripheral nerve entrapment syndromes, and arthropathies may occur. Hypertension, cardiomyopathy, diabetes, and an increased risk of colon cancer are prevalent with acromegaly. Adrenocorticotropic hormone hypersecretion by a pituitary tumor results in Cushing disease, with weight gain, hypertension, striae, hyperglycemia, infertility, osteoporosis, increased skin pigmentation, and psychiatric symptoms. Rarely, pituitary adenomas can present acutely with headaches, visual loss, and confusion, which can progress to obtundation. This potentially life-threatening condition is termed *pituitary apoplexy*. The etiology of apoplexy is thought to involve tumor infarction due to interruption of its blood supply, but the exact mechanism is not known. Symptomatic pituitary apoplexy is a surgical emergency, and patients need to be carefully medically managed with judicious fluid and salt replacement and administration of high-dose corticosteroids. A need for prolonged hormone replacement therapy is often a consequence of apoplexy.

On MRI, pituitary microadenomas are generally seen within the gland according to the distribution of normal cells. For example, prolactinomas tend to be located laterally within the sella. Microadenomas show subtle hypointensity to the normal gland on T1-weighted sequences and are often more difficult to detect on T2 sequences.[432] Immediately after the administration of contrast, adenomas show less enhancement than adjacent normal glands (Fig. 97.11). On delayed views, the tumor enhances more than the normal gland. Indentation of the sellar floor, stalk deviation, and mass effect on adjacent structures also provide evidence of the presence of tumor.

Surgery

There are two primary goals of surgery for macroadenomas: to decompress the visual pathways by reducing tumor bulk and, for secreting tumors, to normalize hypersecretion, with the preservation of remaining normal pituitary function.

The standard surgical approach for the majority of pituitary tumors is transsphenoidal, which is safer and better tolerated than the transcranial (frontotemporal craniotomy) approach.[433–435] The transsphenoidal approach is used for microadenomas that occupy the sella turcica and for many macroadenomas. Image-guided neuronavigation and intraoperative fluoroscopy are essential to reduce the risk of injury to the carotid arteries. Even when the majority of tumor is actually suprasellar, a transsphenoidal resection can be safely accomplished if the tumor consistency is soft (and tumor aspiration and curettage can thus easily be performed) and if the tumor is situated so that it can drop into the sella with progressive

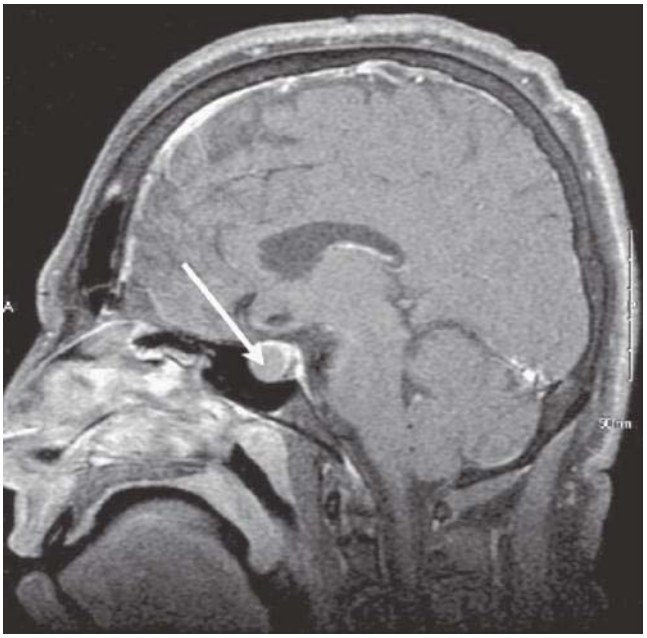

Figure 97.11 Pituitary adenomas are usually isointense to the gland on noncontrast T1-weighted magnetic resonance images; on contrast administration, the normal gland enhances early, as visualized on this sagittal image, whereas the adenoma (*arrow*) continues to remain unenhanced. With late imaging, the adenoma enhances.

resection. Tough, fibrous suprasellar tumors and those that extend laterally into the middle fossa, anteriorly beneath the frontal lobes, or into the posterior fossa may require a craniotomy for resection.[436] A tumor that invades the cavernous sinus is generally not removed. The role of endoscopic transsphenoidal surgery for pituitary adenomas is currently being expanded. Potential advantages include a less invasive surgical approach with a wider field of view and quicker postoperative recovery. Moreover, Cappabianca et al.[436] observed a decreased incidence of complications in a series of 146 consecutively treated patients who underwent an endoscopic endonasal transsphenoidal approach to the sellar region for resection of these tumors, compared with large historical series that employed the traditional microsurgical transsphenoidal approach.

Current surgical cure rates for hormonally active adenomas are 80% to 90% if there is no involvement of the cavernous sinus, suprasellar region, or clivus.[437] Patients with microadenomas have a higher surgical cure rate than patients with macroadenomas.[438] Patients cured of their endocrine disease can expect to have a normal lifespan; however, those with persistent endocrinopathies, and particularly those with acromegaly, may not enjoy a normal lifespan due to the impact of the high hormone levels on multiple organ systems.[437] In patients not biochemically cured with initial surgery, the tumor is often found at the time of second surgery just next to the original site. Patients with persistent acromegaly, however, may not be amenable to biochemical cure with a second surgery because the residual growth hormone secreting cells can be difficult to visualize. Growth through the dura into the adjacent cavernous sinus is often found at repeat surgery even when no tumor is seen preoperatively on MRI.[439] Benveniste et al.[440] found that, although repeated transsphenoidal surgery to treat a recurrent or residual tumor mass was associated with a 93% rate of clinical remission, its use to treat recurrent or persistent hormone hypersecretion produced only a 57% rate of initial endocrinologic remission, with a 37% likelihood of sustaining such remission at a mean of 31 months. Thus, they suggested that for the treatment of residual or recurrent adenomas that cause persistent or recurrent hormone hypersecretion, radiosurgery may be a better option.

Radiation Therapy

RT may be indicated for hormone-secreting adenomas that are not surgically cured and are refractory to pharmacologic management. After subtotal resection of a macroadenoma, more than 50% of patients demonstrate radiographic evidence of progression within 5 years.[441] Younger patients (less than 50 years old) with residual disease have faster tumor regrowth. Ki-67 antigen labeling of more than 1.5% predicts more rapid growth.[442] For these patients, earlier postoperative radiotherapy should be considered.

RT decreases serum growth hormone concentrations to "normal" levels in 80% to 85% of acromegalic patients, but the definition of normalization has varied over time.[443] Growth hormone levels decrease at a rate of 10% to 30% per year, so several years may be required for the levels to normalize.[443] The probability of endocrine cure is highest for tumors with relatively low pre-RT growth hormone elevations (30 to 50 ng/mL); response is less reliable for tumors that produce higher growth hormone levels. In contrast, serum IGF-1 levels remain elevated after radiotherapy, and long-term treatment with somatostatin or its analogs may be required.[444,445] RT controls hypercortisolism in 50% to 75% of adults and 80% of children with Cushing disease. Response occurs within 6 to 9 months of treatment.[446]

Pituitary adenomas may be treated using several different techniques. The most commonly used techniques include 3D-CRT, IMRT, and stereotactic radiotherapy or radiosurgery. Treatment with charged-particle beams has been used at select centers.[447,448] The total dose used for nonfunctioning lesions is 45 to 50 Gy in 25 to 28 fractions of 1.8 Gy. Slightly higher total doses are recommended for secretory lesions. This controls tumor growth in 90% of cases at 10 years.[449–451] Radiation-induced injury to the optic apparatus or adjacent brain with this dose-fractionation scheme is rare, whereas larger fractions or greater total doses lead to a higher incidence of injury. Hypopituitarism may develop, often years after radiation treatment.[451] It is more common in patients who have had both surgery and RT than in those treated with either modality alone. Hypopituitarism is largely correctable by hormone-replacement therapy. One publication suggests that patients treated with surgery and radiation have an elevated risk for late cerebrovascular mortality.[452] Possible contributing factors include hypopituitarism, radiotherapy, and extent of initial surgery. The risk of developing a radiation-induced brain tumor after treatment is 1.3% to 2.7% at 10 years and beyond.[452–454]

Radiosurgery is increasingly being used for treating small residual adenomas.[448,455] In general, patients are eligible for radiosurgery only if the superior extent of the lesions is more than 3 to 5 mm from the optic chiasm. Doses of more than 10 Gy in a single fraction to the optic pathways can cause visual loss.[456] Radiosurgery results in excellent tumor control and appears to cause more rapid biochemical normalization than is seen with conventional RT, with the caveat that it is used primarily for smaller tumors.[457–459] Side effects such as hypopituitarism and cranial nerve injury is also lower with radiosurgery.[460,461]

Reirradiation can be considered for patients with recurrent pituitary adenomas when there has been a long interval after the first course of radiotherapy and other therapeutic methods have been unsuccessful.[462]

Medical Therapy

Medical therapy is very important and effective for patients with secreting pituitary tumors.[463] Dopamine agonists (e.g., bromocriptine or cabergoline) are the most effective therapy for prolactinomas and are often used as primary treatment, with definitive treatment reserved for patients who either cannot tolerate or do not respond to a dopamine agonist. Somatostatin analogs (e.g., octreotide and lanreotide) are effective for patients with acromegaly and are usually reserved when there is persistent growth hormone

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hypersecretion after resection. Control rates with octreotide are approximately 50%; dopamine agonists can control growth hormone production in 10% to 34%.[464] A recently approved growth hormone receptor antagonist (pegvisomant) can be used for patients for whom somatostatin analogs fail. Rates of IGF-1 level normalization as high as 97% have been reported with this agent, but concerns persist that because it acts at the end-organ receptor level, tumor growth may continue in some patients, and the lifetime cost of the agent is prohibitive.[465] Medical therapy for patients with Cushing disease is directed at the adrenal glands to reduce cortisol hypersecretion (ketoconazole). Unfortunately, no known drug effectively reduces pituitary corticotropin production.

CRANIOPHARYNGIOMAS

Clinical and Pathologic Considerations

Craniopharyngiomas are the most common benign nonglial brain tumors in children, occurring primarily in the late 1st and 2nd decades, although they can present at any age.[2,466] Craniopharyngiomas are thought to arise from epithelial cell rests that are remnants of the Rathke pouch at the juncture of the infundibular stalk and the pituitary gland. Most have a significant associated cystic component with only 10% being purely solid. Most craniopharyngiomas become symptomatic because of effects of the combined tumor and cyst on the optic apparatus or hypothalamus or both. They may also compress the pituitary gland or extend superiorly into the third ventricle. Cyst fluid is proteinaceous, and this can be seen on MRI. A CT shows calcification in 30% to 50% of cases.

Common presenting symptoms include headache, visual complaints, nausea, vomiting, and intellectual dysfunction (especially memory loss). Specific visual signs include optic atrophy, papilledema, hemianopsia, unilateral or total blindness, and diplopia with associated cranial nerve palsies. Endocrine abnormalities at presentation can include growth retardation, menstrual abnormalities, and disorders of sexual development or regression of secondary sexual characteristics (or both). DI is uncommon at presentation.[467]

The optimal treatment of any specific patient with a craniopharygioma is complicated, and open to debate. Variables such as patient age, endocrine status, visual status, aeration of the sinuses, extent of solid disease, extent of cystic disease, and involvement of the hypothalamus either unilaterally or bilaterally can all contribute to the decision of optimal therapy. In general, the number of options for therapy for a given patient will vary directly with the number of opinions sought because there are many different approaches and schools of thought as to how best treat this highly variable disease.

Surgery

Craniopharyngiomas are generally resected using a microsurgical subfrontal or pterional approach. More recently, some surgeons have been approaching some lesions using an endoscopic skull base approach from the sphenoid sinus. Larger tumors may require bifrontal or skull-base approaches, including a supraorbital craniotomy. Endoscopically assisted surgery is sometimes used, although outcome advantages have not yet been clearly shown. Although complete resection remains the optimal surgical goal, the risk of devastating long-term effects on hypothalamic function and quality of life cannot be ignored. In some cases, there is no clearly defined plane between tumor and surrounding hypothalamus, which makes aggressive resection dangerous. Aggressive removal is frequently associated with some injury to the pituitary stalk, with subsequent temporary or permanent DI and elements of hypopituitarism.[468–470] These patients require lifelong replacement hormones and inhaled desmopressin acetate spray for the control of DI. Most patients with preoperative visual loss can expect at least some improvement after surgery. The reported mortality rates for craniopharyngioma resection range from 2% to 43%, with severe morbidity in 12% to 61%.[471] Complications are less likely with experienced surgeons. Alternative approaches include placement of an Ommaya reservoir for largely cystic tumors through which one can instill sclerosing agents (e.g., bleomycin, interferons, or radioisotopes.)

Radiation Therapy

Radioisotope Therapy

Predominantly cystic craniopharyngiomas can be treated with stereotactic or endoscopic instillation of colloidal therapeutic radioisotopes, particularly ^{90}Y or ^{32}P.[469,472] The short penetrance of the beta-particles emitted by these isotopes allows the epithelial cells lining the cyst to be treated without significant dose to neighboring structures. Intracystic therapy may have a role in treating cysts that recur after conventional external-beam irradiation, or even as a primary cyst treatment. Although most cysts shrink with intracystic therapy, one-third of patients require further surgery later.

External-Beam Radiation Therapy

Numerous reports demonstrate that subtotal removal and irradiation produce local tumor control and survival rates comparable to those after radical excision.[473–475] The local control rates after complete resection, subtotal resection alone, and subtotal resection with postoperative irradiation are 70%, 26%, and 75%, respectively. A study from Children's Memorial Hospital in Chicago found a 32% rate of recurrence after complete resection and 0% after subtotal resection and adjuvant radiotherapy.[476] Ten-year survival rates range from 24% to 100% for complete resection, 31% to 52% for subtotal resection, 62% to 86% for incomplete resection and irradiation, and 100% after radiotherapy alone.[471,473,474,476,477] Patients who undergo conservative treatment, including biopsy and cyst drainage and irradiation, appear to enjoy a better quality of life and demonstrate less psychosocial impairment than those initially treated with more extensive resections.[473] Furthermore, conservative therapy is associated with less hypothalamic and pituitary dysfunction and a lower incidence of persistent DI than when a total or near-total excision is attempted. More extensive resections, using a subfrontal approach, may be associated with frontal lobe and visual perceptual dysfunction. The negative impact on IQ is greater in patients treated with aggressive resection than in those treated with conservative surgery and postoperative irradiation.[478]

The radiation treatment volume is based on CT and MRI scans, with relatively small margins. Generally, more sophisticated 3D-CRT and IMRT approaches and stereotactic radiotherapy techniques are increasingly being used to spare surrounding normal tissues.[477] One report showed excellent tumor control (100%) with minimal late toxicity when FSRT (mean dose, 52.2 Gy in 29 fractions) was used.[479] No significant effect on cognition or visual injury was reported. The total dose is 50 to 55 Gy, given in daily 1.8-Gy increments. One review suggested better local control when doses of 55 Gy or more are delivered.[480]

Radiosurgery

The use of radiosurgery is limited by the proximity of most lesions to the optic chiasm and brainstem,[481] and should be reserved for those uncommon tumors confined to the pituitary fossa and away from the chiasm and hypothalamus.[482] Kobayashi et al.[483] reviewed long-term results (follow-up of 65.5 months) of radiosurgical treatment for residual or recurrent craniopharyngiomas after microsurgery in 98 consecutive patients and found only a 20.4% tumor progression rate. They used a tumor margin dose of 11.5 Gy at the

retrochiasm and ventral stalk area, which decreased the rate of visual and pituitary function loss so that deterioration both in vision and endocrinologic functions occurred in only six patients (6.1%). Similarly, Albright et al.[469] used radiosurgery as the initial treatment for the solid component of craniopharyngiomas in five children, limiting radiation to the optic chiasm to 8 Gy, and reported no operative morbidity or mortality, whereas 5 of 27 children who underwent microsurgical tumor resection suffered worsened vision postoperatively.

VESTIBULAR SCHWANNOMAS

Clinical and Pathologic Considerations

Schwannomas, also known as *neurilemmomas* or *neurinomas*, are benign neoplasms derived from Schwann cells that show a predilection for sensory nerves. Most intracranial schwannomas arise from the vestibulocochlear nerve, with trigeminal nerves being a distant second in frequency. Previously called *acoustic neuromas*, these neoplasms are more correctly termed *vestibular schwannomas* because they arise from both the superior and inferior portions of the vestibular nerve rather than the cochlear nerve. Vestibular schwannomas are equally common between genders and median age at diagnosis is approximately 50 years, with an overall increased incidence between 45 and 64 years of age. Vestibular schwannomas account for approximately 6% to 8% of intracranial neoplasms. The incidence of vestibular schwannomas is between 0.8 and 1.7 per 100,000, with an increasing incidence since the early 1980s.[484,485] This increased incidence may represent the discovery of asymptomatic lesions by a rising number of cranial imaging studies, predominantly MRI. The rate of incidental vestibular schwannomas detected on MRI ranges from 0.02 to 0.07%.[486,487] More than 90% of vestibular schwannomas are sporadic and unilateral. Bilateral vestibular schwannoma is virtually pathognomonic for NF2 and is one of the key components of the Manchester criteria for the diagnosis of NF2.[488] When associated with NF2, vestibular schwannomas have a significantly earlier disease manifestation and tend to occur in the 2nd or 3rd decade of life.

Vestibular schwannomas arise along the zone of transition between the central and peripheral myelin located near the medial aperture of the internal auditory canal (IAC). Macroscopically, they are typically lobulated, with the eighth cranial nerve located eccentrically along the surface because these tumors grow in an expansile fashion, displacing rather than invading nerves. Vestibular schwannomas in NF2 tend to embed within the seventh and eighth cranial nerve bundles more frequently.[489] As with peripheral schwannomas, a microscopic examination yields Antoni A and B tissue patterns. Vestibular schwannomas are benign, with few case reports of malignant dedifferentiation.

Although vestibular schwannomas arise from the vestibular portion of cranial nerve VIII, cochlear symptoms predominate, with the two most common being hearing loss and tinnitus.[490] Progressive unilateral sensorineural hearing loss is characteristic. An evaluation is typically delayed, with the duration of hypacusis averaging 3.7 years prior to diagnosis. Vertigo and unsteadiness are the most common vestibular symptoms. Facial nerve paresis or spasm may be seen. Large tumors can compress the trigeminal nerve, with paresthesias or neuralgia. Impingement of the brainstem or cerebellum may lead to ataxia and long-tract signs as well as involvement of the lower cranial nerves. Most ominous is the rare patient with nausea and vomiting from fourth ventricular compression and obstructive hydrocephalus.

MRI with thin-section, high-resolution, gadolinium-enhanced T1- and T2-weighted images of the cerebellopontine angle is the study of choice. Vestibular schwannomas typically enhance along the course of the eighth cranial nerve with variable intra- and extracanalicular components. Cystic changes are frequently identified in larger lesions. An MRI allows for the identification of the lesion and potential differentiation from other masses of the cerebellopontine angle such as meningiomas, epidermoid cysts, arachnoid cysts, and, rarely, lipomas. Auditory brainstem response audiometry is less sensitive than MRI.[491] Pure tone and speech audiometry continue to be performed to document hearing loss. Hearing loss is more pronounced at higher frequencies, and the degree of speech discrimination loss is disproportionately worse than the pure tone hearing loss.

Treatment

Treatment revolves around the dual goals of local control and cranial nerve function preservation. Factors that influence treatment choice include tumor size, location, patient age, the presence and degree of symptoms such as tinnitus and vertigo, whether a patient has NF2, the status of contralateral hearing, and patient preference. Consultation with a multidisciplinary team is essential.

Observation

Vestibular schwannomas are typically slow growing, and various studies have shown an increase in size ranging from 0.35 to 2.2 mm per year (mean, 1.42 mm per year).[492] Chalabi et al.[493] reported that with mean follow-up of 4.2 years, 85% of observed vestibular schwannoma were noted to have exhibited measurable growth. Given the slow growth pattern and the recognition that neither surgery nor RT restore hearing lost to a vestibular schwannoma and both pose risks to cranial nerve function, observation is a reasonable choice for some patients. Such an approach requires that the patient be willing to undergo regular annual or semiannual clinical and imaging follow-ups. This course may be selected by many patients with small acoustic neuromas, particularly older patients and patients with multiple medical comorbidities. Patients with functional hearing must understand that further hearing loss, including sudden hearing loss, can occur while under observation.

Surgery

Surgery has the unique advantage of removal of the schwannoma, with a low risk of recurrence following complete resection. Microsurgical resection has been the mainstay of treatment for many years and was previously recommended as the standard of care in a 1991 consensus statement.[494] However, stereotactic radiosurgery has also become accepted as a standard treatment for these tumors.[495] Surgical risks include the inherent risk of general anesthesia, CSF leak, meningitis, headache, hearing loss, and facial nerve paralysis. Hearing preservation is influenced by preoperative hearing acuity, location of the tumor, and size. Loss of facial nerve function is the most significant surgical concern, as well as morbidity. Again, tumor size is a factor, as is the relationship between the facial nerve and tumor. Surgery is made particularly challenging by the increased adherence and infiltration in NF2.[496] The risk of facial nerve injury has decreased since the advent of facial nerve electromyography for intraoperative monitoring. The auditory brainstem response may also be used to evaluate the integrity of the cochlear portion of the eighth cranial nerve intraoperatively, improving the odds of potential avoidance and preservation.

Most modern surgical series achieve complete resection in more than 90% of patients, with some reporting significantly higher rates.[490,497] Subtotal resections are frequently deliberate to preserve hearing or provide emergent, life-saving decompression of the brainstem and fourth ventricle. Results appear to be both surgeon and volume dependent, leading to questions of the widespread applicability of results obtained by subspecialty surgeons in academic institutions.[498] There also appears to be a significant learning curve of 20 to 60 patients with new surgical teams.[499,500] An extensive surgical series of 962 patients undergoing 1,000 vestibular schwannoma operations has been compiled

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by Samii and Matthies,[490] who reported a 98% complete resection rate with fewer than 1% of non-NF2 patients having a recurrence. The facial and cochlear nerves were preserved in 93% and 68% of patients, respectively, and functional preservation was 39% for patients with intact hearing preoperatively. Mortality was 1.1%, although this included several individuals who were disabled with advanced disease prior to surgery. If hearing is to be preserved, the auditory nerve is also identified and preserved; preservation of hearing is more likely in patients lacking severe adhesion in the interface between the cochlear nerve and the tumor.[501] Life-threatening complications of acoustic neuroma resections are rare except in patients with extremely large tumors.[502] The tendency of postoperative CSF leaks to develop in patients (10% to 13%) is independent of the surgical approach employed and tumor size and may stem from factors such as transient postoperative increases in CSF pressure.[249] Postoperative headaches were a significant morbidity in a cohort of 1,657 patients who underwent surgery for acoustic neuroma.[503] Patients who underwent tumor resection by the retrosigmoid approach (82.3%) were significantly more likely to report their worst postoperative headache as "severe" than those resected using the translabyrinthine (75.2%) or middle fossa approaches (63.3%). In another quality-of-life study, hearing loss was perceived as the most disabling symptom among 386 patients who underwent acoustic neuroma surgery.[504]

Radiosurgery

The most substantial experience in radiation-based treatment is with SRS. Both Gamma Knife units and SRS-compatible linear accelerators may be used to perform SRS. The University of Pittsburgh published a review of 162 consecutive patients treated with SRS to a mean dose of 16 Gy with a tumor control rate of 98%.[505] Subsequent surgical resection was required in four patients. Normal facial function was preserved in 79% and normal trigeminal function was preserved in 73% of patients. Because of the unacceptable cranial nerve morbidity in this and other series, the prescription dose for radiosurgery was lowered to 12 to 13 Gy. Results from the decreased prescription dose have a similarly low rate of recurrence, with 97% tumor control at a mean dose of 13 Gy.[506] The risk of facial nerve weakness dropped to 1%, and hearing preservation improved to 71%. These results were confirmed with longer follow-up.[507] Recently, a prospective cohort study of 82 patients with unilateral vestibular schwannomas smaller than 3 cm compared surgery and SRS and provided level 2 evidence favoring SRS over microscopic surgical resection. Tumor control was not statistically different (100% for surgery versus 96% for SRS). Normal facial movement and preservation of serviceable hearing was more frequent in the SRS group at all time points, and no quality-of-life decline was seen in the SRS group.[508] New incomplete trigeminal and facial cranial neuropathies typically develop at approximately 6 or more months after radiosurgery. These tend to be mild and usually improve within a year after onset. Approximately half of patients with useful hearing before radiosurgery maintain their pretreatment hearing level, and hearing lost before treatment is not regained. The risk of treatment-induced cranial neuropathy is directly related to the volume of the lesion, the dose given, and the length of nerve irradiated.

Fractionated Radiation Therapy

Different fractionation regimens have been tried to capitalize on theoretical radiobiologic differences between the neoplastic vestibular schwannoma and the surrounding normal tissue. Multiple fractions also allow for the treatment of lesions that would otherwise not be amenable to treatment with SRS based on size (more than 3 cm) or location (direct compression of the brainstem). Hypofractionation was examined in a series that compared 25 Gy in five fractions and 30 Gy in 10 fractions. Actuarial hearing preservation rate was 90% at 2 years, and no recurrence or facial nerve weakness occurred.[509] A nonrandomized prospective trial from the Netherlands, however, had a nonstatistically inferior outcome in hearing preservation when comparing hypofractionation to SRS at 10 to 12 Gy.[510] A comparison of FSRT (50 Gy in 25 fractions) to SRS in a prospective trial showed comparable high control rates and minimal cranial nerve injury, with the exception of retention of useful hearing, which was 81% versus 33% at 1 year (in favor of FSRT) when followed by serial audiometry.[511] Similar rates of tumor control and hearing preservation have been reported by single-institution experiences elsewhere.[512–514]

Several issues confound radiation outcomes assessments for vestibular schwannomas. First, documentation of recurrences can be confounded by inherently slow growth rates and transient post-procedure lesion enlargement.[515,516] Second, ionizing radiation does carry a small inherent risk of inducing secondary neoplasms or malignant transformation of the vestibular schwannoma.[517,518] The risk of a secondary neoplasm can be particularly concerning in tumor-prone genetic conditions such as NF2. However, given the immense number of individuals who have undergone SRS worldwide, the number of presumed radiation-induced malignancies is only a handful and represents, at most, 1 per 1,000 patients. This is substantially lower than the rate of surgery-related mortality. Malignant transformations can also be seen in resected vestibular schwannoma patients who did not receive radiation.[519] Finally, because of increased adherence of the facial nerve to the tumor, eighth nerve preservation rates are lower when the excision is performed for regrowth after radiation when compared to a non-irradiated control group.[520]

Targeted Therapy for Vestibular Schwannoma

There is significant interest in the development of medical therapy for patients with refractory vestibular schwannoma. Aberrant signaling pathways are known to be present, and there are now reports of the use of targeted agents in this disease. In a single patient case report, the EGFR inhibitor erlotinib was associated with radiographic response of the tumor and improved audiologic function.[521] There are also two reports of the use of bevacizumab in the treatment of vestibular schwannoma in the setting of NF1 in patients with a single hearing ear.[522,523] These studies consisted of a small number of patients, but the demonstration of objective regression of tumors and improvement in hearing was impressive, highlighting the need for larger prospective trials of antiangiogenic agents for this disease. There is also a report of using bevacizumab in treating vestibular schwannoma in the patients who have NF2.[522]

GLOMUS JUGULARE TUMORS

Clinical and Pathologic Considerations

Glomus jugulare tumors (paragangliomas) arise from glomus tissue in the adventitia of the jugular bulb (glomus jugulare) or along the Jacobson nerve in the temporal bone, sometimes multifocally. The tumor invades the temporal bone diffusely, but growth is characteristically slow. Sometimes these tumors are endocrine active, with a carcinoid- or pheochromocytoma-like syndrome.[524] Because glomus jugulare tumors occur in the jugular foramen, they commonly cause lower cranial nerve palsies and early symptoms of hoarseness and difficulty swallowing. Facial weakness, hearing loss, and atrophy of the tongue from hypoglossal palsy can follow. Pulsating tinnitus also may be a presenting symptom, and a red pulsating mass is often visible behind the eardrum. A presumptive diagnosis of glomus tumor can be made by CT or MRI scanning, with jugular schwannoma and meningioma being the main differential diagnoses. On CT scans, glomus tumors show a characteristic salt-and-pepper appearance in involved bone; MRI often discloses large blood vessels within the mass. Glomus tumors give positive results on octreotide scintigraphy. These tumors incite a tremendous blood supply, particularly by way of the ascending pharyngeal

artery. An angiography provides the definitive diagnosis. Because preoperative tumor embolization is essential to the surgical removal of glomus tumors, the diagnostic angiogram should be taken before surgery. Histopathologically, numerous vascular channels are distinctive. The background is composed of clear cells clumped in a fibrous matrix. A small percentage of glomus tumors are malignant. There is a familial form in which the tumors are multiple.

Surgery

The treatment of glomus jugulare tumors is controversial, with advocates for surgery, RT, radiosurgery, and combined approaches. Although surgery can often provide a cure for these benign tumors, especially for small lesions, RT and radiosurgery avoid the morbidities that may follow surgical removal (e.g., lower cranial nerve and facial palsies). Surgery for glomus tumors is most often jointly performed by a neurosurgeon and an otorhinolaryngologist after preoperative embolization, which may decrease intraoperative blood loss during the resection of these extremely vascular tumors. Complications of this procedure can include swallowing and aspiration problems, CSF leak, and facial palsy.

Radiation Therapy

Even though glomus tumors are histologically benign, RT is effective and has been recommended for symptomatic lesions that cannot be totally resected, even as primary treatment.[525] These tumors regress slowly after irradiation, and the success of RT is measured by the amelioration of symptoms and the absence of disease progression. A review of the literature demonstrated local control rates with radiation in excess of 90% with or without surgery.[526] A dose of 45 to 50 Gy over the course of 5 weeks is recommended.

Radiosurgery

A literature review by Gottfried et al.[527] showed that the use of SRS to treat glomus jugulare tumors has increased. Compared with conventional radiotherapy, radiosurgery involves a shorter treatment time, precise stereotactic localization, and irradiation of a small volume of normal tissue, which results in a reduced incidence of complications. Among 142 patients treated radiosurgically in eight series reviewed by Gottfried et al., tumors diminished in 36.5%, tumor size was unchanged in 61.3%, and subjective or objective improvements occurred in 39%. Although a residual tumor was present in all of these patients, only 2.1% experienced progression, the morbidity rate was 8.5%, and no deaths occurred; however, the incidence of late recurrence is unknown. In another study of eight patients who underwent radiosurgery (median dose of 15 Gy to the tumor margin) for recurrent, residual, or unresectable glomus jugulare tumors, all remained stable without cranial nerve palsies at a median follow-up of 28 months.[528] The authors suggested treatment of small glomus tumors (3 cm or less in average dimension) with radiosurgery and treatment of young patients with large tumors (3 cm or more in average dimension) and patients with symptomatic tumors with surgical resection.

A recent meta-analysis based on data from 19 studies revealed radiosurgical tumor control in 97% of patients. In eight studies with a median follow-up time exceeding 36 months, 96% of patients achieved tumor control.[529]

HEMANGIOBLASTOMAS

Clinical and Pathologic Considerations

Hemangioblastomas account for 1% to 2% of intracranial tumors, arising most often in the cerebellar hemispheres and vermis. Although usually solitary, these tumors can be multiple and may also occur in the brainstem, spinal cord, and less often, the cerebrum. Cerebellar hemangioblastomas can be sporadic or occur as part of the autosomal-dominant von Hippel-Lindau complex, which is transmitted with more than 90% penetrance.[530] Other entities associated with von Hippel-Lindau disease are retinal angiomatosis, polycystic kidneys, pancreatic cysts, pheochromocytoma, and renal cell carcinoma. Identification of the *VHL* gene on chromosome band 3p25–26 allows individuals who are at risk for the syndrome, or who have some of its components as an apparent sporadic case, to undergo genetic testing with a high degree of accuracy.[531]

Cerebellar hemangioblastomas usually are recognized in the 3rd decade in patients with von Hippel-Lindau disease and in the 4th decade or later in patients with sporadic tumors. These tumors can cause symptoms and signs of cerebellar dysfunction, especially gait disturbance and ataxia, and hydrocephalus from obstruction of CSF pathways. These tumors tend to enlarge slowly, but patients may become symptomatic from tumor cysts, which can grow quickly.[532]

Hemangioblastomas are composed of capillary and sinusoidal channels lined with endothelial cells. Interspersed are groups of polygonal stromal cells with lipid-laden cytoplasm and hyperchromatic nuclei. An immunohistochemical study of these cells shows expression of neuron-specific enolase, vimentin, and S100 protein, but not epithelial membrane antigen or glial fibrillary acidic protein.[533] Grossly, the tumor is often cystic, containing proteinaceous, xanthochromic fluid and with an orange-red, vascular, and firm mural nodule. The cyst wall is a glial nonneoplastic reaction to fluid secreted by the nodule. Some hemangioblastomas lack cysts, especially in the brainstem and spinal cord, but cystic lesions are more often symptomatic, at least in patients with von Hippel-Lindau disease.[532]

The natural history of spinal hemangioblastomas has been described.[532] The authors reviewed the clinical records and MRIs of 160 consecutively treated patients with 331 spinal hemangioblastomas. Most lesions were located in the posterior cord. Cysts were commonly associated with the lesions, often showing faster growth than the solid portion of the tumor. When symptoms appeared, the mass effect derived more from the cyst than from the tumor. These tumors often have alternating periods of tumor growth and stability, and some remain stable in size for many years. These factors have to be considered in the timing and choice of treatment.

Surgery

A complete resection of a hemangioblastoma is often curative. Patients with preoperative hypertension should be evaluated for the presence of a pheochromocytoma, which can be associated with von Hippel-Lindau disease. Hemangioblastomas are very vascular lesions, and a biopsy of a suspected hemangioblastoma, either through an open approach or stereotactically, is usually ill advised because of the high risk of hemorrhage. Surgical resection should be carried out en bloc with avoidance of entry into the lesion, which can result in fierce bleeding reminiscent of an arteriovenous malformation. Preoperative, transarterial embolization is rarely safe because these tumors often receive supply from distal segments of the intra-axial circulation. Fortunately, these hemangioblastomas can be resected with minimal bleeding if resection is carried out entirely in the gliottic plane that surrounds the mass. This is straightforward in most cerebellar tumors, for which a margin of gliottic tissue can be resected with the lesion with little neurologic risk. In contrast, brainstem hemangioblastomas are immediately adjacent to critical structures.[534,535] Sometimes, dissection immediately adjacent to the tumor can cause significant bleeding, with a high risk of inducing neurologic deficits.

These tumors are often associated with significant cysts. Surgery is the optimal treatment for the rapid relief of mass effect.

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The cyst wall is not lined with tumor cells, and drainage, rather than excision, of the cyst lining is required. The mural tumor nodule must be entirely resected to avoid cyst recurrence. Cysts can be drained before opening the dura completely to provide brain relaxation, but great care must be taken not to disturb the tumor nodule during this maneuver to avoid inducing significant bleeding. The risk of hemorrhage during the resection is minimized by coagulating and dividing arterial feeders, if they can be visualized, before tumor removal. Finally, hemangioblastomas that occur in patients known to have von Hippel-Lindau disease may not need to be resected or otherwise treated unless they have demonstrated active growth or are symptomatic from mass effect or hydrocephalus. Because many of these patients harbor multiple tumors, other approaches, including radiosurgery, should also be considered, although surgery remains the only option that has a proven benefit.

Radiation Therapy

RT is recommended for patients with unresectable, incompletely excised, and recurrent hemangioblastomas and for those who are medically inoperable. Doses of at least 50 to 55 Gy over the course of 5.5 to 6 weeks appear to be warranted.[536] Because of the noninvasive nature of these lesions, conformal radiotherapy or radiosurgery is indicated.

Radiosurgery should be considered for surgically unresectable hemangioblastomas, as adjuvant treatment for incompletely excised tumors, as definitive treatment for multifocal disease, and as salvage therapy for discrete recurrences after surgery.[100,537] Although SRS treatment of hemangioblastomas in von Hippel-Lindau disease has a low risk for adverse radiation effects, it is associated with diminishing control over a long-term follow-up,[538] and SRS should not be used to prophylactically treat asymptomatic tumors and should be reserved for the treatment of tumors that are not surgically resectable.

Chemotherapy

Because stromal cells in hemangioblastomas secrete VEGF, there is much interest in evaluating small-molecule inhibitors of the vascular endothelial growth factor -2 (VEGF-2) (KDR, FLK-1) receptor as medical management for these tumors, especially for patients with von Hippel-Lindau disease, who routinely harbor multiple hemangioblastomas. Unfortunately, the extreme heterogeneity of tumor growth, with periods of spontaneous stability and a slow overall growth rate, makes it extremely difficult to design trials to rigorously test the efficacy of any systemic therapy. In a small study of sunitinib therapy in 15 patients with von Hippel-Lindau disease–associated tumors, one-third of individuals with renal cell carcinoma achieved a partial response but none with hemangioblastomas.[539]

CHORDOMAS AND CHONDROSARCOMAS

Chordomas and chondrosarcomas are rare, locally destructive, slow-growing, malignant bone tumors. Although skull-base chordomas and chondrosarcomas are sometimes pooled together, recent studies have shown important differences between these entities.

Clinical and Pathologic Considerations

Chordomas arise within aberrant chordal vestiges along the pathway of the primitive notochord that extends from the tip of the dorsum sellae to the coccyx.[540] One-third of chordomas arise cranially, with this location more common in women and younger individuals.[541] Chordomas are extradural, pseudoencapsulated, multilobulated tumors, with a gelatinous consistency centered in the bone, classically with soft tissue extension. Microscopically, the typical chordoma is characterized by cordlike rows of *physaliferous* cells with multiple round, clear cytoplasmic vacuoles that impart a bubbly appearance to the cytoplasm. Two pathologic variants have been described. The *chondroid chordoma* has areas with cartilaginous features but a genetic profile distinct from chondrosarcomas.[542] The *dedifferentiated chordoma* contains areas of typical chordoma admixed with components that resemble high-grade or poorly differentiated spindle cell sarcoma. In typical chordomas, mitotic figures and atypia are rare; a higher mitotic rate and Ki-67 more than 6% are associated with a shorter doubling time.[543]

Chondrosarcomas are cartilage-producing neoplasm that arise within any of the complex synchondroses in the skull base, with the most common sites of origin being the temporo-occipital synchondrosis (66%), the spheno-occiput synchondrosis (28%), and the sphenoethmoid complex (6%).[544] Thus, chondrosarcomas predominantly originate in more lateral skull-base structures, unlike most chordomas, which originate in the midline. Chondrosarcomas can be difficult to differentiate from chordomas on a pathologic examination. Immunohistochemical advances have improved differentiation between chordomas and chondrosarcomas. In one series of 200 chondrosarcomas, 99% stained positive for S100, 0% for keratin, and epithelial membrane antigen was expressed in 8%.[544] These immunohistochemical studies allow a chondrosarcoma to be differentiated from a chordoma, which is reactive for keratin and epithelial membrane antigen. The same series confirmed the low-grade nature of base of skull chondrosarcomas because a majority were grade 1, with no grade 3 tumors identified. Mesenchymal chondrosarcomas may have a separate, more aggressive natural history.[545]

Symptoms that prompt evaluation are typically cranial nerve deficits with the precise deficit dependent on the location and extent of the tumor. In one series, the most common presentation was headaches with intermittent abducens nerve palsy.[546] Additional symptoms can be caused by intracranial extension with compression of the brainstem, pituitary gland, or optic apparatus. Neck pain may develop in lower clival tumors, possibly the result of pathologic fracture or periosteal expansion.

The differential diagnosis of cranial chordoma and chondrosarcoma includes basal meningioma, schwannoma (neurilemoma), nasopharyngeal carcinoma, pituitary adenoma, and craniopharyngioma. Chondrosarcomas and chordomas cannot be reliably distinguished from each other based on imaging features or location alone.[547] High-resolution CT images with bone and soft tissue algorithms show a discrete, expansile soft tissue mass with extensive bony destruction.[548] On MRI scanning, both chordomas and chondrosarcomas are hyperintense on T2-weighted sequences, with variegated enhancement. The location may be useful in distinguishing chordomas (midline clivus) from chondrosarcomas (petrous apex), although there is considerable overlap. Given the low risk of nodal or hematogenous dissemination, imaging beyond the primary site other than a chest x-ray is typically not indicated unless metastatic disease is suspected clinically.[549] A baseline endocrine evaluation and neuro-ophthalmologic examination are both recommended if diagnostic imaging or symptoms suggest involvement.

Surgery

Surgery for cranial chordomas and chondrosarcomas provides the backbone of treatment and is obligatory to obtain diagnostic tissue, to enhance the effectiveness of subsequent RT, and to improve the patient's clinical condition. An aggressive initial approach may improve overall outcome.[126] Intracranial chordomas occur at the base of the skull, a region relatively remote from surgical access. Approaches to skull-base chordomas and chondrosarcomas often involve teams that include both neurosurgeons and otolaryngologists. There is developing interest in the use of endoscopy for the primary removal of chordomas or to assist in the removal of these

tumors via traditional open approaches. Although most series remain small, excellent results have been reported in appropriately selected patients not having extension lateral to the carotid arteries.[550,551] A combination of exposures and procedures can be used for extremely large tumors. One goal of surgery is to remove as much tumor from the optic system and brainstem as possible so that very high doses of radiation can be delivered safely. Optimal treatment of these lesions is complete resection, if possible.

A potentially serious complication of the transsphenoidal, transsphenothmoid, and transoral approaches is CSF leakage into the nose or oropharynx and consequent meningitis. Therefore, every attempt is made to keep the dura intact during these procedures. Because dural invasion by cranial chordomas may occur 50% of the time, dural entry during tumor resection is sometimes unavoidable. Careful intraoperative patching of the leak with fat and muscle grafts followed by postoperative spinal CSF drainage should be undertaken to decrease the risk of infection in these cases. This may be more challenging in the setting of a total endoscopic tumor removal, although some techniques appear to be associated with reasonably low rates of CSF leak. Surgical series have reported gross total resection rates of 43% to 72%, with the most recent series using modern imaging and microsurgical techniques reporting the highest gross total resection rate. In this series, there was a 31% recurrence-free survival at 10 years, which was improved for those without previous intervention, and a 35% recurrence after gross total resection.[552,553] The extent of resection correlated with both recurrence rates and survival. Surgical morbidity can be significant, with Gay et al.[554] reporting a significant transient (53%) and permanent (43%) worsening of the Karnofsky performance score following surgery.

Approaches for chondrosarcomas are different because of the paramedian location of the tumors. Like chordomas, chondrosarcomas begin as extradural tumors, and maintaining the intact dural barrier is paramount. A complete tumor excision, which is paramount in chordoma surgery, is less critical for chondrosarcomas because tumor control rates with adjuvant high-dose radiation are high. Surgery is often tailored to emphasize the removal of tumor portions abutting critical structures such as the chiasm or brainstem to allow adequate radiation treatment. Cranial chordomas often recur after surgery and RT. In this situation, reoperation directed toward symptomatic improvement is the only treatment option. Reoperations are complicated by surgical scarring and tissue compromise from irradiation, and CSF leaks and other complications are more frequent.

Radiation Therapy

A radical excision with negative margins is often not feasible, and even gross excision is often obtained piecemeal with the risk of persistent microscopic disease. Because relentless extension is typical of chordomas and chondrosarcomas and recurrence is a strong predictor of OS, adequate local control is paramount in determining outcome. Radiotherapy is a mainstay of treatment in preventing recurrence or progression of tumor.

Local control of chordomas appears to be dose dependent. Conventional radiation at doses of 50 to 55 Gy does not offer satisfactory local control. A median dose of 50 Gy to chordomas of the skull, sacrum, and mobile spine provided only a 27% local control rate with a median time to progression of 35 months.[549] Durable control was worse in base of skull disease, with only 1 out of 13 clival chordomas remaining disease free. FSRT to 37 sphenooccipital chordomas to a mean dose of 66.6 Gy provided local control rates of 82% at 2 years and 50% at 5 years. Despite a median tumor volume of 55.6 mL, complications were limited with one patient developing a pontine infarct 25 months posttreatment. No instances of optic neuropathy were identified. Chondrosarcomas treated with the same fractionation scheme had 100% 5-year local control.[555]

Radiosurgery

SRS has been used to treat chordomas and chondrosarcomas of the skull base, although its application is limited because of size constraints and proximity to critical structures. In one series, candidates were limited to less than 3 cm in greatest diameter and 5 mm from the optic chiasm, with a mean treatment volume of 4.6 mL and a maximum volume of 10.3 mL.[556] With a mean margin dose of 18 Gy, more than 50% of patients in this mixed series of chondrosarcomas and chordomas had symptomatic improvement and, at a mean follow-up of 40 months, 20% had recurred locally outside of the treatment field. Krishnan et al.[557] reported a similar local control rate (24%) with both in-field and out-of-field recurrences, although no recurrences occurred in patients with chondroid chordomas or chondrosarcomas. The risk of significant radiation-related complications was high at 34%, although complications were seen only in patients who had received prior fractionated radiotherapy.

Particle-Beam Therapy

Charged-particle therapy, because of its innate dose-distribution advantages, has been used for many years to escalate dose to chordomas and chondrosarcomas while minimizing radiation-related side effects. The most extensive experience in treating base of skull chordomas and chondrosarcomas with proton therapy arises from the experience at the Harvard Cyclotron Laboratory. Chordoma relapse-free survival was 59% at 4 years and 44% at 10 years, with similar rates seen in other series.[558–561] Mean dose ranged from 67 to 70.7 cobalt gray equivalent (CGE). Female gender, dose heterogeneity, large tumor size (more than 25 to 75 mL), brainstem invasion, and dose constrained by proximity to critical structures were all associated with higher rates of recurrence.[562] In a study of skull-base chordomas in 73 children and adolescents (mean age, 9.7 years), patients were treated with partial or gross surgical excision and postoperative proton beam irradiation.[559] The mean follow-up period was 7.25 years, and the overall patient survival rate was 81% among 42 patients with conventional chordomas, 17 with chondroid chordomas, and 14 with cellular chordomas, 6 of which were poorly differentiated and highly aggressive. The most recent relatively large proton experience for managing skull-based chordomas comes from the Paul Scherrer Institute (PSI) in Switzerland. They have reported an 81% 5-year local control with surgery plus scanning proton beam therapy in 42 patients, possibly the best data reported in this disease to date. Median total dose for chordomas was 73.5 Gy at 1.8 to 2.0 Gy per fraction. Actuarial 5-year freedom from high-grade toxicity was 94%.[563]

Chondrosarcomas of the skull base had remarkably high local control rates of 99% and 98% at 5 and 10 years, respectively. Pituitary dysfunction and hearing loss were the most common side effects, with depression, memory loss, temporal necrosis, hearing loss, and blindness being less common. Given the relative lack of morbidity and the suboptimal local control for chordomas, dose escalation has been proposed. Recent radiotherapeutic advances include spot-scanning proton radiation.[564] Carbon ion radiotherapy—charged-particle therapy using a heavier ion—has also been used with good local control with a short follow-up and better than expected radiographic responses.[565] Amichetti et al.[566] recently conducted a systematic review of the scientific literature published between 1980 and 2008 on data regarding irradiation of chondrosarcoma of the skull base with proton therapy. From 49 reports retrieved, there were no prospective trials and 9 uncontrolled single-arm studies mainly related to advanced and frequently incompletely resected tumors. According to the inclusion criteria, only four articles, reporting the most recent updated results of the publishing institution, were included in the analysis, providing clinical outcomes for 254 patients. The major findings corroborated the high control rates with low morbidity described previously.

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Chemotherapy

In a prospective, phase II clinical study trial, 56 patients with advanced disease were treated with imatinib.[567] In 50 evaluable patients, one partial response (PR) and 35 stable disease (SD, 70%) and a 64% clinical benefit rate (i.e., Response Evaluation Criteria in Sold Tumors [RECIST] complete response + PR + SD ≥6 months) was noted.[567] Patients who have progressed after an initial response to imatinib can respond to combinations of imatinib and sirolimus.[568]

CHOROID PLEXUS TUMORS

Clinical and Pathologic Considerations

Primary tumors of the choroid plexus (CP) are classified according to the WHO as choroid plexus papilloma (CPP, WHO grade I), atypical CPP (grade II), and choroid plexus carcinoma (CPC, grade III).[4] These are rare tumors that occur most often in children younger than 12 years of age. Although the grade might imply a clinical progression, typical CPP are a distinct entity, and almost never progress to CPC. Choroid plexus tumors appear irregular and lobulated, often very red because of underlying vasculature. Histopathologic examinations of papillomas often show an apparently normal choroid plexus, with increased cellular crowding and elongation. CPC show malignant features such as increased cellularity, high mitotic activity, loss of typical cellular architecture, and invasion of the brain parenchyma. Bridging the CPP and CPC is the entity called atypical CPP. Histologically, atypical CPP retains the architecture of the CPP but has high mitotic activity and an increase probability for recurrence after surgical resection. CPCs are commonly seen in families who carry a germline mutation in either the *TP53* gene (Li-Fraumeni syndrome).[569] However, most patients with CPP and sporadic CPC do not harbor a germline TP53 mutation.

In children, CPCs most often occur in the lateral ventricles. In adults, the fourth ventricular papilloma is most common. Third ventricle tumors are exceedingly rare. Because papillomas tend to grow slowly within ventricles, they expand to fill the ventricle and block CSF flow. In addition, papillomas can secrete CSF. CPPs and CPCs can produce hydrocephalus secondary to obstruction of the CSF, CSF overproduction by the tumor, or damage to the CSF resorptive bed from recurrent hemorrhages. As a result, increased ICP without focal findings is the most common presentation. Fourth ventricular tumors can also be associated with focal findings of ataxia and nystagmus. Although CPPS rarely seed throughout the CSF spaces, seeding from carcinomas is frequent and often symptomatic. CPCs will show invasion of the surrounding brain with resultant increased signal on T2-weighted MRI, and signs of rich vascularity (flow voids), often arising medially from the choroidal vessels.

Choroid plexus tumors are seen easily by MRI. Imaging demonstrates a lobulated, well-circumscribed, enhancing, intraventricular lesion, often with associated hydrocephalus. Calcification is not common. Choroid carcinomas may show areas consistent with necrosis and brain invasion.[570]

Surgery

The complete treatment of CPPs is total excision. Hydrocephalus is the rule and simplifies the exposure once the ventricle is opened. Tumor-associated branches of the choroidal vessels are coagulated and divided as early as is feasible in the procedure because this greatly reduces hemorrhaging. Smaller tumors are removed intact and larger tumors are removed piecemeal. Perioperative CSF drainage is used to prevent subdural hygromas. In half of patients, hydrocephalus is relieved by tumor resection, but persistent hydrocephalus requires shunting. The ability to perform a complete resection depends on histologic type, with nearly a 100% complete resection rate for papillomas versus as low as a 33% complete resection rate for CPCs.[571] A meta-analysis of all individual cases of CPC reported as of 2004 (347 patients) showed that in the subgroup of incompletely resected carcinomas, 22.6% of patients required a second surgery.[572] The prognosis for these patients appeared better than for those with incomplete resections who did not undergo a second surgery (2-year OS times were 69% and 30%, respectively).

In the pediatric age group, when the diagnosis of CPC is suspected, the primary tumor resection should not be attempted because these tumors are extremely vascular, leading to the loss of multiple blood volumes and not infrequent intra-operative deaths. Cases of suspected CPC should be treated with an open biopsy, followed by ICE chemotherapy to devascularize the tumor. Subsequent postchemotherapy surgery is much safer and results in a greatly reduced blood loss.

Radiation Therapy

Because CPPs are often cured by a complete resection, radiotherapy is infrequently employed. Further, in a study of 41 patients, Krishnan et al.[573] noted that reoperation for recurrence was required only half the time after the initial subtotal resection, suggesting that adjuvant radiotherapy may not be necessary after the initial subtotal resection in all patients. Because local control outcome at first relapse was poor after the subtotal resection, they concluded that the most reasonable role for RT is after a subtotal resection of a recurrence.

Chemotherapy

Chemotherapy is not used for CPPs, although it has been attempted for CPCs. As with many of the less common CNS tumors, there are no firm guidelines. Anecdotal reports have cited moderate responses to the platinum compounds, as well as to alkylating agents, etoposide, methotrexate, and possibly anthracyclines. A Pediatric Oncology Group study of eight infants with CPC suggests that radiation can be forestalled by using chemotherapy in some infants with these tumors.[574] In a meta-analysis conducted by Wrede et al.,[575] CPCs were analyzed; 104 cases with CPC received chemotherapy and had a statistically better survival than those without chemotherapy.

SPINAL AXIS TUMORS

Clinical and Pathologic Considerations

Most primary spinal axis tumors produce symptoms and signs as a result of cord and nerve root compression rather than parenchymal invasion. The frequency of primary spinal cord tumors is between 10% and 19% of all primary CNS tumors. Parenthetically, the majority of neoplasms that affect the spine are extradural metastases, whereas most primary tumors are intradural. Of the intradural neoplasms, extramedullary schwannomas and meningiomas are the most common. Schwannomas and meningiomas are normally intradural, but occasionally, may present as extradural tumors. Other intradural, extramedullary neoplasms include vascular tumors, chordomas, and epidermoids. Intramedullary tumors include ependymomas, comprising approximately 40% of intramedullary tumors; the remainder are astrocytomas, oligodendrogliomas, gangliogliomas, medulloblastomas, and hemangioblastomas. Approximately half of spinal tumors involve the thoracic spinal canal (the longest spinal segment), 30% involve the lumbosacral spine, and the remainder involve the cervical spine, including the foramen magnum. Schwannomas occur with greatest frequency in the thoracic spine, although they can be found at

other levels. They often extend through an intervertebral foramen in a dumbbell configuration. Meningiomas are dural based and arise preferentially at the foramen magnum and in the thoracic spine. Most patients are women. Astrocytomas are distributed throughout the spinal cord, and most ependymomas involve the conus medullaris and the cauda equina. Spinal chordomas are characteristically sacral and only rarely affect the cervical region or the rest of the mobile spine.

Patients may present with a sensorimotor spinal tract syndrome, a painful radicular spinal cord syndrome, or a central syringomyelic syndrome. In the sensorimotor presentation, symptoms and signs reflect compression of the cord. The onset is gradual during weeks to months, initial presentation is asymmetric, and motor weakness predominates. The level of impairment determines the muscle groups involved. Because of external compression, dorsal column involvement results in paresthesia and abnormalities of pain and temperature on the side contralateral to the motor weakness.

Radicular spinal cord syndromes occur because of external compression and infiltration of spinal roots. The main symptom is sharp, radicular pain in the distribution of a sensory nerve root. The intense pain is often of short duration, with pain that is more aching in nature persisting for longer periods. Pain may be exacerbated by coughing and sneezing or other maneuvers that increase ICP. Local paresthesia and numbness are common, as are weakness and muscle wasting. These findings often precede cord compression by months. Often, the pain is difficult for the clinician to differentiate from ordinary musculoskeletal symptoms, which causes diagnostic delay.

Intramedullary tumors, in particular, can give rise to syringomyelic dysfunction by destruction and cavitation within the central gray matter of the cord. This produces lower motor neuron destruction with associated segmental muscle weakness, atrophy, and hyporeflexia. There is also a dissociated sensory loss of pain and temperature sensation with the preservation of touch. As the syrinx increases in size, all sensory modalities are affected.

Surgery

The operating microscope is essential for spinal cord tumor surgery. Ultrasonography can be used to examine the spinal cord through either intact or open dura to find the level of maximum tumor involvement or to differentiate tumor cysts from solid tumors. Intraoperative monitoring of somatosensory-evoked potentials is commonly used, although some surgeons think that changes in somatosensory-evoked potentials may occur only after irretrievable damage has occurred, and this remains a topic of controversy. Motor-evoked potentials are used in some centers to guide resection and have retrospectively been shown by some to decrease long-term motor deficits.

MRI is invaluable for the diagnosis, localization, and characterization of spinal tumors. For extremely vascular tumors—notably, hemangioblastoma—angiography may provide important preoperative delineation of the tumor blood supply. CT scanning is useful for tumors of the bony axis. Determination of the spinal level of the tumor and its exact relation to the cord is important. Corticosteroids are given before, during, and after spinal cord tumor surgery to help control spinal cord edema.

Meningiomas and schwannomas occur in the intradural, extramedullary spinal compartment. Most of these tumors can be completely resected through a laminectomy. They can be easily separated away from the cord, which is displaced but not invaded by tumor. Schwannomas arise most often in the dorsal spinal rootlets, and their removal includes the rootlets involved. They can grow along the nerve root in a dumbbell fashion through a neural foramen. Some of these can be removed by extending the initial laminectomy exposure laterally, whereas others require a separate operation (e.g., a thoracotomy, costotransversectomy, or a retroperitoneal approach). Strictly anteriorly situated cervical tumors can successfully be removed via an anterior approach using a corpectomy of the appropriate vertebral levels, followed by strut grafting after the tumor resection.

The most common intramedullary tumors are ependymomas and astrocytomas. Except for malignant astrocytomas, resection is the principal treatment for these tumors. Intramedullary tumors are approached through a laminectomy. After dural opening, a longitudinal myelotomy is made, usually in the midline or dorsal root entry zone. The incision is deepened several millimeters to the tumor surface. Dissection planes around the tumor are sought microsurgically and, in the case of ependymomas, usually found and extended gradually around the tumor's surface, whereas removal of the central tumor bulk (by carbon dioxide laser or ultrasonic aspirator) causes the tumor to collapse. Such tumors are usually completely removed, with good long-term outcomes.[576] Some patients later develop a spinal deformity, requiring stabilization procedures.[577] Tumors without clear dissection planes (usually astrocytomas) cannot be removed completely, but bulk reduction can cause long-term palliation. If a frozen-section analysis shows a tumor to be a malignant glioma, a less aggressive surgery is typically performed due to the increased risk of morbidity with little benefit achieved from an extensive debulking procedure.

Radiation Therapy

RT is recommended for unresectable and incompletely resected neoplasms of the spinal axis. In general, doses of 50 to 54 Gy (1.8 Gy per day) are used so that the risk of injury to the cord from radiation is minimized. When lesions involve only the cauda equina or when complete, irreversible myelopathy already has occurred, higher doses are used.

Ependymomas have a longer natural history than astrocytomas. Recurrence of ependymomas may be delayed for as long as 12 years.[578,579] RT is not necessary when ependymomas are removed completely in an en bloc fashion.[576] All nonirradiated patients with incompletely excised lesions reported by Barone and Elvidge[580] and by Shuman et al.[581] experienced recurrence. Postoperative irradiation appears to improve tumor control for incompletely resected ependymomas. Five- and 10-year survival rates in irradiated patients with localized ependymomas range from 60% to 100% and 68% to 95%, respectively, whereas 10-year relapse-free survival rates vary from 43% to 61%.[582] The tumor grade has a significant effect on outcome. Waldron et al.[579] found that for patients with well-differentiated tumors, the 5-year cause-specific survival was 97% compared with 71% for patients with intermediate or poorly differentiated tumors ($p = 0.005$). Myxopapillary ependymomas that arise in the conus medullaris and filum terminale have a better prognosis than the cellular ependymomas that arise in the cord.[583] Local recurrence is the predominant pattern of treatment failure, occurring in 25% of irradiated patients.[579]

The 5- and 10-year survival rates for irradiated patients with low-grade astrocytomas of the spinal cord vary from 60% to 90% and 40% to 90%, respectively; 5- and 10-year relapse-free survival rates range from 66% to 83% and 53% to 83%, respectively.[577,579] Fifty percent to 65% of astrocytomas are controlled locally. Good neurologic condition at the time of irradiation, lower histologic grade, and younger age are favorable factors.[584] Patterns of recurrence for malignant astrocytomas of the spine have been analyzed by MRI.[585] Despite surgery and full-dose radiation, spinal or brain dissemination is the predominant mode of failure.

Chemotherapy

There are no significant controlled clinical trials of chemotherapy for primary spinal axis tumors. Drugs active against intracranial tumors logically may be assumed to be equally efficacious against histologically identical tumors in the spinal cord. Temozolomide is being increasingly used in this setting.

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The full reference list can be accessed at lwwhealthlibrary.com/oncology.

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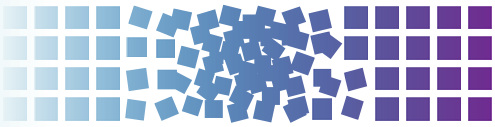

Lee J. Helman and David Malkin

INTRODUCTION

Tumors of childhood are clinically, histopathologically, and biologically distinct from that of adult-onset malignancies. Childhood cancers tend to have short latency periods, are often rapidly growing and aggressively invasive, are rarely associated with exposure to carcinogens, and are generally more responsive to standard modalities of treatment, in particular chemotherapy. Most childhood tumors occur sporadically in families with, at most, a weak history of cancer. In at least 10% to 15% of cases, however, a strong familial association is recognized or the child has a congenital or genetic disorder that imparts a higher likelihood of specific cancer types.[1] Examples of genetic disorders that render a child at increased risk of tumor development include xeroderma pigmentosa, Bloom syndrome, or ataxia telangiectasia, which predispose to skin cancers, leukemias, or lymphoid malignancies, respectively. In all three cases, constitutional gene alterations that disrupt normal mechanisms of genomic DNA repair are blamed for the propensity to cell transformation. Other hereditary disorders, including Beckwith-Wiedemann syndrome (BWS), von Hippel-Lindau disease, Rothmund-Thomson syndrome, and the multiple endocrine neoplasias types 1 and 2, are associated with their respective tumor spectra through constitutional activation of molecular pathways of deregulated cellular growth and proliferation. The cancers that occur in these syndromes are generally secondary phenotypic manifestations of disorders that have distinctive recognizable physical stigmata. On the other hand, some cancer predisposition syndromes are recognized only by their malignant manifestations, with nonmalignant characteristics being virtually absent. These include hereditary retinoblastoma, Li-Fraumeni syndrome (LFS), familial Wilms tumor, and familial adenomatous polyposis coli. Each of these presents with distinct cancer phenotypes and unique molecular defects (Table 98.1). Careful attention to detailed cancer family histories continues to lead to the discovery of new cancer predisposition syndromes and the coincident identification of novel cancer genes.[2]

The study of pediatric cancer and rare hereditary cancer syndromes and associations has led to the identification of numerous cancer genes, including dominant oncogenes, DNA repair genes, and tumor suppressor genes. These genes are important not only in hereditary predisposition, but also in the normal growth, differentiation, and proliferation pathways of all cells. Alterations of these genes have been consistently found in numerous sporadic tumors of childhood and led to studies of their functional role in carcinogenesis. The numerous properties of transformed malignant cells in culture or in vivo can be explained by the complex abnormal interaction of numerous positive and negative growth-regulatory genes. Pediatric cancers offer unique models in which to study these pathways in that they are less likely to be disrupted by nongenetic factors. The embryonic ontogeny of many childhood cancers suggests that better understanding of the nature of the genetic events leading to these cancers will also augment the understanding of normal embryologic growth and development.

This chapter begins with an outline of tumor suppressor genes—the most frequently implicated class of cancer genes in childhood malignancy. This leads into a discussion of molecular features of retinoblastoma, the paradigm of cancer genetics, followed by an analysis of the molecular pathways associated with other common pediatric cancers. Evaluations of the importance of molecular alterations in familial cancers, as well as new approaches in molecular therapeutics are also addressed.

TUMOR SUPPRESSOR GENES

Faulty regulation of cellular growth and differentiation leads to neoplastic transformation and tumor initiation. Many inappropriately activated growth-potentiating genes, or *oncogenes*, have been identified through the study of RNA tumor viruses and the transforming effects of DNA isolated from malignant cells. However, activated dominant oncogenes themselves do not readily explain a variety of phenomena related to transformation and tumor formation. Among these is the suppression of tumorigenicity by the fusion of malignant cells with their normal counterparts. If these malignant cells carried an activated dominant oncogene, it would be expected that such a gene would initiate transformation of the normal cells, likely leading to either embryonic or fetal death. The observation is more readily explained by postulating the existence of a factor in the normal cell that acts to suppress growth of the fused malignant cells. Malignant cells commonly exhibit specific chromosomal deletions (Table 98.2). The best example of this occurs in retinoblastoma, a rare pediatric eye tumor in which a small region of the long arm of chromosome 13 is frequently missing. The presumed loss of genes in specific chromosomal regions argues strongly against the concept of a dominantly acting gene being implicated in the development of the tumor. Comparisons between the frequencies of familial tumors and their sporadic counterparts led Knudson[3] to suggest that the familial forms of some tumors could be explained by constitutional mutations in growth-limiting genes. The resulting inactivation of these genes would facilitate cellular transformation.[4] Such growth-limiting genes were termed *tumor suppressor genes*.

Whereas acquired alterations of dominant oncogenes most commonly occur in somatic cells, mutant tumor suppressor genes may be found either in germ cells or somatic cells. In the former, they may arise de novo or be transmitted from generation to generation within a family. The diversity of functions, cellular locations, and tissue-specific expression of the tumor suppressor genes suggest the existence of a complex, yet coordinated, cellular pathway that limits cell growth by linking nuclear processes with the intra- and extracytoplasmic environment. This discussion is limited to those genes for which pediatric tumors are frequently associated.

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TABLE 98.1

Hereditary Syndromes Associated with Childhood Cancer Predisposition

Syndrome	OMIM Entry[a]	Major Tumor Types	Mode of Inheritance	Genes
Hereditary Gastrointestinal Malignancies				
Adenomatous polyposis of the colon	175100	Colon, thyroid, stomach, intestine, hepatoblastoma	Dominant	*APC*
Juvenile polyposis	174900	Gastrointestinal	Dominant	*SMAD4/DPC4*
Peutz-Jeghers syndrome	175200	Intestinal, ovarian, pancreatic	Dominant	*STK11*
Genodermatoses with Cancer Predisposition				
Nevoid basal cell carcinoma syndrome	109400	Skin, medulloblastoma	Dominant	*PTCH*
Neurofibromatosis type 1	162200	Neurofibroma, optic pathway glioma, peripheral nerve sheath tumor	Dominant	*NF1*
Neurofibromatosis type 2	101000	Vestibular schwannoma	Dominant	*NF2*
Tuberous sclerosis	191100	Hamartoma, renal angiomyolipoma, renal cell carcinoma	Dominant	*TSC1/TSC2*
Xeroderma pigmentosum	278730, 278700, 278720, 278760, 278740, 278780, 278750, 133510	Skin, melanoma, leukemia	Recessive	*XPA, B, C, D, E, F, G, POLH*
Rothmund-Thomson syndrome	268400	Skin, bone	Recessive	*RECQL4*
Leukemia/Lymphoma Predisposition Syndromes				
Bloom syndrome	210900	Leukemia, lymphoma, skin	Recessive	*BLM*
Fanconi anemia	227650	Leukemia, squamous cell carcinoma, gynecologic system	Recessive	*FANCA, B, C, D_2, E, F, G*
Shwachman-Diamond syndrome	260400	Leukemia/myelodysplasia	Recessive	*SBDS*
Nijmegen breakage syndrome	251260	Lymphoma, medulloblastoma, glioma	Recessive	*NBS1*
Ataxia telangiectasia	208900	Leukemia, lymphoma	Recessive	*ATM*
Genitourinary Cancer Predisposition Syndromes				
Simpson-Golabi-Behmel syndrome	312870	Embryonal tumors, Wilms tumor	X-linked	*GPC3*
Von Hippel-Lindau syndrome	193300	Retinal and central nervous hemangioblastoma, pheochromocytoma, renal cell carcinoma	Dominant	*VHL*
Beckwith-Wiedemann syndrome	130650	Wilms tumor, hepatoblastoma, adrenal carcinoma, rhabdomyosarcoma	Dominant	*CDKN1C/ NSD1*
Wilms tumor syndrome	194070	Wilms tumor	Dominant	*WT1*
WAGR syndrome	194072	Wilms tumor, gonadoblastoma	Dominant	*WT1*
Costello syndrome	218040	Neuroblastoma, rhabdomyosarcoma, bladder carcinoma	Dominant	*H-Ras*
Central Nervous System Predisposition Syndromes				
Retinoblastoma	180200	Retinoblastoma, osteosarcoma	Dominant	*RB1*
Rhabdoid predisposition syndrome	601607	Rhabdoid tumor, medulloblastoma, choroid plexus tumor		*SNF5/INI1*
Medulloblastoma predisposition	607035	Medulloblastoma	Dominant	*SUFU*
Sarcoma/Bone Cancer Predisposition Syndromes				
Li-Fraumeni syndrome	151623	Soft tissue sarcoma, osteosarcoma, breast, adrenocortical carcinoma, leukemia, brain tumor	Dominant	*TP53*
Multiple exostoses	133700, 133701	Chondrosarcoma	Dominant	*EXT1/EXT2*
Werner syndrome	277700	Osteosarcoma, meningioma	Recessive	*WRN*
Endocrine Cancer Predisposition Syndromes				
MEN1	131000	Pancreatic islet cell tumor, pituitary adenoma, parathyroid adenoma	Dominant	*MEN1*
MEN2	171400	Medullary thyroid carcinoma, pheochromocytoma, parathyroid hyperplasia	Dominant	*RET*

[a] Online Mendelian Inheritance in Man (OMIM), http://omim.org/search/advanced/geneMap.
WAGR, Wilms tumor, aniridia, genitourinary abnormalities, mental retardation; MEN, multiple endocrine neoplasia.
Adapted from Hahn H, Wojnowski L, Zimmer AM, et al. Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome. *Nat Med* 1998;4:619–622.

TABLE 98.2

Common Cytogenetic Rearrangements in Solid Tumors of Childhood

Solid Tumor	Cytogenetic Rearrangement	Genes[a]
Ewing sarcoma	t(11;22) (q24;q12), +8	*EWS(22) FLi-1(11)*
Neuroblastoma	del1p32–36, DMs, HSRs, +17q21-qter	*N-MYC*
Retinoblastoma	del13q14	*Rb*
Wilms tumor	del11p13, t(3;17)	*WT1*
Synovial sarcoma	t(X;11) (p11;q11)	*SSX(X) SYT(18)*
Osteogenic sarcoma	del13q14	*?*
Rhabdomyosarcoma	t(2;13) (q37;q14), t(2;11),3p-,11p-	*PAX3(2) FOXO1(13)*
Peripheral neuroepithelioma	t(11;22) (q24;q12), +8	*EWS(22) FLi-1(11)*
Astrocytoma	i(17q)	*?*
Meningioma	delq22, -22	*MN1, NF2, ?*
Atypical teratoid/rhabdoid tumor	delq22.11	*SNF 5*
Germ cell tumor	i(12p)	

[a] Chromosomal location in parentheses.

RETINOBLASTOMA: THE PARADIGM

Retinoblastoma is the prototype cancer caused by mutations of a tumor suppressor gene. It is a malignant tumor of the retina that occurs in infants and young children, with an incidence of approximately 1:20,000.[5] Approximately 40% of retinoblastoma cases are of the heritable form in which the child inherits one mutant allele at the retinoblastoma susceptibility locus (*Rb1*) through the germ line, and a somatic mutation in a single retinal cell causes loss of function of the remaining normal allele, leading to tumor formation. Tumors are often bilateral and multifocal. The disease is inherited as an autosomal-dominant trait, with a penetrance approaching 100%.[6] The remaining 60% of retinoblastoma cases are sporadic (nonheritable), in which both *Rb1* alleles in a single retinal cell are inactivated by somatic mutations. As one can imagine, such an event is rare, and these patients usually have only one tumor that presents itself later than in infants with the heritable form. Fifteen percent of unilateral retinoblastoma is heritable[6] but by chance develops in only one eye. Survivors of heritable retinoblastoma have a several 100-fold increased risk of developing mesenchymal tumors such as osteogenic sarcoma, fibrosarcomas, and melanomas later in life.[7] Several genetic mechanisms have been implicated in elimination of the second wild-type *Rb1* allele in an evolving tumor. These include chromosomal duplication or nondisjunction, mitotic recombination, or gene conversion.[8]

The *Rb1* gene maps to chromosome 13q14 and encodes a 105kD phosphoprotein.[9,10] The second target gene that led to disease was actually the second copy of the Rb1 locus. Reduction to homozygosity of the mutant allele (or loss of heterozygosity [LOH] of the wild-type allele) would lead to the loss of functional Rb1 and account for tumor development. As well as being altered in retinoblastoma, *Rb1* and its protein product are altered in osteosarcomas, small cell lung carcinomas, and bladder, breast, and prostate carcinomas.[10,11] *Rb1* plays a central role in the control of cell-cycle regulation, particularly in determining transition from G1 through S (the DNA synthesis) phase in virtually all cell types.

Although it is clear that *Rb1* and its protein product play some role in growth regulation, the precise nature of this role remains obscure. In the developing retina, inactivation of the *Rb1* gene is necessary but not sufficient for tumor formation.[12] Retinoblastomas develop as a result of a complex interplay of aberrant expression of other cell-cycle control genes. In particular, a tumor surveillance pathway mediated by Arf, MDM2, MDMX, and p53 (see later discussion) is activated after a loss of *Rb1* during development of the retina. *Rb1*-deficient retinoblasts undergo p53-mediated apoptosis and exit the cell cycle. Subsequently, amplification of the *MDMX* gene and increased expression of MDMX protein are strongly selected for during tumor progression as a mechanism to suppress the p53 response in *Rb1*-deficient retinal cells.[13] Not only do these observations provide a provocative biologic mechanism for tumor formation in retinoblastoma, but it also offers potential molecular targets for novel therapeutic approaches to this tumor.[14,15] Some *Rb1* mutations appear to lead to an attenuated form of the disease, an observation that highlights the variable penetrance in families.[16,17] Outside the retina, *Rb1* inactivation is often a rate-limiting step in tumorigenesis generated by multiple genetic events. The molecular characteristics and potential functional activities of *Rb1* are outlined in detail elsewhere in this volume.

The patterns of inheritance and presentation of retinoblastoma have been well described and the responsible gene has been identified. The basic mechanisms by which the gene is inactivated are understood, and provocative evidence indicates that the intricate functional interactions of pRB with its binding partners and other cell cycle targets will provide targets for development of novel small molecule therapies.

SDH DEFICIENT GIST

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors found in the gastrointestinal (GI) tract, and occur in both adults and children. Strikingly, unlike most adult GIST tumors that harbor either *KIT* or platelet-derived growth factor receptor A (*PDGFRA*) mutations and are susceptible to tyrosine kinase (TK) inhibitors directed against these kinases, 85% of pediatric GIST tumors do not harbor such mutations, and not surprisingly are much less responsive to TK inhibitors. Thus, it appears that a histologically similar tumor in a pediatric population is remarkably biologically distinct from the same tumor in the adult population. It was recently demonstrated that the pediatric-type GIST tumor is characterized by metabolic derangement of the mitochondrial enzyme, succinate dehydrogenase (SDH).[18] Many of these patients have been found to carry germ-line mutations of SDH subunits B, C, or D (SDH_x) mutations. These germ-line

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mutations were first described in Carney-Stratakis syndrome, an inherited predisposition to GIST and paragangliomas.[19] It is noteworthy that these SDH-deficient pediatric predominant GISTs have a marked predilection for gastric location and are more frequent in the female population.[20]

Further characterization of molecular differences between the typical adult GIST, *KIT*, or *PDGFRA* activated tumors and SDH-deficient, pediatric-type GIST tumors revealed marked epigenetic distinctions between these two types of GISTs. Specifically, the SDH-deficient tumors were found to have marked global hypermethylation compared to the TK-mutant GISTs. Of specific note was enrichment for hypermethylation within DNase hypersensitivity sites.[21]

Thus it is clear that most pediatric GISTs are driven by a distinct mechanism related to SDH-deficient function compared to the adult GISTs, which are driven by the mutation activation of KIT or PDGFRA kinases. Furthermore, many of these tumors are associated with germ-line mutations of SDH_x, and may or may not be associated with paragangliomas. The finding of global hypermethylation in the pediatric-type GISTs was associated with very stable genomes, suggesting these tumors are epigenetically driven tumors.[21] Overall, these data clearly suggest that despite histologically similar tumors, the GISTs that occur in the younger population have a distinct behavior that will require management that is quite different than the management of adult, TK-mutating GISTs.

NEUROFIBROMATOSES

The neurofibromatoses comprise two similar entities. Neurofibromatosis type 1 (NF1) is one of the most common autosomal-dominantly inherited disorders, affecting about 1 in 3,500 people,[22] half of which arise from new spontaneous mutations. Carriers of mutant NF1 are predisposed to a variety of tumors, including Schwann cell–derived tumors (neurofibromas and malignant peripheral nerve sheath tumors, gliomas including optic nerve gliomas and malignant gliomas, and pheochromocytomas).[23,24] Occurring with less frequency are leukemias, osteosarcomas, rhabdomyosarcomas, GISTs, and Wilms tumors.

Using a standard linkage analysis, the neurofibromatosis type 1 (NF1) gene was mapped to chromosomal band 17q11, and subsequently cloned.[25,26] The NF1 gene is unusual in that it contains three embedded genes, OMGP, EV12A, and EV12B, in a single intron.[27] This gene encodes a 2818 amino acid protein, termed *neurofibromin*, that is ubiquitously expressed. One region of the gene shows extensive structural homology to the GTPase activating domain of mammalian GTP-ase–activating protein (GAP) proteins: loss of the protein's activity results in failure of hydrolysis of GTP to guanosine diphosphate (GDP) by the ras oncoprotein. Loss of neurofibromin function usually results from mutations in one allele of the gene leading to premature truncation of the protein, followed by absence or mutations of the second allele in tumors. This loss of function is thought to lead to elevated levels of the GTP-bound RAS protein that transduces signals for cell division. Downstream modulators of RAS activation in NF1-associated tumors include activation of mammalian target of rapamycin (mTOR) and mitogen activated protein kinase kinase (MEK).[28,29] Thus, although targeting RAS remains elusive, mTOR and MEK inhibitors are currently either available or are being tested in the clinic. Neurofibromatosis type 2 (NF2) is much less frequent than NF1, occurring in only 1 in 1 million persons. Although it is also inherited as an autosomal-dominant disorder with high penetrance, the new mutation rate in NF2 is low.[30] It is clinically characterized by bilateral vestibular schwannomas, spinal nerve root tumors, meningiomas, and ependymomas.

The NF2 locus was mapped to chromosome 22, band q12,[31] and its 69-kDa encoded protein, termed merlin, has been shown to be expressed in various tissues, including the brain, although not as ubiquitously as NF1.[32,33] The mechanism of tumor formation in NF2 appears to be in concordance with the Knudson two-hit model, although the mechanism of action of the NF2 protein has not yet been elucidated. Merlin is a member of the Band 4.1 family of proteins that link cell surface proteins to the cytoskeleton.[34] Merlin has been shown to inhibit the number of signaling pathways critical for normal growth including mTOR, Rac1, and Hippo/YAP, with loss of Merlin leading to activation of these pathways, again suggesting potential therapies aimed at inhibition of these pathways.[34]

NEUROBLASTOMA

Nonrandom chromosomal abnormalities are observed in more than 75% of neuroblastomas.[35] The most common of these is deletion or rearrangement of the short arm of chromosome 1, although loss, gain, and rearrangements of chromosomes 10, 11, 14, 17, and 19 have also been reported. The allelic losses indicate loss of function of as yet unknown tumor suppressor genes in these regions. It is believed that a tumor suppressor gene, such as CHD5 and the kinesin KIF1Bbeta that lies on band p36 of chromosome 1 is critically important in the pathogenesis and aggressive nature of neuroblastoma.[36,37] It has been shown that the loss of chromosome 1p is a strong prognostic factor in patients with neuroblastoma, independent of age and stage.[38] Although it is as yet unclear which gene(s) in this region may be directly implicated in neuroblastoma development, aberrant expression of one candidate—*p73*—which is a member of the *p53* tumor suppressor family, has been suggested to play a role in the neuroblastoma cell growth as well as chemotherapy resistance.[39] *p73* gives rise to multiple functionally distinct protein isoforms as a result of alternative promoter utilization and alternative mRNA splicing.[40,41] Alternative splicing of the *p73* mRNA results in more than seven protein isoforms that differ in the coding sequences of the COOH terminus (TA-p73 α, β, γ, δ, ε, ζ, η).

In addition to these COOH-terminal splice forms, three additional forms, Np73α, ΔNp73β, and ΔNp73γ, are transcribed from an alternative promoter located in intron 3. Higher levels of ΔNp73 are associated with an overall worse clinical prognosis, presumably because of the *antiapoptotic* properties of ΔNp73 and its ability to inactivate both TAp73 and p53.[42,43]

Two other unique cytogenetic rearrangements are highly characteristic of neuroblastomas.[44] These structures, homogeneous staining regions (HSR) and double-minute chromosomes (DM), contain regions of gene amplification. The *N-myc* gene, an oncogene with considerable homology to the cellular protooncogene *c-myc*, is amplified within HSR and DM. Virtually all neuroblastoma tumor cell lines demonstrate amplified and highly expressed *N-myc*,[45] and *N-myc* amplification is thought to be associated with rapid tumor progression. Expression of *N-myc* is increased in undifferentiated tumor cells compared with much lower (or single-copy) levels in more differentiated cells (ganglioneuroblastomas and ganglioneuromas). *N-myc* expression is diminished in association with the in vitro differentiation of neuroblastoma cell lines.[46] This observation formed the basis for current therapeutic trials demonstrating a survival advantage to patients treated with *cis*-retinoic acid.[47] Furthermore, a close correlation exists between *N-myc* amplification and advanced clinical stage.[48]

Neuroblastoma cells that express the high-affinity nerve growth factor receptor trkA[49] can be terminally differentiated by nerve growth factor and may demonstrate morphologic changes typical of ganglionic differentiation. Tumors showing ganglionic differentiation and *trk* gene activation have a favorable prognosis.[49] In contrast, trkB receptor expression is associated with poor-prognosis tumors and appears to mediate resistance to chemotherapy.[50,51] Resistance to multidrug chemotherapeutic regimens (i.e., multidrug resistance) is characteristic of aggressive, poorly responsive *N-myc*–amplified neuroblastomas. It is interesting to note that expression of the multidrug resistance–associated protein, found

to confer multidrug resistance in vitro, is increased in neuroblastomas with *N-myc* amplification, decreased after differentiation of tumor cells in vitro, and associated with poor outcome independent of *N-myc* amplification.[52] Gain of chromosome segment 17q21-qter, in which *BICR5*, which encodes Survivin (a member of the apoptosis inhibiting proteins NM23 and PMMID) has been posited as a neuroblastoma-associated candidate gene, has been shown to be the most powerful prognostic factor yet.[53]

A small subset of neuroblastomas is inherited in an autosomal-dominant fashion. Until recently, the only gene definitively associated with neuroblastoma risk was *PHOX2B*, also linked to central apnea.[54] De novo or inherited missense mutations in the TK domain of the anaplastic lymphoma kinase (*ALK*) gene on chromosome 2p23 have been observed in the majority of hereditary neuroblastoma families, as well as in somatic tumor cells.[55–58] Current phase I/II clinical trials with ALK inhibitors substantiate the value of such target identification for novel therapies.[59] The role of other molecular alterations in neuroblastoma continues to be elucidated. In addition to chromosomal loss on chromosome 1p36, unbalanced LOH at 11q23 is independently associated with decreased event-free survival. Alterations at 11q23 occur in almost one-third of neuroblastomas, being most commonly associated with stage 4 disease and age at diagnosis greater than 2.5 years. Both 1p36 LOH and 11q23 LOH were independently associated with decreased progression-free survival in patients with low- and intermediate-risk disease.[60]

Yet another valuable biologic marker of clinical significance is telomerase expression and telomere length. In particular, short telomere length is predictive of favorable prognosis, irrespective of disease stage, whereas long or unchanged telomeres are predictive of poor outcome.[61,62] Telomerase expression, as measured by human telomerase reverse transcriptase (hTERT), has been shown to be negative in good-risk neuroblastomas, although it is high in tumors with unfavorable histology.[62] The combined use of these markers—chromosomes 1p and 11q, *N-Myc* amplification, *trkA*, and telomerase expression—as prognostic indicators provides a powerful armamentarium with which to develop rational stratified treatment programs for neuroblastomas.

EWING SARCOMA FAMILY OF TUMORS

Ewing sarcoma (ES) is one of the first examples in which the application of molecular diagnostics led to improved tumor classification. ES was first described by James Ewing[63] as a bone tumor characterized by small, blue, round cells and minimal mitotic activity. Turc-Carel et al.[64] identified a recurring reciprocal t(11;22) chromosomal translocation in these tumors in 1983. Investigators subsequently demonstrated a cytogenetically identical t(11;22) in adult neuroblastoma or peripheral primitive neuroectodermal tumor (pPNET), so named because of its histologic similarity to neuroblastoma.[65] Based on the presence of the identical translocation, it was hypothesized that pPNET was related to ES. This translocation breakpoint has been molecularly characterized as an in-frame fusion between a new ES gene, *Ewing's sarcoma breakpoint region 1 (EWS)*, on chromosome 22 and an ETS transcription family member, friend leukemia virus integration 1 (FLI-1), on chromosome 22.[66–68]

In addition to this fusion transcript being identified in pPNET, other variants, notably the chest-wall Askin tumor and soft tissue ES—previously treated as an RMS because of its location in soft tissue—were also shown to bear the identical fusion transcript. In total, five translocations also have been identified, invariably fusing the *EWS* gene to an ETS family member.[69–72] More than 90% of the ES family of tumors (ESFT) carry the *EWS-ETS* fusion gene, and a search for *EWS-ETS* by either reverse transcriptase-polymerase chain reaction or fluorescence in situ hybridization should be considered standard practice in the diagnostic evaluation of suspected ESFTs. Interestingly, although it was suggested that the specific fusion protein expressed in ESFT has prognostic significance,[73] several prospective studies in the United States and Europe demonstrated no prognostic impact.[74,75] The nature of the novel fusion transcription factor and its downstream targets is currently under intense investigation. One target of the EWS-ETS fusion is repression of the transforming growth factor-β type II receptor,[76] a putative tumor suppressor gene.

Expression profiling analysis has also revealed that p53 is transcriptionally upregulated by the *EWS-ETS* fusion gene.[77] This is of particular interest because it is now known that expression of *EWS-ETS* can lead to apoptosis, and that additional alterations such as loss of *p53* or *p16* signaling, or both, appear to be necessary components of EWS-ETS–induced transformation.[78] Investigators have now taken advantage of RNA interference technology to inhibit EWS–FLI-1 in Ewing cell lines to identify genes regulated by the fusion in the proper context. Using this approach, *NKX2.2* and *NR0B1* have been found to be a target gene of EWS–FLI-1 that is necessary for oncogenic transformation.[79,80] Recent findings suggest that GGAA microsatellites might mark genes that are upregulated by EWS–FLI-1 binding.[81]

RHABDOMYOSARCOMA

The two major histologic subtypes of RMS, embryonal and alveolar, have both unique histologic appearance as well as distinctive molecular genetic abnormalities, while sharing a common myogenic lineage. Embryonal tumors comprise two-thirds of all RMS, and are histologically characterized by a stroma-rich spindle-cell appearance. Alveolar tumors comprise about one-third of RMS, and are histologically characterized by densely packed small round cells often lining a septation reminiscent of a pulmonary alveolus, giving rise to its name. Both histologic subtypes express muscle-specific proteins, including α-actin, myosin, desmin, and MyoD,[82–84] and they virtually always express high levels of insulin-like growth factor 2 (IGF2).[85,86]

At the molecular level, embryonal tumors are characterized by LOH at the 11p15 locus, which is of particular interest because this region harbors the IGF2 gene.[87,88] The LOH at 11p15 occurs by loss of maternal and duplication of paternal chromosomal material.[89] Although LOH is normally associated with loss of tumor suppressor gene activity, in this instance, LOH with paternal duplication may result in activation of IGF2. This occurs because IGF2 is now known to be normally imprinted; that is, this gene is normally transcriptionally silent at the maternal allele, with only the paternal allele being transcriptionally active.[90,91] Thus, LOH with paternal duplication potentially leads to a twofold gene-dosage effect of the IGF2 locus. Furthermore, in alveolar tumors where LOH does not occur, the normally imprinted maternal allele has been shown to be reexpressed.[92,93] Thus, LOH and loss of imprinting (LOI) may in this case lead to the same functional result—namely, biallelic expression of the normally monoallelically expressed IGF2. However, loss of an as yet unidentified tumor suppressor activity due to LOH also remains a possibility.

Alveolar RMS is characterized by a t(2;13)(q35;q14) chromosomal translocation.[94] Molecular cloning of this translocation has identified the generation of a fusion transcription factor, fusing the 5′ DNA-binding region of *PAX-3* on chromosome 2 to the 3′ transactivation domain region of *FOXO1* gene on chromosome 13.[95,96] A variant t(1;13)(q36;q14) has been identified in a small number of alveolar RMS tumors that fuses the 5′ DNA-binding region of the *PAX-7* gene on chromosome 1 with the identical 3′ transactivation domain of the FOXO1 gene.[97] Fluorescence in situ hybridization (FISH) or reverse transcription polymerase chain reaction (RT-PCR) can be used to identify these *PAX3/7-FOXO1* fusions in approximately 90% of tumors, and are diagnostic of alveolar rhabdomyosarcoma (ARMS). As noted previously, the fusion protein generated by the translocation leads to a novel transcription factor.

The recent application of molecular techniques have now clearly shown that PAX3/7-mutation positive RMS is clearly distinct from fusion-negative tumors. At the RNA expression level,

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fusion-positive tumors cluster together and have an expression profile that is distinct from fusion-negative tumors. Furthermore, the fusion-positive tumors have a much more aggressive clinical behavior.[98] Using current next-generation sequencing techniques, it has now also been shown that the fusion-negative tumors have significantly more mutations per tumor compared to fusion-positive tumors.[99,100] Recurrent mutations identified in fusion-negative RMS included both previously identified (*H, K and NRAS, FGFR4, phosphatidyl–4, 5 bisphosphate 3–kinase catalytic subunit alpha [PIK3CA], and NF1)* as well as a ubiquitin ligase *FBXW7*, and a transcriptional repressor *BCOR*.

Notably, many of the identified mutations in fusion-negative RMS were shown to be transcriptional targets of PAX fusion proteins, suggesting either accumulation of point mutations or upregulation of the same genes by aberrant transcription activation lead to similar tumors. And despite the relative paucity of mutations compared to adult tumors, there is accumulating evidence that targeting members of the RAS/Phosphatidyl – 4, 5 bisphosphate 3 – kinase catalytic subunit alpha (PIC3CA) pathway represents a rational approach to development of new therapeutic approaches to these tumors.[101]

HEREDITARY SYNDROMES ASSOCIATED WITH TUMORS OF CHILDHOOD

LI-FRAUMENI SYNDROME

Although a detailed description of the many syndromes that are associated with the development of childhood cancers are beyond the scope of this chapter, several are discussed here to illustrate the breadth of phenotypes and underlying genetic mechanisms that should be considered in their management. A few hereditary cancer syndromes are associated with the occurrence of childhood as well as adult-onset neoplasms. The paradigm LFS cancer was first described in 1969 from an epidemiologic evaluation of more than 600 medical and family history records of patients with childhood sarcomas.[102] The original description of a kindred with a spectrum of tumors that includes soft tissue sarcomas, osteosarcomas, breast cancer, brain tumors, leukemia, and adrenocortical carcinoma (ACC) has been overwhelmingly substantiated by numerous subsequent studies,[103] although other cancers, usually of particularly early age of onset, are also observed.[104] Germ-line alterations of the *TP53* tumor suppressor gene are associated with LFS.[105,106] These are primarily missense mutations that yield a stabilized mutant protein. The spectrum of mutations of *TP53* in the germ line is similar to somatic mutations found in a wide variety of tumors. Carriers are heterozygous for the mutation, and in tumors derived from these individuals, the second (wild-type) allele is frequently deleted or mutated, leading to functional inactivation.[107]

Several comprehensive databases document all reported germline (and somatic) *TP53* mutations and are of particular value in evaluating novel mutations as well as phenotype–genotype correlations.[108] Only 60% to 80% of *classic* LFS families have detectable alterations of the gene. It is not yet determined whether the remainder is associated with the presence of modifier genes, promoter defects yielding abnormalities of *TP53* expression, or simply the result of weak genotype–phenotype correlations (i.e., the broad clinical definition encompasses families that are not actual members of LFS). Other candidate predisposition genes, such as *p16, p15, p21, BRCA1, BRCA2*, and *PTEN*, associated with multisite cancer associations have generally been ruled out as potential targets. The role of the hCHK2 checkpoint kinase as an alternative mechanism for functional inactivation of TP53 in LFS has been suggested,[109] although its place as a major contributor to the phenotype has been controversial.[110]

Germ-line *TP53* alterations have also been reported in some patients with cancer phenotypes that resemble the classic LFS phenotype. Between 3% and 10% of children with apparently sporadic RMS or osteosarcoma have been shown to carry germ-line *TP53* mutations.[111,112] These patients tend to be younger than those who harbor wild-type *TP53*. Germ-line TP53 mutations are observed in more than 60% of children with the anaplastic variant of RMS,[113] and are also overrepresented in children with the Sonic hedgehog (SHH) subgroup of medulloblastomas.[114] It appears as well that more than 75% of children with apparently sporadic ACC carry germ-line *TP53* mutations, although in some of these cases, a family history develops that is not substantially distinct from LFS.[115,116] These important findings indicate a broader spectrum of patients at risk of germ-line *TP53* mutations and refined criteria for *TP53* mutation analyses.[117,118] A striking genotype–phenotype correlation has been observed in a unique subgroup of ACC patients in Brazil in whom the same germ-line *TP53* mutation at codon 337 has been observed in 35 unrelated kindred.[119] The functional integrity of the mutant protein appears to be regulated by alterations in cellular pH,[120] which suggests potential biologic mechanisms in ACC cells by which the *TP53* mutation leads to malignant transformation. All these observations suggest that germ-line *TP53* alterations may be associated with early-onset development of the childhood component tumors of the syndrome.[121] The variability in age of onset and type of cancer among LFS families suggests modifier effects on the underlying mutant *TP53* genotype. An analysis of mutant genotype-to-phenotype correlations reveals intriguing observations. Nonsense, frameshift, and splice mutations yield a truncated or nonfunctional protein commonly associated with early-onset cancers, particularly brain tumors. Missense mutations in the *TP53* DNA-binding domain are frequently observed in the setting of breast and brain tumors, whereas adrenocortical cancers are the only group that is associated with mutations in the non–DNA-binding loops. Age of onset modifiers have also now been established. The protein murine double-minute-2 (MDM2) is a key negative regulator of TP53 and targets TP53 toward proteasomal degradation. The MDM2 single nucleotide polymorphism 309 increases Sp1 transcription factor binding, leading to increased *MDM2* expression levels. Coinheritance of the MDM2 single nucleotide polymorphism 309 T/G isoform is associated with an earlier onset of cancer.[122] The earlier age of onset of cancers with subsequent generations in mutant *TP53* LFS families suggests genetic anticipation. This observation can be partially explained by several molecular mechanisms including accelerated telomere attrition from generation to generation, absence of the PIN3 polymorphism, or excessive DNA copy number variation in *p53* mutation carriers, all of which may be useful predictive markers of tumor age of onset.[122–124] Thus, although germ-line *p53* mutations establish the baseline risk of tumor development in LFS, a complex interplay of modifying genetic cofactors likely defines the specific phenotypes of individual patients.

Beckwith-Wiedemann Syndrome

BWS occurs with a frequency of 1 in 13,700 births. More than 450 cases have been documented since the original reported associations of exomphalos, macroglossia, gigantism, and other congenital anomalies. With increasing age, phenotypic features of BWS become less pronounced. Laboratory findings may include, at birth, hypoglycemia, polycythemia, hypocalcemia, hypertriglyceridemia, hypercholesterolemia, and high serum α-fetoprotein levels. Early diagnosis of the condition is crucial to avoid deleterious neurologic effects of neonatal hypoglycemia and to initiate an appropriate screening protocol for tumor development.[125] The increased risk for tumor formation in BWS patients is estimated at 7.5% and is further increased to 10% if hemihyperplasia is present. Tumors occurring with the highest frequency include Wilms tumor, hepatoblastoma, neuroblastoma, and ACC.[126]

The genetic basis of BWS is complex. Various 11p15 chromosomal or molecular alterations have been associated with the BWS phenotype and its tumors.[127] It is unlikely that a single gene is responsible for the BWS phenotype. Because it appears that

abnormalities in the region impact an imprinted domain, it is more likely that normal gene regulation in this part of chromosome 11p15 occurs in a regional manner and may depend on various interdependent factors or genes. These include the paternally expressed genes *IGF2* and *KCNQ10T1* and the maternally expressed genes *H19*, *CDKN1C*, and *KCNQ1*. BWS children who develop rhabdomyosarcoma or hepatoblastoma have epigenetic changes in domain 1, whereas those with Wilms tumor have domain 2 changes or uniparental disomy.[128]

Chromosomal abnormalities associated with BWS are extremely rare, with only 20 cases having been associated with 11p15 translocations or inversions. The chromosomal breakpoint in each of these cases is always found on the maternally derived chromosome 11. This parent-of-origin dependence in BWS suggests that the chromosome translocations disrupt imprinting of a gene in the 11p15 region. On the other hand, BWS-associated 11p15 duplications (approximately 30 reported cases) are always paternally derived, and the duplication breakpoints are heterogeneous.[129] Paternal uniparental disomy, in which two alleles are inherited from one parent (the father), has been reported in approximately 15% of sporadic BWS patients.[130] The insulin/IGF2 region is always represented in the uniparental disomy, although the extent of chromosomal involvement is highly variable. Alterations in allele-specific DNA methylation of *IGF2* and *H19* reflect this paternal imprinting phenomenon.[130] A minority of BWS patients have demonstrable constitutional DNA sequence alterations, the most common of these being *CDKN1C* mutations.[131] Of BWS patients, 25% to 50% exhibit biallelic rather than monoallelic expression of *IGF2*. Another 50% have epigenetic mutations resulting in LOI of *KCNQ10T0*. Of interest, epigenetic changes, such as methylation and chromatin modification, occur in many pediatric and adult cancers,[132] indicating the value of the BWS model in understanding the broad scope of molecular changes in cancer. Despite the associated cytogenetic and molecular findings for some patients, no single diagnostic test exists for BWS. This observation is not unlike that described for LFS, or perhaps for other multisite cancer phenotypes, in which the clarity of the phenotype is often weak, making the genetic link cloudy and the likelihood of multiple pathways to tumor formation strong.

Gorlin Syndrome

Nevoid basal cell carcinoma syndrome, or Gorlin syndrome, is a rare autosomal-dominant disorder characterized by multiple basal cell carcinomas; developmental defects, including bifid ribs and other spine and rib abnormalities; palmar and plantar pits; odontogenic keratocysts; and generalized overgrowth.[133] The SHH signaling pathway directs embryonic development of a spectrum of organisms. Gorlin syndrome appears to be caused by germline mutations of the tumor suppressor gene *PTCH*, a receptor for SHH.[134,135] Medulloblastomas develop in approximately 5% of patients with Gorlin syndrome. Furthermore, approximately 10% of patients diagnosed with medulloblastoma by the age of 2 years are found to have other phenotypic features consistent with Gorlin syndrome and also harbor germ-line *PTCH* mutations.[136] Although Gorlin syndrome develops in individuals with germ-line mutations of *PTCH*, a subset of children with medulloblastoma harbor germ-line mutations of another gene, *SUFU*, in the SHH pathway, with accompanying LOH in the tumors. Of further note, mice with heterozygous *PTC* deletions develop RMS.[137] Although RMS is rarely seen with Gorlin syndrome, the mouse studies suggest a possible link between PTC signaling and RMS.[138]

MALIGNANT RHABDOID TUMORS

Malignant rhabdoid tumors are unusual pediatric tumors that occur as primary renal tumors, but have also been described in lung, liver, soft tissues, and the central nervous system, where they are often termed *atypical* and *teratoid rhabdoid tumors*.[139] Recurrent chromosomal translocations of chromosome 22 involving a breakpoint at 22q11.2, as well as complete or partial monosomy 22, have been observed, strongly suggesting the presence of a tumor suppressor gene in this area. The *hSNF5/INI1* gene has been isolated and has been shown to be the target for biallelic, recurrent inactivating mutations.[140] The encoded gene product is thought to be involved in chromatin remodeling. Studies have not only demonstrated the presence of inactivating mutations in the majority of malignant rhabdoid tumors (renal or extrarenal), but also in chronic myelogenous leukemia,[141] as well as in a wide variety of other childhood and adult-onset malignancies.[142] An intriguing feature in some individuals with malignant rhabdoid tumors is the observation of germ-line mutations, suggesting that this family of tumors may occur as a result of a primary inherited defect in one allele of the *INI1* gene.[143] Further studies of the function of this gene will be important in determining its role in tumorigenesis of this wide spectrum of neoplasms.

DICER1 SYNDROME

DICER1 syndrome is a very recently characterized phenotype of distinctive dysontogenetic, hyperplastic, or overtly malignant tumors. The most frequent of these is the rare childhood lung malignancy pleuropulmonary blastoma (PPB). Other manifestations include ovarian Sertoli-Leydig cell tumors (SLCT), nodular thyroid hyperplasia, pituitary blastoma, pineoblastoma, papillary and follicular thyroid carcinoma, cervical rhabdomyosarcoma, cystic nephroma, and possibly, Wilms tumor.[144] Germ-line *DICER1* mutations have been identified in children and young adults affected with one or several of these tumors, and somatic *DICER1* mutations have been found in sporadic component tumors. DICER1 is an endoribonuclease that processes hairpin precursor microRNAs (miRNA) into short, functional miRNAs. Mature 5′ miRNAs as well as other components of the RNA-induced silencing complex (RISC) downregulate targeted miRNA.

Unlike the classical Knudson *two-hit* mechanism that inactivates tumor suppressor genes, the effect of DICER1 loss-of-function appears to result from an initial inactivating mutation that reduces by half the amount of wild-type DICER1 protein, followed by a second hit that specifically eliminates production of 5′ mature miRNAs. Disease penetrance is highly variable. The risk of potentially lethal tumors such as PPB and pineoblastoma in *DICER1*-mutation carriers indicates a need for clinical surveillance, particularly targeting the lungs, abdomen, and brain.

PREDICTIVE TESTING FOR GERM-LINE MUTATIONS AND CHILDHOOD CANCERS

Several important issues have arisen as a result of the identification of germ-line mutations of tumor suppressor genes in cancer-prone individuals and families. These include ethical questions of predictive testing in such families and in unaffected relatives and the selection of patients to be tested, as well as the development of practical and accurate laboratory techniques, the development of pilot testing programs, and the role of clinical interventions based on test results. This chapter was not meant to discuss these problems in detail, but one would be remiss to ignore their significance.

For several reasons, testing cannot as yet be offered to the general pediatric population, particularly in light of the demonstrably low carrier rate of the abnormal tumor suppressor genes and the general lack of standardized methods of preclinical screening of carriers. Exceptions to these limitations include screening of gene carriers in families with retinoblastoma, BWS, multiple

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endocrine neoplasia, familial adenomatous polyposis, LFS, hereditary paraganglioma syndromes, and von Hippel-Lindau disease. For some of these diseases, clinical surveillance tools are available, whereas for others, risk-reductive surgery has also been shown to be of value.[145–148] In general, it has been demonstrated that genetic testing does not lead to clinical levels of anxiety, depression, or other markers of psychological distress in children who are tested or their parents.[149,150] However, certain circumstances or personality traits are associated with a greater likelihood of an individual experiencing psychological distress after a positive result.[149] Parents now routinely discuss the options of prenatal diagnosis and preimplantation genetic diagnosis. Multidisciplinary teams must be engaged to provide parents and families the necessary tools with which to approach these ethically challenging decisions.[151,152] The development of screening programs should address aspects of cost, informed consent (particularly where it affects children), socioeconomic impact on the individual tested, consistency in providing results, and counseling. Concerns of risk of employment, health insurance, or life insurance discrimination exist but may be alleviated by congressional legislation to ban such practices.[153]

MOLECULAR THERAPEUTICS

With the identification of alterations in a variety of molecular signaling pathways increasingly identified with application of newer molecular analytic techniques, it has become increasingly apparent that these alterations may potentially represent the *Achilles heel* for these tumors. New agents targeting the TK enzymes as well as agents that alter the epigenetic state of tumors are at various stages of development in early clinical studies of pediatric tumors. A particular issue for the development of targeted therapy for pediatric tumors is the relative effectiveness of standard cytotoxic agents in many tumors. Thus, the identification of single targeted agent activity and the incorporation of such agents into combinations with cytotoxic agents is an important challenge. Ongoing studies incorporating mTOR inhibitors (rhabdomyosarcoma) or IGFIR inhibitors with combination chemotherapy (Ewing sarcoma) will provide important information on whether these approaches will improve outcome. The most important challenge will be to correctly identify the targetable altered pathways that are critical for malignant behavior of specific pediatric tumors and then rapidly test them in a rational approach to improve the outcomes of pediatric cancer patients.

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99 Solid Tumors of Childhood

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INTRODUCTION

Solid tumors account for about one-third of the estimated 14,000 cases of cancer diagnosed each year in patients under 20 years in the United States (Fig. 99.1).[1]

Since 1975, the overall mortality rates for childhood cancer have decreased by 50% and survival rates for childhood cancer now exceed 80%; however, this progress has been most evident for children with hematologic malignancies, and mortality rates for children affected by some of the most common pediatric solid tumors such as neuroblastoma and sarcomas have remained virtually unchanged over the past 20 years.[2] The reasons for the lack of progress in patients with solid tumors when compared to those with hematologic malignancies is unclear but might be related to the relatively limited numbers of patients available to conduct randomized trials and the scarcity of recurrent and actionable genetic aberrations reported to date.[2,3]

MULTIDISCIPLINARY CARE IS ESSENTIAL FOR CHILDREN AND ADOLESCENTS WITH SOLID TUMORS

The management of pediatric solid tumors is complex and requires a coordinated multidisciplinary approach that incorporates multiple subspecialties including surgery, radiology, pathology, radiotherapy, oncology, and physical therapy.[4]

Local control of the tumor may be achieved with the use of surgery, radiation therapy, or both. The use of neoadjuvant chemotherapy offers the theoretical advantage of decreasing the risk of micrometastatic dissemination as well as primary tumor size and, therefore, limiting the extent of surgery and the radiation doses.[4] In addition, the administration of preoperative chemotherapy facilitates the evaluation of histologic response in the resected surgical specimen providing important prognostic and therapeutic information.[5]

Surgery plays a dual role in the management of pediatric solid tumors because it provides tissue for diagnosis and biologic studies as well as a mechanism for removing the primary tumor. The role of debulking surgery is controversial.[6,7] Surgery can also be used later in the course of treatment to remove residual tumor, and the results of the surgery may provide important information regarding the need for additional radiotherapy or chemotherapy.[8–10]

The radiation oncologist should evaluate the patient prior to the initiation of any therapy and to help select the radiation plan and optimal treatment modality. The patient's age, extent of disease, and disease status all play a role in selecting the appropriate radiotherapeutic approach from the available techniques including intensity modulated radiation therapy (IMRT), three-dimensional (3D) conformal radiotherapy, proton beam radiation therapy (PBRT), and brachytherapy.

NEUROBLASTOMA

Neuroblastomic tumors comprise a spectrum of tumors that arise from primitive sympathetic ganglion cells and include neuroblastomas, ganglioneuroblastomas, and ganglioneuromas (Fig. 99.2). They can originate anywhere along the sympathetic ganglia; about half arise in the adrenal medulla. This group of tumors is known for a broad spectrum of clinical behaviors ranging from a completely benign mass to spontaneous regression to widely disseminated, aggressive, and often fatal disease.[11–14] Tumors can be subdivided into distinct risk categories based on clinical and biologic features.[11,15] Historically, treatment has been tailored based on determination of the risk of recurrence for a given patient. A combination of clinical and biologic features of disease have been used to classify patients as low, intermediate, and high risk.[11,15]

Epidemiology

Neuroblastoma is the third most common childhood cancer after leukemia and brain tumors. From 2006 to 2010, the age-adjusted incidence of neuroblastoma was 10.1 per 1 million children aged 0 to 14 years.[1,16] During the first year of life, neuroblastoma accounts for the majority of cancer cases with an incidence almost double that of leukemia; 16% of infant neuroblastomas are diagnosed during the first month of life.[16]

There are approximately 650 children diagnosed each year with neuroblastoma in the United States[16] and their median age at diagnosis is 19.1 months (573 days).[17] Neuroblastoma is slightly more common in Caucasians and males, with the greatest sex-specific incidence difference occurring during infancy.[16]

The majority of neuroblastomas are sporadic. About 1% to 2% of cases are associated with a history of neuroblastoma in immediate or extended family members.[18–23] An early analysis of these family pedigrees suggests an autosomal-dominant mode of inheritance with incomplete penetrance.[24] Although many of these familial neuroblastoma patients present before 1 year of age, and more frequently have multifocal primaries,[14,25] there is also great clinical and biologic heterogeneity among patients in the same kindred.[19–21,26] Recently, germ-line mutations in the anaplastic lymphoma kinase (ALK) gene were identified as the cause of most cases of hereditary neuroblastomas.[25,27–30] Another less common cause of hereditary neuroblastoma involves germ-line mutations of the pairedlike homeobox 2B (PHOX2B) gene.[31–33] These children often have concomitant neural crest disorders (neurocristopathies),[34] including Hirschsprung disease,[32,34,35] and congenital hypoventilation syndrome.[33,34,36] Other genetic conditions that may predispose for the development of neuroblastoma include neurofibromatosis,[32,37,38] Turner syndrome,[39] and Beckwith-Wiedemann syndrome.[40]

Pathology

Neuroblastic tumors, which include ganglioneuromas, ganglioneuroblastomas, and neuroblastomas (see Fig. 99.2), are derived

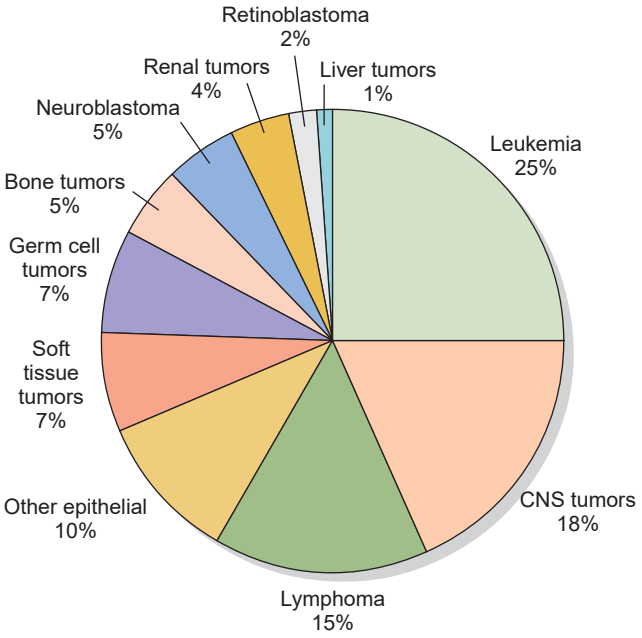

Figure 99.1 Age adjusted Surveillance, Epidemiology, and End Results (SEER) Cancer Incidence rates by the International Classification of Childhood Cancer category 0–19 years of age, all races, both sexes, SEER 2002–2010. CNS, central nervous system.

from primitive cells involved in the development of the sympathetic nervous system.[41] The most undifferentiated neuroblastic tumors are composed of neuroblasts with very few schwannian (stromal) cells. Immunohistochemistry may be useful in distinguishing neuroblastoma from other small round blue cell tumors, and immunoreactivity is commonly observed to neuron-specific enolase, synaptophysin, chromogranin A, NB84, and S100. As tumors become more differentiated, the ratio of Schwann to neuroblastoma cells increases, and the neuroblasts appear more mature. Ganglioneuroblastoma can be subdivided into either a stroma-rich, intermixed variant, or nodular variant. Ganglioneuroma is predominantly composed of Schwann cells studded with maturing or fully mature ganglion cells and is considered a benign tumor. These tumors were originally classified according to an "age-linked" classification system by Shimada et al.,[42] which was subsequently clarified and adopted for international use by the International Neuroblastoma Pathology Committee (INPC).[43–45] These tumors are morphologically classified into four histologic subtypes according to the balance between neural-type cells (primitive neuroblasts, maturing neuroblasts, and ganglion cells) and the Schwann-type cells (Schwannian blasts and mature Schwann cells) and the degree of surrounding Schwannian stroma: (1) neuroblastoma (Schwannian stroma-poor), undifferentiated, poorly differentiated, and differentiating; (2) ganglioneuroblastoma, intermixed (Schwannian stroma-rich); (3) ganglioneuroma (Schwannian stroma dominant), maturing and mature; and (4) ganglioneuroblastoma, nodular (composite Schwannian stroma rich/stoma dominant and stroma poor).[43] The grade of neuroblastic differentiation and mitosis-karyorrhexis index (MKI; defined as the number of tumor cells in mitosis and in the process of karyorrhexis) and age of patient are then used to assign to either a *favorable* or *unfavorable* prognostic group (Fig. 99.3).[44]

Neuroblastoma tumors are classified into favorable histology (FH) and unfavorable histology (UH) lesions based on the degree of stromal development, morphology, MKI, and age.

The INPC system's prognostic significance was confirmed in a retrospective review of two Children's Cancer Group (CCG) protocols that demonstrated a threefold difference in the 5-year event-free survival (EFS) for patients with FH versus UH features.[46]

Molecular Biology

Amplified segments of DNA in the form of homogeneously staining regions of chromosomes (HSR) or extrachromosomal DNA called double-minute chromosomes (DMs) are often found in neuroblastomas (Fig. 99.4). The gene that was consistently amplified in these segments was identified by Schwab and colleagues[47] as a myc-related gene, which was eventually called MYCN (v-myc avian myelocytomatosis viral oncogene neuroblastoma-derived homolog). Amplification of MYCN is predominantly associated with advanced stage disease, rapid tumor progression, and poor prognosis.[48,49] A 5- to 500-fold amplification of the MYCN gene is found in about 22% of all patients with neuroblastoma,[11] and in approximately 30% to 40% of patients with advanced stage disease (see Fig. 99.4).[48,49] Amplification (increased gene copy number) results in persistently high levels of the MYCN protein, a DNA-binding transcription factor known to cause malignant transformation in tumor models. The negative prognostic significance of MYCN amplification (>10 copies per haploid genome) is independent of age, stage, or other genetic alterations and remains an important part of the current international risk group classification system.[50,51]

The DNA content (ploidy) of tumors is either near diploid or hyperdiploid.[50,52] Near-diploid tumors in patients less than 18 to 24 months of age are associated with an adverse prognosis, whereas hyperdiploid tumors in this age group are associated with low-stage disease and a favorable outcome.[53,54] In the new International Risk Group pretreatment classification system, ploidy is only used to stratify patients less than 18 months old with metastatic disease who do not have *MYCN*-amplified tumors.[51]

Chromosomal deletions or gains are also frequently identified. Loss of heterozygosity (LOH; loss of one allele) at chromosome 1p36 and 11q occurs in 23% and 34% of tumors respectively.[55] A gain of chromosome 17 or 17q is present in 50% to 60% of neuroblastomas and is associated with an aggressive phenotype.[56] Trk, a family of neurotropin receptors, is important in the development of the central and peripheral nervous system. Elevated expression of TrkA or TrkC is seen in favorable, low-stage tumors. Low expression of TrkA or elevated expression of full-length TrkB is seen in advanced-stage *MYCN*-amplified tumors.[57] Additionally, TrkB expression has been linked to chemotherapeutic resistance.[57,58] Among the most common mutations in sporadic neuroblastoma is in the α-thalassemia/mental retardation syndrome X-linked (ATRX) gene, which is seen in children over 12 years of age (44%).[59] The evaluation of a chromosomal translocation in a neuroblastoma patient led to the identification of a family of genes on chromosome 1, now called neuroblastoma breakpoint family (NBPF).[60] Copy number variation in this region (1q21.1) has also been shown to be highly associated with neuroblastoma, probably through disruption of one of the NBPF genes, NBPF23.[61] A number of technologic advances have enabled the analysis of whole genome or exomes of tumors to provide insight into the pathogenesis of neuroblastoma and are under investigation to refine risk assignment.[62–68] For example, risk polymorphisms at 6p22, identified by genome-wide association studies (GWAS), have been associated with sporadic neuroblastoma, and homozygosity for any of these alleles is associated with high-risk features.[69] Similarly, common variations in the BRCA1-associated RING domain 1 (BARD1) gene have been implicated in the development of high-risk disease.[70] By expanding these GWAS studies, this same group also identified risk alleles within the LMO1 (LIM domain only 1) gene at 11p15.4 that were more common in tumors with high-risk disease ($p < 0.0001$), suggesting that LMO1 may be a neuroblastoma oncogene.[71]

Diagnosis

About two-thirds of neuroblastomas originate in the abdomen, with 50% beginning in the adrenal gland. Other common sites include the chest (16%), neck (3%), and pelvic sympathetic ganglia (3%). Approximately 50% of tumors have spread to the bone or bone

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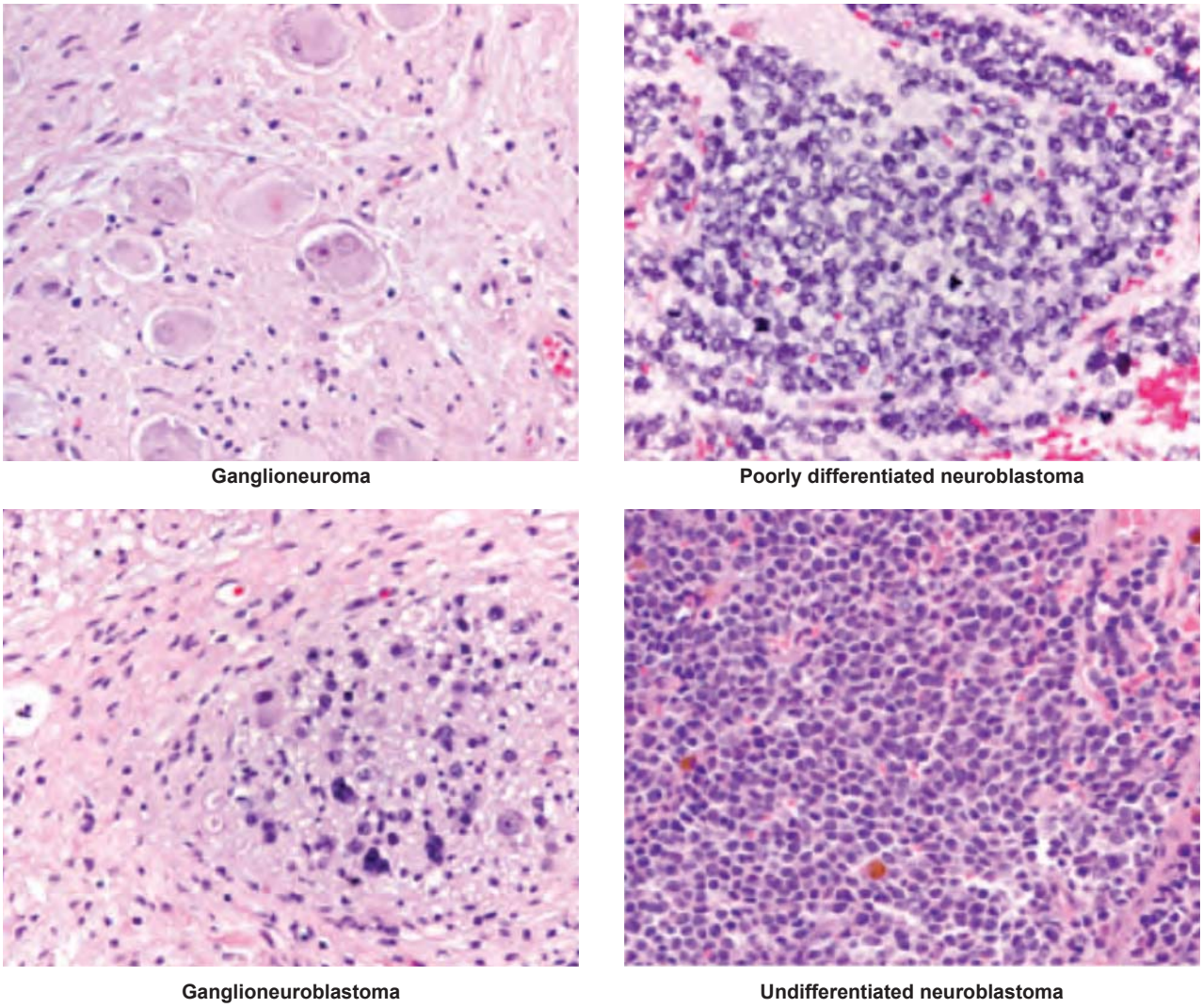

Figure 99.2 The principal histopathologic subtypes of neuroblastoma (hematoxylin and eosin). (Courtesy of Dr. Jesse Jenkins, Department of Pathology, St. Jude Children's Research Hospital.)

marrow at presentation.[51] The pattern of metastatic spread varies with age and histology of the tumor. Infants with favorable histologies are more likely to have liver and skin metastases. Unfavorable histology tumors are more likely to spread to the bone marrow and bones.[72] At presentation, abdominal tumors can cause increased abdominal girth, a palpable mass, diarrhea, or constipation. Many thoracic tumors are detected incidentally; however, large thoracic or cervical tumors can cause Horner syndrome, superior vena cava syndrome, or mechanical airway obstruction. Children with bone metastases may have a limp, complain of pain, or develop periorbital swelling, ecchymoses, or proptosis. Fever and hypertension are often present.

The evaluation of a child suspected of having neuroblastoma begins with a careful history and physical examination. A detailed head and neck exam, looking for skull metastases; "raccoon eyes,"[73] proptosis, or other eye abnormalities; and Horner syndrome should be performed. Attention to neurologic function is critical due to the paraspinal location of many tumors, which can result in spinal cord compression and permanent loss of function if not treated expeditiously.[74] Similarly, blindness has developed in children with periorbital disease while staging evaluations were being completed.[75] In infants, the examination must include a search for skin and subcutaneous metastases that appear as reddish-purple, raised lesions.[76] Because neuroblastomas produce catecholamine metabolites, the measurement of these can often help narrow the diagnosis.[77,78] Urine samples for quantitative excretion of vanillylmandelic acid (VMA) and homovanillic acid (HVA) are elevated in more than 80% of patients[79] and should be part of the diagnostic evaluation for every patient. These markers are also useful to evaluate the effectiveness of therapy and to monitor for disease recurrence.[80] Ultrasound is often the initial radiologic study to identify the tumor. However, magnetic resonance imaging (MRI) or computerized tomography (CT) of the chest, abdomen, and pelvis is required to clearly define the location and extent of the primary tumor. Radiographically, a neuroblastoma typically appears as a heterogeneous mass with calcifications. Adrenal and retroperitoneal tumors characteristically involve and displace the major vessels.

Patients with paraspinal primary tumors may have asymptomatic extension through the spinal foramina. Due to the increased risk of neurologic compromise, these patients should undergo further imaging of the spinal canal using MRI with contrast. Imaging of the head should be considered in any child with palpable skull lesions, ptosis, or orbital ecchymosis.[75]

Tumors can secrete a vasoactive intestinal polypeptide resulting in intractable diarrhea and abdominal distention that resolves after

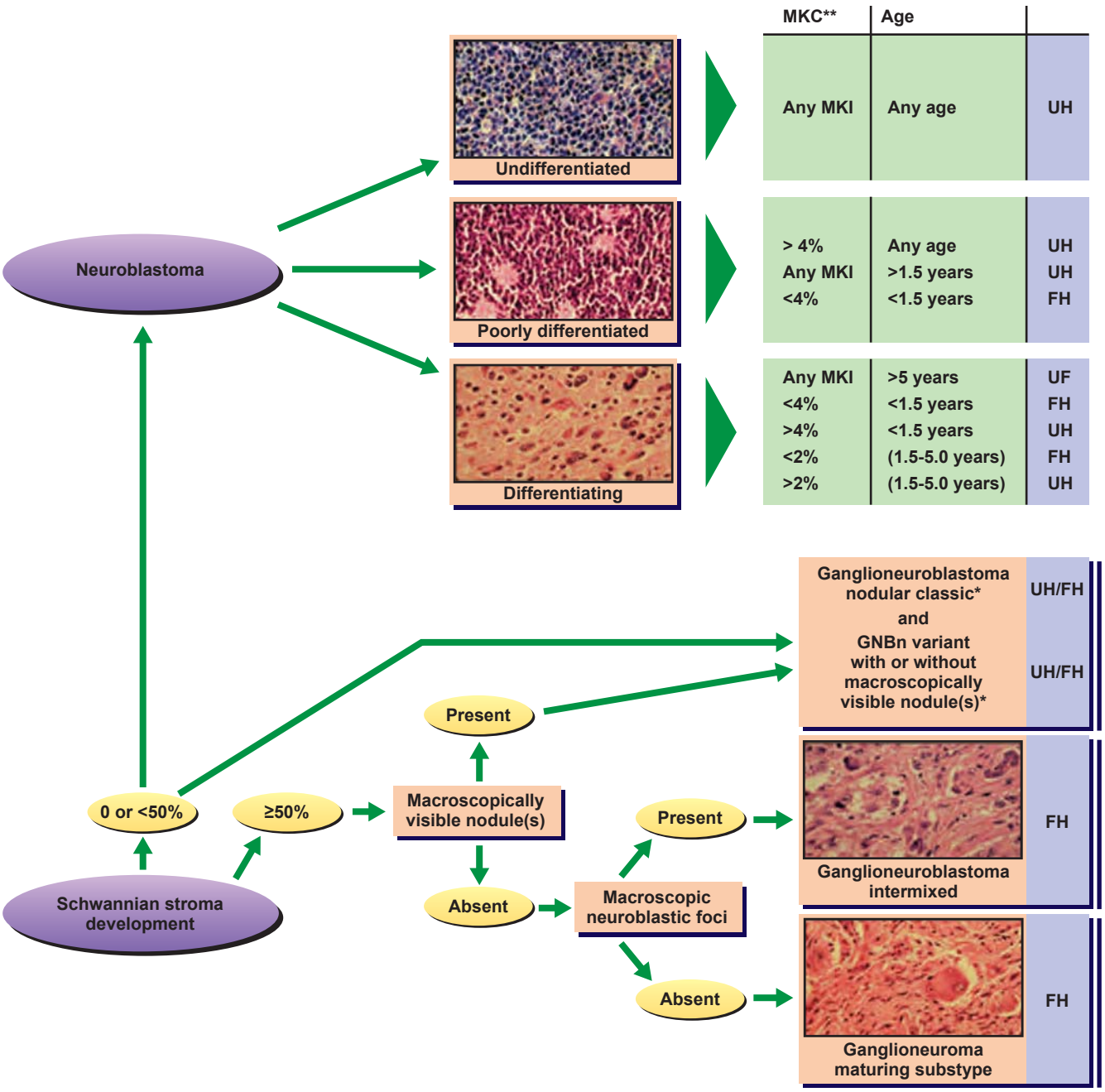

Figure 99.3 The International Neuroblastoma Pathology classification schema. Neuroblastoma tumors are classified into favorable (FH) and unfavorable (UH) histology lesions based on degree of stromal development, morphology, mitosis-karyorrhexis index (MKI), and age. GNBn, ganglioneuroblastoma. (From Park JR, Eggert A, Caron H. Neuroblastoma: biology, prognosis, and treatment. *Hematol Oncol Clin North Am* 2010;24:65–86, with permission.)

resection. In the largest review of 22 patients, most presented with significant weight loss and metabolic abnormalities, and 16 out of 22 had 1 to 21 months of gastrointestinal (GI) symptoms prior to the diagnosis of neuroblastoma. Although usually associated with differentiating tumors of low stage, 6 of these 22 were high-risk patients who developed diarrhea upon initiation of chemotherapy.[81] Opsoclonus-myoclonus-ataxia (OMA) syndrome, often called *dancing eyes*,[82] *dancing feet* is seen in up to 3% of children with neuroblastoma and presents with chaotic, multidirectional eye movements, spontaneous limb jerking, and ataxia. Many patients also have significant behavioral problems, sleep disturbances, and learning defects.[83,84] Although this process is thought to arise as a consequence of immune-mediated cross-reactivity between neuroblasts and the central nervous system, no specific antibody or lymphocyte marker has been identified.[85] The tumors associated with OMA patients typically are of favorable biology and limited stage, and thus, patients with this condition have an excellent cancer prognosis.[86,87] Although the movements may resolve over time, most children have persistent neurologic deficits, including speech and cognitive delays and behavioral problems.[88,89]

Spinal cord involvement with infiltration of the intervertebral foramina is often seen in the initial evaluation of neuroblastoma[90]; however, only about 5% of children at the time of diagnosis develop symptoms related to spinal cord compression.[74,91] Spinal cord compression is considered an oncologic emergency because prolonged compression of the spinal cord can lead to irreversible loss of function. This can be treated with urgent radiation therapy, neurosurgery, or chemotherapy. Although there is no current

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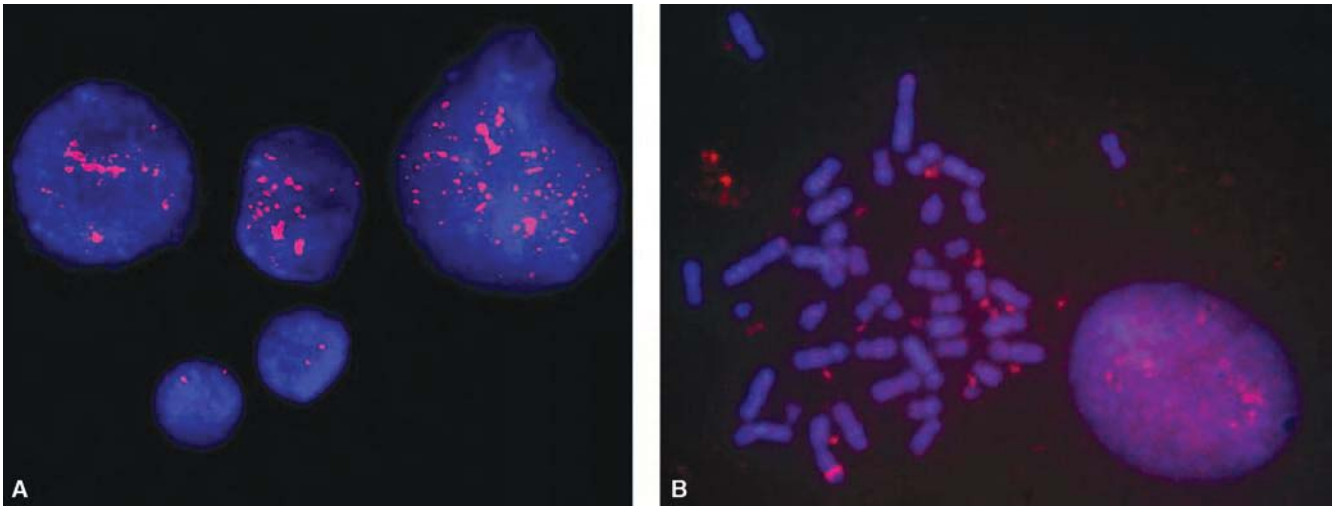

Figure 99.4 **(A)** shows upper three cells with MYCN amplification, having likely both, homogeneously stained regions (HSR) and double minutes (DM); the lower cells are normal with two copies each of the MYCN signals. **(B)** shows a metaphase plate with DM scattered outside the chromosomes. (Courtesy of Dr. Susana Raimondi, Department of Pathology, St. Jude Children's Research Hospital.)

consensus as to the best initial treatment,[92] a retrospective analysis suggests that each of these modalities are equally effective in the short term. However, there are significant differences in late effects.[90,93–95] The management of this rare presentation requires an experienced multidisciplinary team and the treatment recommendations should be made on a case-by-case basis specific to the severity and duration of symptoms and an assessment of the anticipated short- and long-term risks of the specific intervention.[90,92]

Metaiodobenzylguanidine (MIBG) is an analog of norepinephrine and is taken up by tissues that express the norepinephrine transport protein, which includes almost 90% of neuroblastomas.[96,97] With a sensitivity of about 90% and a specificity of 100%, MIBG has replaced technetium-99m bone scans and has been recommended as a standard agent for staging patients with neuroblastoma[96,98,99] because of its sensitivity and specificity; all patients should undergo MIBG scans at the time of diagnosis and at subsequent intervals after the start of treatment to assess response. In the 10% of patients who have negative MIBG scans, fludeoxyglucose (^{18}F-FDG) positron-emission tomography (PET) scans can be considered.[98] Newer PET agents, such as fluorine-18-L-dihydroxyphenylalanine (^{18}F-Dopa) are also under investigation.[100] Bone marrow involvement should be further evaluated by bilateral aspiration and a biopsy.[99,101]

Other malignancies for which neuroblastoma can occasionally be confused include other *small, round, blue cell* tumors such as rhabdomyosarcoma, the Ewing sarcoma family of tumors, non-Hodgkin lymphoma, and acute leukemia. If the diagnosis is to be based on material from bone marrow, it is recommended that these cells be confirmed to be neuroblasts by immunohistochemistry staining with synaptophysin[102] or chromogranin[103] and that urine HVA and/or VMA be >3.0 SD above mean per milligram creatinine for age.[99]

Screening

Because infants have the best prognosis and because more than 80% of patients with neuroblastoma have elevated catecholamine markers that can be easily measured, screening infants was thought to be a promising approach.[104] Unfortunately, two carefully controlled trials definitively demonstrated that screening did not reduce mortality and, in fact, because many infant neuroblastomas spontaneously regress, led to over diagnosis and treatment of patients that needed no intervention.[105–108]

Staging

The International Neuroblastoma Staging System (INSS), developed in 1988[109] and revised in 1993[99] (Table 99.1) was developed to facilitate comparison among trials and is the current staging system used in North American and European cooperative group trials. This system is a postsurgical staging system, in that it uses the extent of initial surgical resection to stage patients. The INSS is significantly affected by the experience of the surgeon, leading to variability in stage assignments. To better define homogenous pretreatment patient cohorts and compare trials conducted in different regions of the world, another staging system, based on preoperative, image-defined risk factors (Tables 99.2A and 99.2B), called the International Risk Group classification system.[110] The predictive prognostic significance of this system will be tested in future prospective clinical trials.

Management by Risk Group

The treatment of neuroblastoma incorporates a number of clinical and biologic variables, in addition to the stage of disease, to evaluate the risk of recurrence and to thus determine the intensity of treatment. The Children's Oncology Group's (COG) risk categories incorporate the patient's age at presentation, stage of the tumor, histologic appearance, quantitative DNA content, and presence or absence of *MYCN* amplification within the tumor cells (Table 99.3).

The age at presentation is an important prognostic factor, because infants tend to have tumors with more favorable features, including stage and histology. A multivariate analysis of 3,666 patients enrolled in cooperative group studies demonstrated 82% 4-year EFS for children less than 18 months versus 42% for those more than 18 months.[17] Adolescents and young adults rarely present with neuroblastoma, but their tumors tend to be indolent and fatal.[110–112]

The variables selected by other pediatric oncology cooperative groups to define the risk categories have not been uniform. To be able to compare trials worldwide, an international conference developed a consensus approach to pretreatment risk stratification, now called the International Neuroblastoma Risk Group (INRG) Classification System (see Tables 99.2A and 99.2B).[51] This new classification system is different than that of the COG in that it

TABLE 99.1

International Neuroblastoma Staging System Criteria[99]

Stage	Definition
1	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached to and removed with the primary tumor may be positive)
2A	Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically
2B	Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor; enlarged contralateral lymph nodes must be negative microscopically
3	Unresectable unilateral tumor infiltrating across the midline[a] with or without regional lymph node involvement; or localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement[b]
4	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined for stage 4S)
4S	Localized primary tumor (as defined for stage 1, 2A, or 2B), with dissemination limited to skin, liver, and/or bone marrow[c] (<10% involvement) (limited to infants <1 year of age)

Note: Multifocal primary tumors should be staged according to the greatest extent of disease and followed by the subscript "M."
[a] The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.
[b] Patients are upstaged to International Neuroblastoma Staging System stage 3 if there is proven malignant effusion within the abdominal cavity or bilateral thoracic cavity.
[c] Marrow involvement in stage 4S should be minimal (i.e., <10%) of total nucleated cells identified as malignant on bone marrow biopsy or on marrow aspirate. More extensive marrow involvement would be considered to be stage 4. The metaiodobenzylguanidine (MIBG) scan, if performed, should be negative in the marrow.
From Brodeur GM, Prichard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncol* 1993;11:1466–1477.

uses the new INRG staging system,[113] and adds the presence/absence of 11q aberrations. As new trials are developed, the value of this new risk group classification system will be validated.

Low Risk

Children with low-risk neuroblastomas generally include patients of any age with localized, resectable tumors (Table 99.4). Low-risk tumors comprise about 50% of all neuroblastomas. Surgery is the primary treatment, and overall survival rates are greater than 90%.[114–116] Recently, a prospective study of 87 infants less than 6 months of age and with small (volume ≤16 mL) isolated adrenal masses who were observed only showed that 81% (67 out of 83) were spared surgery with a median follow-up of 3.2 years and an overall survival at 3 years of 100%.[117] Thus, expectant observation without surgery in this subgroup of patients should be considered standard treatment and, in fact, the authors recommend extending this approach to "all localized noninfiltrative neuroblastoma tumors [corresponding to the International Neuroblastoma Risk Group Staging System stage L1; see Table 99.2][113] in infants less than 1 year of age."[117]

A 4S neuroblastoma is a *special*[118] entity because the high rate of spontaneous regression allows for a delay or elimination of chemotherapy for up to 80% of children.[119,120] Two subsets are more likely to require chemotherapy: those tumors with unfavorable biologic features (i.e., *MYCN* amplification) and tumors in infants younger than 2 months who have extensive liver disease. Untreated, the latter case leads to a higher incidence of mortality, because massive hepatomegaly prevents adequate chest wall expansion.[120,121] These children should receive chemotherapy or low-dose radiation (4.5 to 6 Gy in three to four fractions) to decrease tumor size.

TABLE 99.2A

International Neuroblastoma Risk Group (INRG) Staging System[113]

Stage	Description
L1	Localized tumor not involving vital structures as defined by the list of Image Defined Risk Factors and confined to one body compartment
L2	Local–regional tumor with presence of one or more Image Defined Risk Factors
M	Distant metastatic disease (except Stage MS)
MS	Metastatic disease in children <18 months with metastases confined to skin, liver, and/or bone marrow

Intermediate Risk

Children with intermediate-risk tumors comprise 10% to 15% of new cases and are defined as: infants (<365 days old) with INSS 3 or 4 tumors without *MYCN* amplification and favorable histology, children (≥365 days old) with INSS 3 tumors without *MYCN* amplification and favorable histology, or INSS 4 with unfavorable histology and/or diploid DNA (Table 99.5).[122] Surgery is an important component of treatment, as is the addition of moderately intensive chemotherapy (i.e., carboplatin, etoposide, adriamycin, cyclophosphamide). Radiotherapy is reserved for children with progression or those who have unresectable tumors after chemotherapy. This approach has led to excellent survival of 50% to 100%, depending on the clinical and biologic characteristics.[123–125] In a recent report from the COG, 479 children were treated according to a biologically based risk assignment system. Of these, 323 with favorable biologic characteristics received four courses of chemotherapy and 141 children with unfavorable biology received eight courses. This approach resulted in a 3-year EFS of 88±2% and 3-years overall survival of 96±1%.[122]

High Risk

Approximately 50% to 60% of children present with metastatic disease at diagnosis and the vast majority of these are classified as

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TABLE 99.2B

International Neuroblastoma Risk Group (INRG) Consensus Pretreatment Classification System.[51]

INRG Stage	Age (months)	Histologic Category	Grade of Tumor Differentiation	*MYCN*	11q Aberration	Ploidy	Pretreatment Risk Group
L1/L2		GN maturing; GNB intermixed					A Very low
L1		Any, except GN maturing or GNB intermixed		N/A Amplified			B Very low K High
L2	<18	Any, except GN maturing or GNB intermixed		N/A	No Yes		D Low G Intermediate
	≥18	GNB nodular; neuroblastoma	Differentiating Poorly differentiated or undifferentiated	N/A N/A Amplified	No Yes		E Low H Intermediate N High
M	<18 <12 12–<18 <18 ≥18			N/A N/A N/A Amplified		Hyperdiploid Diploid Diploid	F Low I Intermediate J Intermediate O High P High
MS	<18			NA Amplified	No Yes		C Very low Q High R High

GNB, ganglioneuroblastoma; GN, ganglioneuroma; N/A, not applicable.

high-risk patients.[126] As seen in Table 99.6, children with high-risk disease predominantly include children more than 18 months of age with INSS 4 disease. Other subsets of high-risk patients include children of any age with INSS 2a/b or 3 disease and *MYCN* amplification, children ≥18 months with INSS 3 and unfavorable histology, and infants (<1 year of age) with *MYCN* amplification. The treatment consists of four general components: (1) induction chemotherapy, (2) surgical resection of all gross disease, (3) consolidation, which generally includes myeloablative chemotherapy with stem-cell rescue and radiation therapy to the tumor bed, and then (4) treatment of minimal residual disease (MRD). Despite this intensive therapy, almost half of all high-risk patients eventually relapse and die of their disease.[126]

Induction Chemotherapy. Standard chemotherapy regimens for high-risk disease include combinations of the epipodophyllotoxins etoposide or teniposide, platinum-based agents, cyclophosphamide, and doxorubicin; other active agents such as ifosfamide and topotecan have also been included in some regimens.[127–134] A retrospective analysis in 1991 of 44 published clinical trials showed a convincing correlation between dose intensity and response and progression-free survival, particularly with cisplatin and teniposide.[135] Although a later review did not corroborate these findings,[136] the general approach worldwide has been to increase the intensity of induction chemotherapy. In the only recent randomized trial comparing induction regimens, 262 patients were assigned to either rapid treatment (N = 130) with cisplatin (C), vincristine (O), carboplatin (J), etoposide (E), and cyclophosphamide (C) (COJEC), or standard treatment (N = 132) with vincristine, cisplatin (P), etoposide, cyclophosphamide (OPEC) alternating with vincristine, carboplatin, etoposide, cyclophosphamide (OJEC). The rapid regimen was given every 10 days and the standard regimen was given every 21 days. The same cumulative doses of each drug, except vincristine, were administered and the relative dose-intensity of the rapid regimen was 1.94 compared to the standard regimen.[137] There was no difference in the overall survival (OS) at 5 and 10 years, although the EFS at 5 years was significantly better in the rapid group (30.2% versus 18.2%; p = 0.022). Also, patients progressed to consolidation a median of 55 days earlier, prompting the authors to speculate that this might have contributed to the better outcome in the rapid group.[137] The lack of difference in OS and relatively inferior outcomes, compared to other induction regimens,[133] make interpretation of these results difficult. What is clear is that response, as measured by MIBG scans at the end of induction, strongly correlate with overall outcome[138–140] and that further refinement in induction therapy may focus on those *ultra* high-risk patients who have inferior early response as measured by MIBG or more sensitive quantitative methods.[141,142]

Surgery. The role and timing of surgery in the management of children with high-risk neuroblastoma is uncertain.[143] Although some reports of patients with stage 3 or 4 disease have found that gross total resection of the primary tumor and metastatic local–regional disease has been associated with improved local tumor control and increased overall patient survival,[144–147] other reports have not.[7,148,149] Nevertheless, the COG as well the major European cooperative group, SIOPEN,[137] currently recommend gross total resection of the primary tumor and regional disease in patients with high-risk neuroblastoma. The resection is often delayed until near the end of induction, even though tumor volume reduction appears to plateau after the second or third course of chemotherapy,[150] and there are some data that early resection may provide a survival benefit.[151] The goal of surgical local control is to remove all visible tumors without jeopardizing vital organs or delaying chemotherapy; however, most primary tumors invade local structures and surround major blood vessels, making resection difficult. Complete removal at the time of diagnosis is possible for less than 20% of patients.[146,152] Resection of invasive tumor is associated with complications, including the removal of normal organs, hemorrhage, renal injury,[153] vascular injury, chylous ascites and chronic diarrhea. After treatment with chemotherapy, complete removal becomes possible in more than 50%.[7]

TABLE 99.3

Children's Oncology Group Risk Categories for Neuroblastoma

INSS Stage	Age	MYCN	INPC Histology	Ploidy	Other	Risk Group Assignment
1	Any	Any	Any	Any		Low
2A/2B	Any	Not amp	Any	Any	Resection ≥50%, asymptomatic	Low
2A/2B	Any	Not amp	Any	Any	Resection ≥50% symptomatic	Intermediate
2A/2B	Any	Not amp	Any	Any	Resection <50%	Intermediate
2A/2B	Any	Not amp	Any	Any	Biopsy only	Intermediate
2A/2B	Any	Amp	Any	Any	Any degree of resection	High
3	<547 d	Not amp	Any	Any		Intermediate
3	≥547 d	Not amp	Favorable	Any		Intermediate
3	Any	Amp	Any	Any		High
3	≥547 d	Not amp	Unfavorable	Any		High
4	<365 d	Amp	Any	Any		High
4	<365 d	Not amp	Any	Any		Intermediate
4	365–<547 d	Amp	Any	Any		High
4	365–<547 d	Any	Any	DI = 1		High
4	365–<547 d	Any	Unfavorable	Any		High
4	365–<547 d	Not amp	Favorable	DI > 1		Intermediate
4	≥547 d	Any	Any	Any		High
4S	<365 d	Not amp	Favorable	DI > 1	Asymptomatic	Low
4S	<365 d	Not amp	Any	DI = 1	Asymptomatic or symptomatic	Intermediate
4S	<365 d	Missing	Missing	Missing	Too sick for biopsy	Intermediate
4S	<365 d	Not amp	Any	Any	Symptomatic	Intermediate
4S	<365 d	Not amp	Unfavorable	Any	Asymptomatic or symptomatic	Intermediate
4S	<365 d	Amp	Any	Any	Asymptomatic or symptomatic	High

Amp, amplified; DI, DNA Index.

Consolidation. Early studies using high-dose chemotherapy showed a strong correlation of increasing dose intensity with progression-free survival in metastatic neuroblastoma.[135,154] The critical importance of dose intensity ultimately led to the integration of high-dose chemotherapy and autologous stem cell transplant (ASCT) into modern treatment regimens for high-risk neuroblastoma.[155–157] Although with it comes unique challenges and risks, the use of myeloablative ASCT allows for the delivery of chemotherapeutic doses not usually feasible due to the dose-limiting toxicity of myelosuppression. Although there is no consensus on the optimal conditioning regimen, many nonrandomized studies supported the use of ASCT as consolidation[158] and several randomized trials have subsequently made ASCT following intensive induction chemotherapy part of the *standard of care* in the treatment of children with high-risk neuroblastoma.[129,158–160]

One of the most common myeloablative regimens used worldwide is a combination of busulfan and melphalan (BuMel). A recent retrospective analysis of nearly 3 decades of European experience with ASCT for neuroblastoma revealed a survival benefit for BuMel over other melphalan-based conditioning regimens.[161] The 343 patients treated with this regimen in first remission had a 5-year OS rate of 48%.[162] Specifics regarding transplant-related complications were not reported. A recent, smaller retrospective study from Spain using a BuMel conditioning reported a progression-free survival of 57% among 36 patients at a mean follow-up of 55 months.[162] There were no toxic deaths reported and no reported veno-occlusive disease (VOD). Risk of complications from this regimen may be age dependent; a report of infants treated with a busulfan-melphalan regimen (with or

TABLE 99.4

Children's Oncology Group Definition of Low-Risk Neuroblastoma

Stage	Age	MYCN	Ploidy	INPC Histology	Other
1	Any	Any	Any	Any	
2A/B	Any	Not amplified	Any	Any	Resection ≥50%; asymptomatic
4s	<365 d	Not amplified	DI>1	Favorable	asymptomatic

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TABLE 99.5

Children's Oncology Group Definition of Intermediate-Risk Neuroblastoma

Stage	Age	MYCN	Ploidy	INPC Histology	Other
2A/B	Any	Not amplified	Any	Any	Resection ≥50%; symptomatic
2A/B	Any	Not amplified	Any	Any	Resection <50%
2A/B	Any	Not amplified	Any	Any	Biopsy only
3	<547 d	Not amplified	Any	Any	
3	≥547 d	Not amplified	Any	Favorable	
4	<365 d	Not amplified	Any	Any	
4	365–<547 d	Not amplified	DI > 1	Favorable	
4s	<365 d	Not amplified	DI = 1	Any	With or without symptoms
4s	<365 d	Missing	Missing	Missing	Too ill for biopsy
4s	<365 d	Not amplified	Any	Any	Symptomatic
4s	<365 d	Not amplified	Any	Unfavorable	With or without symptoms

DI, DNA Index.

without cyclophosphamide) reported VOD in 9 of 12 children treated, including one death.[163] However, this regimen utilized oral busulfan, which has been shown to have a higher risk of complications.

Another common myeloablative regimen is a combination of carboplatin or cisplatin, etoposide, and melphalan (CEM), and it has been used in some of the largest reported US trials.[160] The SIOP Europe Neuroblastoma Group (SIOPEN) randomized patients to receive BuMel or CEM in the HR-NBL1 trial with the primary aim to demonstrate superiority based on EFS. This randomization was closed early due to superiority of the BuMel regimen. A significant difference in EFS in favor of BuMel (3-years EFS 49% versus 33%) was observed as well as for OS (3-years OS 60% versus 48%; p = 0.004). This difference was mainly related to the relapse and progression incidence, which was significantly (p =0.001) lower with BuMel (48% versus 60%).[164] Although these data clearly show the superiority of the BuMel conditioning regimen over CEM when used with the Rapid COJEC induction regimen of HR-NBL1,[137] it remains to be demonstrated if this would hold true if BuMel were used after other induction regimens.

TABLE 99.6

Children's Oncology Group Definition of High-Risk Neuroblastoma

Stage	Age	MYCN	Ploidy	INPC Histology
2A/B	Any	Amplified	Any	Any
3	Any	Amplified	Any	Any
3	≥547 d	Not amplified	Any	Unfavorable
4	<365 d	Amplified	Any	Any
4	365–<547 d	Amplified	Any	Any
4	365–<547 d	Any	DI = 1	Any
4	365–<547 d	Any	Any	Unfavorable
4	≥547 d	Any	Any	Any

Because undetected neuroblastoma cells present in autologous bone marrow can contribute to relapse,[165] the COG evaluated the role of purging stem cell products with a cocktail of five monoclonal antibodies targeting neuroblasts, attached to magnetic beads. All children received identical induction chemotherapy and myeloablation. Enrollees were randomly assigned to receive nonpurged peripheral blood stem cells (PBSC; N = 243) or purged PBSC (N = 243); 192 from the nonpurged and 180 from the purged group received ASCT. The 5-year EFS was 36% in the unpurged group (95% confidence interval [CI] 30 to 42) and 40% (33 to 46) in the group who received purged PBSCs (p = 0.77).[166] Thus nonpurged PBSCs are acceptable for support of children receiving myeloablation during consolidation.

Based on improvements in EFS seen in patients treated with myeloablative consolidation and ASCT, several nonrandomized studies, which were further dose intensified using tandem, nonoverlapping myeloablative conditioning regimens. suggested this approach may further improve outcome.[167–172] A pilot study of tandem transplant following intensive induction chemotherapy was developed by COG (ANBL00P1) to test the feasibility and toxicity for use as one of the arms of a randomized phase III study.[172] Patients received the first consolidation with thioTEPA and cyclophosphamide and, upon recovery, received a second ABMT with carboplatin, etoposide phosphate, and melphalan. Forty-one patients were enrolled; eight did not receive any ASCT. Of the 33 who received the first ASCT, 26 went on to receive the second. The 3-year EFS was 44.8±9.5% and this tandem ASCT combination was used as the experimental arm in a randomized phase III trial comparing single versus tandem ASCT (ANBL0532).[172] This trial completed accrual in February of 2012 and results are still pending.

Radiation Therapy. ASCT for neuroblastoma has been performed using various combinations of chemotherapy with and without total body irradiation (TBI). Although the optimum chemotherapeutic combination has not been conclusively demonstrated, the incorporation of TBI has not improved survival, but rather increases transplant-related complications.[161,173] However, because primary site failure persists as a component of tumor progression, primary site irradiation has been used as part of consolidation therapy for patients with high-risk neuroblastoma,[129,174–177] including those enrolled on the recent COG ANBL0532 study.[178] In the setting of recurrent or incurable disease, radiotherapy can

also be used to treat bone and soft tissue metastasis to control pain and to prevent a loss of organ function.[179]

Treatment of Minimal Residual Disease. Finally, additional treatment with biologics (i.e., 13-*cis*-retinoic acid) in the setting of minimal residual disease appears to improve survival. CCG randomized children after consolidation with high-dose chemotherapy and ASCT to receive 6 months of *cis*-retinoic acid. The 3-year EFS was significantly better among those who received *cis*-retinoic acid than those assigned to receive no further therapy (46% versus 29%, respectively; p = 0.027).[129] Targeted immunotherapy, using a chimeric monoclonal antibody, ch14.18, against the tumor-associated disialoganglioside, GD2, has been ongoing for more than 2 decades. A recent randomized study from the COG found that the administration of ch14.18, in combination with interleukin-2, granulocyte macrophage colony stimulating factor, and *cis*-retinoic acid following recovery from consolidation was associated with an improved 2-year EFS (66±5% versus 46±5%) and OS (86±4% versus 75±5%) (p = 0.02).[180] This study has established a new standard of care for all children with high-risk disease in the future.

WILMS TUMOR

Wilms tumor is the most common primary malignant renal tumor of childhood. Advances in therapy since the inception of the prospective randomized trials conducted by various multi-institutional cooperative groups have led to an OS rate of 90%, although success has been achieved at a cost of serious chronic health conditions 25 years after diagnosis in 25% of survivors.[181]

Epidemiology and Genetics

Among North American children less than 15 years of age, the incidence of Wilms tumor is 1 in 10,000 children, or approximately 500 new cases per year.[182] Wilms tumor accounts for 6% of all childhood tumors, but more than 90% of all renal cancers in patients under the age of 20 years.[183] The risk for developing Wilms tumor is higher in African Americans and lower among Asian populations.[183] Although unilateral disease is more common, with males presenting at a slightly earlier age (37 months) than females (43 months), approximately 6% of patients harbor bilateral disease at diagnosis, with males presenting slightly earlier (24 months) than females (31 months).[184]

A variety of syndromes and congenital abnormalities are associated with the development of Wilms tumor. A deletion of chromosome 11p13 that encompasses the *WT1* and *PAX6* gene results in Wilms tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR syndrome), whereas *WT1* point mutations at 11p13 are implicated in Denys-Drash syndrome (DDS), and Frasier syndrome.[185–187] A second Wilms tumor locus, *WT2*, is located at 11p15 and has been linked to an increased incidence of Wilms tumor in Beckwith-Wiedemann syndrome (BWS).[186,188] Specific to the *WT2* locus, at least 10 imprinted genes that have preferential expression by the maternal or paternal allele have been identified, including *IGF2*, *H19*, *p57*, and *LIT1*.[186] Other overgrowth syndromes associated with an increased susceptibility for the development of Wilms tumor include Simpson-Golabi-Behmel syndrome, Sotos syndrome, and Perlman syndrome. Patients with isolated hemihypertrophy, aniridia, Bloom syndrome, Alagille syndrome, trisomy 18, or Li-Fraumeni syndrome are also at risk for developing Wilms tumor.[186,189,190] Familial predisposition accounts for 1% to 2% of cases, and putative familial Wilms tumor genes have been mapped to 17q12-21 (*FWT1*) and 19q13.4 (*FWT2*).[185] Children with hereditary syndromes that predispose to the development of Wilms tumor should receive abdominal screening ultrasounds every 3 months until 8 years of age.[190] Although they appear to respond to treatment similarly as other children, patients with a predisposition (*WT1* mutation) and Wilms tumor have a higher incidence of bilateral disease, intralobar nephrogenic rests, and end-stage renal disease.[187]

Pathology

Most Wilms tumors are solitary and composed of varying proportions of blastemal, stromal, and epithelial cells that recapitulate normal kidney development. Anaplasia, either *focal* or *diffuse*, is characterized by the presence of large nuclei, irregular mitotic features, and hyperplasia and is associated with adverse clinical outcomes.[191,192] In focal anaplasia, cells with anaplastic nuclear changes are confined to sharply restricted foci within the primary tumor.[192] A recent analysis has demonstrated that the presence of anaplasia even in stage I disease adversely affects clinical outcome.[193]

Wilms tumor must be distinguished histologically from other pediatric renal tumors, including renal cell carcinoma,[194] clear cell sarcoma,[195] and rhabdoid tumor of the kidney,[196] as well as neuroblastoma. Clear cell sarcoma and malignant rhabdoid tumor of the kidney were previously considered to belong to the *unfavorable histology* group of Wilms tumors, but are now considered to be distinct categories of renal tumors. Rhabdoid tumor of the kidney tends to metastasize to the lung and brain. Primary rhabdoid tumors of the kidney and brain (atypical teratoid or rhabdoid tumors) share deletions of chromosome band 22q11.2, the site of *SMARCB1* (also known as *INI1*, *BAF47*, and *SNF5*), a putative tumor suppressor gene.[197,198] Another important renal neoplasm, congenital mesoblastic nephroma, is important to recognize because it is usually curable by nephrectomy alone. These tumors are typically identified in the first months of life, with a median age at diagnosis of 2 months.[199] They are associated with the t(12;15) translocation that produces the *ETV6-NTRK3* fusion gene product.[200]

Precursor lesions, or nephrogenic rests, have been identified in the renal parenchyma of approximately 36% of patients with Wilms tumor.[201,202] Nephroblastomatosis is a term that describes kidneys with multifocal or diffuse nephrogenic rests. Perilobar rests, located along the perimeter of a renal lobe, are strongly associated with synchronous bilateral Wilms tumors, BWS, and hemihyperplasia, whereas intralobar rests (within the renal lobe) show a strong association with metachronous tumors and WAGR and DDS.[203] Children with nephroblastomatosis have an increased risk of developing Wilms tumor and require close monitoring.[204]

Biologic prognostic factors, such as gain of 1q, LOH at chromosomal regions 1p and 16q, supplement stage, and histology when assigning risk. Gain of 1q is associated with increased risk of relapse in patients with favorable histology Wilms tumor.[205] LOH of chromosomes 1p and 16q is present in approximately 5% of favorable histology tumors and is associated with increased risk of relapse and mortality.[206] The recently closed COG trial incorporated these factors prospectively into treatment decisions with augmented therapy for patients with LOH and favorable histology; a data analysis is pending. In a retrospective analysis of patients with very low risk disease (stage 1, FH, <550 g, <24 months of age) who were treated with surgery alone, *WT1* mutations, and 11p15 LOH were associated with a higher risk of relapse.[207]

Clinical Presentation and Natural History

Most children with Wilms tumor come to medical attention because of abdominal swelling or the presence of an abdominal mass that may be noted by the caregiver during bathing or dressing the child. Abdominal pain, gross hematuria, and fever may be present at diagnosis.[208] Hypertension is present in approximately 20% of cases.[209]

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Clinical Evaluation and Staging

With gentle palpation to maintain tumor capsule integrity, location and size of the abdominal mass and its movement with respiration should be noted. A varicocele may be associated with the presence of a tumor thrombus in the renal vein or inferior vena cava.[210] An evaluation for signs of associated syndromes, such as aniridia, hemihypertrophy, and genitourinary abnormalities, is mandatory. Laboratory studies should include a complete blood cell count, liver function tests, renal function tests, serum chemistries including calcium, and a urinalysis.

Diagnostic Imaging

Imaging should define the local and distant extent of the tumor, evaluate the renal veins and surrounding vessels to detect the presence of a tumor thrombus, and assess the contralateral kidney to rule out bilateral lesions, all of which are important prior to surgery.

The initial radiographic study is often an abdominal ultrasound examination to determine the consistency of the mass and the organ of origin, and to assess patency of the inferior vena cava. CT or MRI is appropriate for imaging of the primary tumor and to provide adequate visualization of the contralateral kidney, liver, and abdomen to define metachronous and metastatic tumors in the abdomen and pelvis. MRI may be superior to other imaging modalities in delineating nephroblastomatosis.[211]

Both plain radiographs of the chest (chest x-ray) and a chest CT should be performed to determine the presence of pulmonary metastases. In many cases, nodules detected by more sensitive CT scans do not necessarily represent a metastatic tumor, and current studies focused on determining which patients truly require intensified therapy with radiation.[212] Imaging of the brain or skeleton is usually reserved for patients with rhabdoid tumors of the kidney and clear cell sarcoma of the kidney.

Staging

The staging criteria developed by the National Wilms Tumor Study Group (NWTSG) is shown in Table 99.7. The NWTSG approach allows for an adequate assessment of the extent of disease and histologic characteristics of the tumor and facilitates the collection of tumor tissue for biologic studies prior to therapy. In contrast, the SIOP approach (staging not shown) uses preoperative chemotherapy, decreasing the volume of tumor, and thereby decreasing the perioperative risk of tumor spillage. As a result of these treatment philosophies, children enrolled on NWTSG trials receive radiation therapy more often, whereas patients on the SIOP trials receive more cumulative doses of anthracyclines.[213–215]

TABLE 99.7

National Wilms Tumor Study Group Staging for Renal Tumors

Stage	Description
I	a. Tumor is limited to the kidney and completely excised b. The tumor was not ruptured before or during removal c. The vessels of the renal sinus are not involved beyond 2 mm d. There is no residual tumor apparent beyond the margins of excision
II	a. Tumor extends beyond the kidney but is completely excised b. No residual tumor is apparent at or beyond the margins of excision c. Tumor thrombus in vessels outside the kidney is stage II if the thrombus is removed en bloc with the tumor
III	Residual tumor confined to the abdomen: a. Lymph nodes in the renal hilum, the periaortic chains, or beyond are found to contain tumor b. Diffuse peritoneal contamination by the tumor c. Implants are found in the peritoneal surfaces d. Tumor extends beyond the surgical margins either microscopically or grossly e. Tumor is not completely resectable because of local infiltration into vital structures
IV	Presence of hematogenous metastases or metastases to distant lymph nodes
V	Bilateral renal involvement at the time of initial diagnosis

From Metzger ML, Dome JS. Current therapy for Wilms' tumor. *Oncologist* 2005;10:815–826, with permission.

Treatment

Tumor stage and histology are the main prognostic indicators that determine the treatment regimen for Wilms tumor, whereas patient age at presentation and biologic factors (LOH 1p and 16q) were tested prospectively in the most recent COG (NWTS) trials (results pending). Surgery and chemotherapy comprise the main therapy for Wilms tumors, with the addition of radiation for metastatic or histologically aggressive tumors.

Surgery

Surgical resection is the primary method for achieving local control and is usually performed at the time of diagnosis in North American studies (COG/NWTS). A review of children treated on the NWTS-IV demonstrated an increased incidence of local recurrence in those cases in which lymph node biopsies were not obtained.[216] Presumably, these children were understaged and thus undertreated. Additionally, this review clearly demonstrated that operative rupture, whether localized to the renal fossa or diffusely spread within the peritoneal cavity, was associated with an increased incidence of local recurrence.[216]

Routine exploration of the contralateral kidney is not necessary if imaging is satisfactory and does not suggest a bilateral process.[217,218] Nephrectomy alone followed by observation for patients less than 2 years of age with stage I favorable histology tumors that weigh less than 550 g resulted in only an 85% EFS, and even though the study was stopped early, the OS was still 100% for this group of patients.[219,220] Further analysis identified a *WT1* mutation and an 11p15 LOH as risk factors associated with relapse in this patient group.[207] Prenephrectomy chemotherapy and nephron sparing techniques may benefit patients with bilateral disease at diagnosis.[221] Children with syndromic, unilateral Wilms tumor who have an increased risk of late renal failure (i.e., DDS or WAGR) may also benefit from partial nephrectomy or nephron sparing surgery.[222,223] Preoperative treatment of Wilms tumor should be considered in children with a solitary kidney, bilateral renal tumors, tumor in a horseshoe kidney, tumor thrombus in the inferior vena cava above the level of the hepatic veins,[224,225] and respiratory distress as a result of extensive metastatic disease.[226] In children with bilateral disease or involvement of a solitary kidney, preoperative chemotherapy is intended to permit maximal conservation of uninvolved renal parenchyma. In the current COG renal tumor protocol, children who present with bilateral renal masses receive two to four cycles of chemotherapy without biopsy

(COG-AREN0534, NCT00945009). A biopsy is reserved for those patients whose tumors do not show appropriate volume reduction. Definitive surgery should be performed by week 12.

The role of surgical resection in the management of solitary pulmonary metastases was evaluated by the NWTSG in 211 patients with no therapeutic benefit identified when surgical resection was added to pulmonary radiotherapy and chemotherapy.[227] Four-year survival rates were identical in the two groups.

Chemotherapy

Vincristine and dactinomycin, with the addition of doxorubicin for more advanced disease, form the backbone of most combinations for the treatment of Wilms tumor.[213] The recommended standard drug regimens and duration of therapy according to stage and intergroup studies are listed in Table 99.8. The five consecutive NWTSG studies have provided the foundation for current care in North America and the results of various US and European trials are depicted in Table 99.9.[222,228–233,236,237]

NWTS-1 documented that postoperative radiation therapy is not required for children with stage I/II favorable histology or stage I anaplastic histology tumors when postnephrectomy chemotherapy with vincristine and dactinomycin is administered; NWTS-2 showed that doxorubicin improved relapse-free survival for group II through IV patients, NWTS-3 upstaged all patients with lymph node involvement to stage III, and provided evidence that patients with stage III favorable histology who receive adjuvant chemotherapy and radiation require augmented therapy with either doxorubicin or higher radiation therapy dose (20 Gy) for successful outcomes. Concerns about late effects of radiation resulted in the selection of the three-drug regimen (vincristine, dactinomycin, and doxorubicin) with lower radiation therapy dose (10 Gy) as standard therapy in this group.[234,235] Furthermore, this trial showed that the addition of cyclophosphamide to the standard three-drug regimen (vincristine, dactinomycin, and doxorubicin) does not improve the outcome of patients with stage IV/favorable histology tumors, but was potentially useful in patients with anaplastic tumors. In NWTS-4, the addition of cyclophosphamide proved to benefit patients with stage II through IV anaplastic tumors. In addition, therapy was reduced for low-risk patients, and *pulse-intensive* regimens were shown to maintain excellent survival with less toxicity than previous regimens.[236] Anaplasia, either diffuse or focal, adversely affects the outcome even after the administration of conventional chemotherapy with vincristine, dactinomycin, and doxorubicin. Cyclophosphamide and etoposide, when added to vincristine, dactinomycin, and doxorubicin, improved the survival of patients with stage II through IV diffuse anaplasia in NWTS-5.[193] A trial incorporating carboplatin to improve outcomes was stopped early due to toxicity of this regimen (UH1). Results of NWTS-5 indicate that patients with stage I anaplasia had lower than expected survival and will require augmented therapy in upcoming studies.

Salvage therapy is successful in up to 80% of patients with low-stage favorable histology tumors initially treated with vincristine, dactinomycin, and no radiotherapy.[237] Late recurrence more than 5 years after the diagnosis is rare, but is associated with a similar outcome to earlier recurrence.[238] At the time of relapse, approximately 50% of unilateral Wilms tumor patients who have received standard chemotherapy, three-drug chemotherapy, and radiation can be successfully re-treated.[239–241] Alternating cyclophosphamide/etoposide and carboplatin/etoposide, surgery and radiation led to 48% OS in high-risk patients with 53% OS in high-risk patients with lung-only relapse on the NWTS-5 relapse protocol.[239] The benefit of high-dose chemotherapy with autologous stem cell rescue (single or tandem transplants) is uncertain,[242] although it has been shown that patients with residual disease do not do as well.[243] Alternative agents, including topotecan[244] and irinotecan,[245] have shown some promising antitumor activity in phase 1/2 studies and require further evaluation in clinical trials.

Radiation Therapy

Successive NWTSG trials refined the dosages and indications to decrease radiation exposure while maintaining local control and control of metastatic pulmonary lesions (see Table 99.7). Patients with stage I and II favorable histology tumors do not require abdominal radiotherapy, whereas those with stage III favorable histology or stages I to III focal or diffuse anaplastic disease require adjuvant radiotherapy to the flank or abdomen. The tumor and renal beds with a 1- to 2-cm margin are treated in cases of positive resection margins, nodal involvement, or local spillage during surgery. Care must be taken to provide a uniform dosage to the vertebral column to limit the risk of scoliosis. Whole-abdominal radiotherapy is used for patients with preoperative rupture, diffuse spill during surgery, or when peritoneal metastasis is present. A dose of 10.8 Gy is sufficient for local control in stage III favorable histology patients if they also received chemotherapy with vincristine, dactinomycin, and doxorubicin.[246] Most patients receive radiotherapy within 8 to 12 days of nephrectomy without compromise in local–regional control.[247] Currently, it is recommended that radiotherapy start within 14 days of a nephrectomy.

Whole-lung irradiation (12 Gy) has been recommended for patients who present with pulmonary metastases, which has

TABLE 99.8

Treatment Regimens for Wilms Tumor with Favorable or Standard Histologic Features from Recently Completed NWTSG and SIOP Studies

	NWTS-5		SIOP93-01		
			Chemotherapy		
Stage	Chemotherapy	Radiation Therapy	Preoperative	Postoperative	Radiation Therapy
I	VA × 18 weeks	—	VA × 4 weeks	VA × 4 weeks	—
II	VA × 18 weeks	—	VA × 4 weeks	VDA × 27 weeks	Node negative: none Node positive: 15 Gy
III	VDA × 24 weeks	10.8 Gy	VA × 4 weeks	VDA × 27 weeks	15 Gy
IV	VDA × 24 weeks	12 Gy lung (if lung metastasis) 10.8 Gy flank (if local stage III)	VDA × 6 weeks	CR after 9 weeks: VCA × 27 weeks	None if lung lesions disappear by week 9, otherwise 12 Gy No CR after 9 weeks: ICED × 34 weeks

V, vincristine; A, dactinomycin; D, doxorubicin; CR, complete remission; I, ifosfamide; C, carboplatin; E, etoposide.
From Metzger ML, Dome JS. Current therapy for Wilms' tumor. *Oncologist* 2005;10: 815–826, with permission.

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TABLE 99.9

Summary of Patient Outcomes from Recently Reported Large Studies of Wilms Tumor

Study	Stage	Relapse-Free Survival/ Event-Free Survival (%)	Overall Survival (%)	Reference
NWTS-3		16 yr:	16 yr:	237
	I	92.5	97.6	
	II	89.6	92.9	
	III	80.4	86.2	
	IV	76.5	79.5	
NWTS-4	I	94.9 (2 yr)	98.7 (2 yr)	238,234
	II	83.6 (8 yr)	93.8 (8 yr)	
	III	88.9 (8 yr)	93 (8 yr)	
	IV	80.6 (2 yr)	89.5 (2 yr)	
	V (synchronous, FH)	74 (8 yr)	89 (8 yr)	223
	V (synchronous, AH)	40 (8 yr)	45 (8 yr)	223
NWTS-5		4 yr:	4 yr:	235
	I (age <24 mos, tumor weight <550 g)	84	98	
	I/II, no LOH	91	98	
	I/II, LOH 1p and 16q	75	91	
	III/IV, no LOH	83	92	
	III/IV, LOH 1p and 16q	66	78	
	V, any LOH	61	81	
	I, diffuse anaplasia	68	79	
	II, diffuse anaplasia	83	82	
	III, diffuse anaplasia	65	67	
	IV, diffuse anaplasia	33	33	
	V, diffuse anaplasia	25	42	
SIOP-9		2 yr:	5 yr:	230
	I (FH)	100	100	
	I (includes AH)	88	93	
	II N0	84	88	
	II N1 and III	71	85	
	Unfavorable (AH, WT, CCSK, and rhabdoid)	71	71	
SIOP93-01	Nonanaplastic	5 yr:	5 yr:	
	I	88.3	97	231
	II	91.9 (5 yr)	96.7 (5 yr)	232
	III	84.3 (5 yr)	91.5 (5 yr)	232
UKW3	Nonanaplastic	5 yr:	5 yr:	
	I	85.7	96.4	233
	II	83.3	93.8	
	III	79.6	89.9	
	IV	71.7	81.1	
	V	70.8	78.6	

FH, favorable histology; AH, anaplastic histology; N0, no lymph node involvement; N1, lymph node involvement; WT, Wilms tumor; CCSK, clear cell sarcoma of kidney; UKW, United Kingdom Children's Cancer Study Group Wilms Tumor trial.

historically been defined by the presence of nodules on chest x-ray. However, CT now allows for the identification of much smaller lesions. Ehrlich et al.[212] found that, in those patients whose pulmonary lesions were biopsied, only 82% of isolated lesions and 69% of multiple pulmonary lesions were actually tumor. Review of NWTS-4 and -5 identified patients with favorable histology and CT-only pulmonary lesions treated with standard three-drug therapy demonstrated no difference in EFS with or without the addition of radiation.[248] For the SIOP 93-01 study, pulmonary radiation was successfully limited to those patients with unresectable, persistent nodules or high-risk primary tumor histology after receiving prenephrectomy chemotherapy for 6 weeks and postnephrectomy chemotherapy for 9 weeks.[249] To develop a response-based approach to pulmonary radiation therapy, a recently closed COG trial evaluated the feasibility of eliminating radiotherapy in children whose chest CT scans demonstrated complete resolution of pulmonary nodules at week 6 of therapy. The results of this strategy are pending.

RETINOBLASTOMA

Retinoblastoma is a rare childhood cancer of the developing retina that often presents with leukocoria and is fatal if untreated. Early detection and judicious therapy based on laterality and stage of disease has resulted in survival rates exceeding 90% in the United States for localized (intraocular) disease.

Epidemiology

The incidence rate in the United States for the period 1975 to 1995 is estimated at 3.7 cases per million (about 300 new cases per year) with no racial predilection or significant difference between males and females.[183] Worldwide, approximately 9,000 new cases are diagnosed each year.[250] Over 85% of patients in the United States present with localized intraocular disease at diagnosis. Two-thirds of cases are diagnosed before 2 years of age and 95% before age 5 years. For unilateral disease, the median age at diagnosis is 2 years; for bilateral disease, the median age at diagnosis is less than 12 months. Approximately one-quarter of cases are bilateral and, therefore, have the heritable form of the disease (either sporadic or familial). In the remaining 75% of cases (unilateral), 10% to 15% of patients are found to have the heritable form of the disease. Parents and siblings of patients with retinoblastoma should undergo a thorough ophthalmoscopic examination while genetic testing is pending.

Biology and Genetics

Retinoblastoma is caused by the biallelic inactivation of the retinoblastoma gene, *RB1*, a tumor suppressor gene that is located on the long arm of chromosome 13 (13q14).[251] Mutations in the *RB1* gene are transmitted as a highly penetrant, autosomal-dominant trait.[252] Patients with 13q- syndrome may have other associated abnormalities, such as distinct facial features, mental retardation, and growth failure.[253] Additionally, these patients have more episodes of neutropenia and GI toxicity than other children with retinoblastoma when receiving systemic chemotherapy (Brennan, unpublished). Recently, rare cases of MYCN-amplified retinoblastoma without *RB1* mutation have been reported.[254] Despite the loss of a tumor suppressor gene, the retinoblastoma genome is relatively stable, with epigenetic dysregulation of cancer pathways, including upregulation of the proto-oncogene SYK, driving further tumorigenesis.[255] Regardless of therapy, patients with heritable retinoblastoma are at an increased risk of developing secondary malignancies, particularly sarcomas, brain cancer, and melanoma[256,257]; those with familial, heritable retinoblastoma may be at an even higher risk.[258] The cumulative incidence of secondary malignancies 50 years after diagnosis in a large US cohort was 36% for hereditary retinoblastoma survivors and 5.7% for nonhereditary survivors; however, the mortality was 25.5% for hereditary retinoblastoma survivors and 1.0% for nonhereditary survivors.[259]

Pathology

Retinoblastoma is composed of uniform small, round, or polygonal cells, which have a scanty, poorly staining cytoplasm. The sparse cytoplasm is located at one side of the cell, suggesting the appearance of an embryonal retinal cell. The nucleus is large and deeply staining, giving the characteristic *small, round, blue cell* appearance. Three types of cellular arrangements may be identified: the Homer-Wright rosette, the Flexner-Wintersteiner rosette, and the fleurette. Calcification and necrosis are often observed.[260]

Clinical Presentation

Leukocoria, an abnormal white reflection from the retina compared with the normal *red reflex*, is the most common presenting sign of retinoblastoma.[261] Strabismus, conjunctival erythema, and decreased visual acuity are other common presenting complaints. Esotropia or exotropia may be present on examination, with decreased visual acuity as a result of involvement of the macula by the tumor or the presence of tumor cells and debris in the vitreous.[262] The eye may be red and painful because of uveitis after spontaneous necrosis of a retinal tumor or from neovascular glaucoma.

Evaluation

The diagnosis of retinoblastoma is based on clinical history (including family history) and results of examination of both eyes under general anesthesia. Once confirmed, the staging evaluation of a child with retinoblastoma should include an ultrasound of the orbits and MRI of the brain and orbits.[263,264] An MRI is useful to evaluate the extent of extraocular (orbital or pineal gland) disease. Although optic nerve enhancement may be concerning for the extraorbital spread of disease, pathology (enucleation) remains the gold standard.[265] Retinoblastoma may metastasize to the central nervous system, bones, or bone marrow.[266,267] A further evaluation for metastatic disease with lumbar puncture, bone marrow aspiration/biopsy, and bone scan is reserved for patients with involvement of extraretinal structures, including the orbit or optic nerve, or when symptoms, signs, pathology, or diagnostic imaging studies suggest the involvement of distant sites.[268,269]

Staging

The patient is staged according to the burden of local and metastatic disease, whereas the eye is *grouped* according to the extent of intraocular disease present. Martin and Reese[270] first proposed a staging system for patients with retinoblastoma in 1942. Two decades later, the Reese and Ellsworth[271] grouping (R-E) attempted to predict the risk of enucleation with external-beam radiotherapy alone (Group Ia through Vb; Table 99.10). Although the R-E group (per eye) is still widely referenced today, the clinical value of utilizing a radiation-based system in an era of multimodal therapy that does not account for the presence of subretinal seeds and was developed before the introduction of indirect ophthalmoscopy is unclear. In 2001, the COG incorporated a new classification system (Group A through E) for grouping intraocular retinoblastoma that could be utilized among all institutions participating

TABLE 99.10

Reese-Ellsworth Grouping System for Retinoblastoma

Group	Description
Group I	
A	Solitary tumor, <4 disc diameters in size, at or behind the equator
B	Multiple tumors, <4 disc diameters in size, all at or behind the equator
Group II	
A	Solitary tumor, 4–10 disc diameters in size, at or behind the equator
B	Multiple tumors, 4–10 disc diameters in size, behind the equator
Group III	
A	Any lesion anterior to the equator
B	Solitary tumors >10 disc diameters behind the equator
Group IV	
A	Multiple tumors, some >10 disc diameters
B	Any lesion extending anteriorly to the ora serrata
Group V	
A	Massive tumors involving more than one-half the retina
B	Vitreous seeding

Adapted from Stannard C, Lipper S, Sealy R, Sevel D. Retinoblastoma: correlation of invasion of the optic nerve and choroid with prognosis and metastases. *Br J Ophthalmol* 1979;63:560–570.

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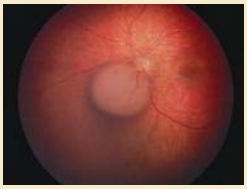
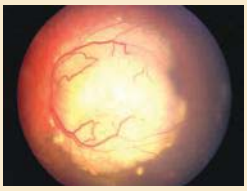
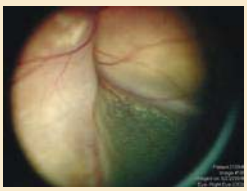
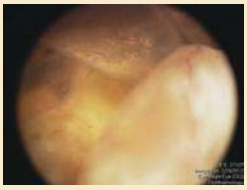
TABLE 99.11

International Classification System for Intraocular Retinoblastoma

Group	Defining Features	
Group A *Small tumors away from fovea and disk*	■ Tumors <3 mm and ■ Located at least 3 mm from fovea and 1.5 mm from the optic disc	
Group B *All remaining tumors confined to the retina*	■ All other tumors confined to retina not in Group A ■ Subretinal fluid <3 mm from the base of tumor	
Group C *Local subretinal fluid or seeding*	■ Local subretinal fluid alone >3 mm to <6 mm from tumor ■ Vitreous seeding or subretinal seeding 3 mm from the tumor	
Group D *Diffuse subretinal fluid or seeding*	■ Subretinal fluid alone >6 mm from tumor ■ Vitreous seeding or subretinal seeding >3 mm from tumor	
Group E *Presence of poor prognosis features*	■ More than two-thirds of globe filled with tumor ■ Tumor in anterior segment or the ciliary body ■ Iris neovascularization, neovascular glaucoma ■ Tumor necrosis, phthisis bulbi	

in clinical trials (Table 99.11). This classification system, which identifies the likelihood of treatment failure requiring enucleation or EBRT (group A = very low risk; group E = very high risk), was originally developed at Children's Hospital Los Angeles and is now used internationally.[272] The St. Jude Children's Research Hospital developed a staging system that is defined by the extent of tumor involving the retina and globe, or by the presence of extrachoroidal disease.[273] Another staging system developed by Chantada et al.[274] provides a common international classification for patients with extraocular retinoblastoma.

Treatment

Patients with suspected retinoblastoma should be referred to a pediatric ophthalmologist with expertise in the management of intraocular tumors and a pediatric oncologist experienced in the treatment of retinoblastoma. Treatment aims to preserve life and useful vision and is based on laterality and the stage of disease. Protective eyewear is recommended for all patients. With the high percentage of survivors, treatment decisions must carefully weigh the functional outcome and potential long-term sequelae of local and systemic therapies.[275]

Enucleation

In view of the excellent response of this tumor to globe-sparing interventions, enucleation is considered in selective situations. Recently, more aggressive attempts at ocular salvage, regardless of visual potential, are being attempted (see the following). However, the most strongly considered indications for enucleation include (1) a unilateral retinoblastoma that completely fills the globe or that has damaged and disrupted the retina or vitreous so extensively that restoration of useful vision is not possible (and attempts at ocular salvage may place the patient at risk for metastatic disease); (2) a tumor that is present in the anterior chamber; (3) a painful glaucoma with a loss of vision after rubeosis iridis; (4) a progressive retinoblastoma disease unresponsive to all other forms of local therapy; and (5) cases with permanent vision loss in which extraocular tumor is suspected. The standard surgical technique is modified to allow excision of the longest possible segment of optic nerve in continuity with the globe.[276] After removal, the globe and

optic nerve are inspected for evidence of extraocular extension of the tumor. A hydroxyapatite implant is placed in the muscle funnel, which improves the cosmetic result and the promotion of normal development of the bony orbit.[277]

Local Therapies

When combined with chemoreduction, local therapies, including laser, cryotherapy, and radioactive plaque brachytherapy, consolidate treatment and improve disease control for patients with R-E group I through IV disease.[278,279] Patients with R-E group V disease may require additional therapy, including external-beam radiation.[280]

Laser is useful for tumors ≤2.5 mm in thickness and ≤4.5 mm in diameter (<4 disc diameters).[278] Like laser therapy, cryotherapy produces an avascular scar, but is more often utilized for tumors 2.5 mm in diameter and 1.0 mm thick located anterior to the equator and confined to the sensory retina.[281] Radioactive plaque brachytherapy using iodine-125 radiation may be utilized for tumor consolidation, but is more commonly used in the setting of localized recurrence for tumor control or for salvage.[282,283] Plaque brachytherapy does not appear to have the risk of secondary malignancies seen in external-beam radiation.[283]

Radiation Therapy

External-beam radiation therapy is indicated for patients with advanced disease that has not responded adequately to chemotherapy and local treatments but who has useful vision, or patients with extraorbital extension of disease, usually after enucleation. Patients requiring radiation receive doses between 40 and 45 Gy, depending on tumor size, patient age, and the presence of vitreous seeding.[284] Modern radiotherapy uses megavoltage accelerators and CT- and MRI-based treatment planning to allow for sparing of the lens and bony orbits. Together with daily image guidance for field placement, these techniques may further reduce known radiotherapy-related late effects to surrounding structures.[285] Proton therapy can potentially decrease dosage to the surrounding bony orbits by more than 60% and avoid significant dosage to the hypothalamus compared with intensity-modulated radiotherapy, with equivalent target coverage.[286]

The preservation of some useful vision in these patients is an obvious advantage of such a treatment approach, but the risk of secondary malignancies after external-beam radiation in young children is well defined.[259,287] Kleinerman et al.[288] published long-term results of 1,601 survivors of retinoblastoma and reported a 3.1-fold increase in the risk of secondary cancers for hereditary retinoblastoma survivors who received radiation therapy. Among hereditary retinoblastoma survivors, radiation therapy increased the cumulative incidence of second malignancies to 38% in patients who received radiation compared with 21% of hereditary patients without radiation. The authors also found the risk of osteosarcomas was higher in patients who received chemotherapy with radiation, compared with radiation alone.

After enucleation, local irradiation to include the orbit and the optic nerve up the chiasm is recommended for all patients with extension of retinoblastoma into the orbit. Presentation with exophthalmos, inability to retain the prosthesis, or a palpable mass through the eyelids suggests the presence of orbital extension of the tumor. The identification of an extraocular mass, histologic confirmation of tumor cells at the cut end of the optic nerve at the time of enucleation, or rupture of the globe during removal is associated with orbital contamination with the tumor.

Chemotherapy

The use of neoadjuvant chemotherapy in treating retinoblastoma was prompted by success in salvaging patients with recurrent extraocular disease.[289,290] Since the 1990s, there have been a series of studies documenting promising rates of vision-sparing therapy without external-beam radiotherapy in intraocular disease.[280,291,292] Most chemoreduction regimens are platinum based and utilize carboplatin over cisplatin (due to reduced ototoxicity). Other active agents include vincristine, etoposide, cyclophosphamide, doxorubicin, and topotecan.[291,293–297] Cyclosporin A has also been used as a multidrug-resistant reversal agent.[298] Despite the initial response, which is most pronounced in patients with R-E group I through IV tumors, chemotherapy alone rarely achieves durable disease control and is, therefore, paired with local therapies, as described previously. Patients with R-E group V disease, especially those with vitreous seeds, are at highest risk for chemoreduction failure and tumor recurrence/resistance.

Because tumor shrinkage may prevent enucleation, patients with bilateral disease are candidates for conservative treatment with chemoreduction. Indeed, in bilateral retinoblastoma cases, the assessment of which eye is most likely to be capable of functional vision is difficult until a response to chemotherapy is determined. Adjuvant chemotherapy is utilized after enucleation in patients who have high-risk histologic features, including invasion of the choroid (massive), anterior chamber, or ciliary body/iris, postlaminar optic nerve involvement with concomitant invasion of the choroid, or involvement of sclera or tumor present at the cut end of the optic nerve.[295,299]

Other recent areas of clinical investigation include locally delivered chemotherapeutic agents to increase drug concentration within the eye such as periocular chemotherapy (subconjunctival or subtenon injection),[300] supraselective intra-arterial chemotherapy,[301,302] and intravitreal chemotherapy.[303] Because several reports have shown promising results, the techniques of intra-arterial and intravitreal chemotherapy will be prospectively evaluated in upcoming COG clinical trials to carefully evaluate the feasibility and toxicity of each regimen. At the same time, preclinical retinoblastoma research is providing novel therapies that target key pathways in retinoblastoma tumorigenesis.[255,304,305] Pairing the right drug and delivery technique will translate into therapy that can save globes and vision while reducing the long-term effects of radiation and chemotherapy.

Metastatic Disease

Although most patients in the United States present with localized (intraocular) disease, 10% to 15% of patients will have evidence of extraocular spread at diagnosis. Patients with heritable disease (*RB1* mutation) have a 3% to 9% risk of developing trilateral disease, or disease invading the pineal gland, although this may be lower in this era of improved MRI (tumor versus cyst of pineal gland) and systemic chemotherapy.[264]

Therapy for patients with metastatic disease requires a multimodal approach with intensive systemic chemotherapy, external beam radiation, and, for patients with distant systemic or central nervous system (CNS) metastatic disease, autologous bone marrow transplantation.[289,306–308] Despite aggressive therapy, survival for patients with metastatic retinoblastoma outside of the CNS is 50%, with survival decreasing to <10% for those with CNS metastatic disease.

Late Effects and Long Term Follow-Up

Although patients treated for intraocular retinoblastomas have an excellent prognosis, those with germ-line RBI mutations remain at risk for secondary malignancies. Patients treated with chemotherapy are at risk for late effects, including secondary leukemia with exposure to etoposide[309,310] and ototoxicity with exposure to carboplatin.[311,312] There is also a growing awareness of the other long-term consequences that reach beyond the genetic and physical effects of therapy, such as health-related quality of life and neurocognitive and psychosocial outcomes.[313,314] Further investigation through COG and other large retinoblastoma treatment centers is underway.

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PEDIATRIC BONE SARCOMAS: OSTEOSARCOMA AND EWING SARCOMA

Osteosarcoma and Ewing sarcoma account for approximately 5% of all pediatric malignancies.[183,315,316] Although the biologic properties of these tumors are distinct, their treatment principles are quite similar.[317] Osteosarcoma has a bimodal age distribution, with the first peak in the 2nd decade of life and the second peak occurring in older adults over the age of 65 years. A comprehensive discussion of osteosarcoma is provided in Chapter 91. This section will focus on the main features of osteosarcoma in the younger population, including its unique molecular, genetic, and cytogenetic features.

Osteosarcoma

Epidemiology

Osteosarcoma is the most common primary, malignant bone tumor in children and adolescents, accounting for 4% of all childhood cancers. In the United States, approximately 400 new cases are diagnosed in those younger than 20 years of age.[183] The peak age of incidence corresponds with the time of most rapid bone growth, 16 years in boys and 12 years in girls.[318] The disease is slightly more prevalent in males and in African Americans.[318]

Biology and Molecular Genetics

The majority of osteosarcomas are sporadic. However, certain conditions, such as previous exposure to ionizing radiation[319] and alkylating agents,[320] predispose individuals to the development of osteosarcoma. Additionally, a large proportion of patients older than 40 years who develop osteosarcoma also have Paget disease of bone.[321] Osteosarcoma is also associated with several cancer-predisposition syndromes, including hereditary retinoblastoma, Li-Fraumeni syndrome (LFS), and Rothmund-Thomson syndrome (RTS).

Patients with hereditary retinoblastoma have germ-line mutations in the *RB1* gene and somatic mutations in retinal cells, resulting in retinoblastoma. The majority of secondary nonocular malignancies in these patients are sarcomas, and more than a third of these are osteosarcomas, half of which occur within a previously irradiated field.[257,287,322] LFS is a familial cancer syndrome in which affected family members have a wide spectrum of cancers, including osteosarcoma.[323] Many of these patients carry germ-line mutations in the *p53* tumor suppressor gene.[324,325] Screening of a large series of children with osteosarcoma showed that approximately 3% to 4% carried constitutional germ-line mutations in *p53*.[326] RTS is an autosomal-recessive condition characterized by a distinctive rash (poikiloderma), small stature, skeletal anomalies, sparse hair, and increased risk for OS. In a cross-sectional study of 41 patients with RTS, 13 patients (30%) had osteosarcoma.[327] Two-thirds of patients had constitutional mutations in the *RECQL4* gene, and the presence of mutations correlated with osteosarcoma risk.[328] Because these genetic conditions are known to predispose individuals to osteosarcoma, careful detailing of family history in a patient with newly diagnosed osteosarcoma is important to identify underlying genetic risk and for genetic counseling of family members.

In view of these genetic predisposition syndromes, it is not surprising that the *RB1* and *p53* genes are also frequently altered in sporadic osteosarcoma tumors. Approximately 70% of primary osteosarcoma tumors have alterations in the *RB1* gene.[329–331] Regulators of the *RB1* pathway, including cyclin-dependent kinases 4 and 6, cyclin D1, and $p16^{INK4a}$, are also altered in some cases of osteosarcoma.[332–334] Inactivating mutations of the *p53* gene occur in approximately 50% of all sporadic cancers. The overall frequency of *p53* mutations in osteosarcoma ranges from 15% to 30% depending on the detection methods used.[335] Other members of the *p53* pathway, including $p14^{ARF}$ and MDM2, are altered in some cases of sporadic osteosarcoma.[336,337] Unlike *p53* and *RB1*, the *RECQL4* gene has not been found to be mutated in cases of sporadic osteosarcoma.[338]

Clinical Presentation and Natural History

Most patients with osteosarcoma present with pain and soft-tissue swelling. Approximately 5% to 10% may present with a pathologic fracture of the affected bone.[339,340] Systemic symptoms such as fever, weight loss, and malaise are generally absent. A physical examination usually demonstrates a firm, tender mass and restricted range of motion in the affected extremity. Laboratory evaluation results are usually normal except for elevated alkaline phosphatase (in approximately 40%),[341] elevated lactate dehydrogenase (in approximately 30%),[342] and elevated erythrocyte sedimentation rate, none of which are specific for osteosarcoma.

Osteosarcoma in the pediatric population preferentially involves the metaphyseal region of the long bones, in contrast to Ewing sarcoma, which typically arises in the diaphyseal region of the long bones. The primary site of disease in 80% of patients with osteosarcoma is an extremity, most commonly (in descending order) the distal femur, proximal tibia, and proximal humerus. Unlike Ewing sarcoma, osteosarcoma rarely affects the axial skeleton.[343] Approximately 20% of patients with osteosarcoma present with clinically detectable metastatic disease, most frequently to the lungs and less often to bones.

Diagnostic and Staging Evaluation

The initial evaluation of a suspected bone tumor involves obtaining a patient's complete history and performing a physical examination. Radiographic studies allow for an assessment of the anatomic site, the extent of local invasion, and the pattern of extension.[344] If the tumor involves an extremity, then plain radiographs should encompass both proximal and distal joint regions, and they should be taken in two planes. Characteristic findings suggestive of osteosarcoma on a radiograph include a mixed lytic and sclerotic appearance, periosteal new bone formation with lifting of the cortex and the formation of the Codman triangle, and ossification of the soft tissue in a radial or *sunburst* pattern (Fig. 99.5).[343] Even if the plain radiographs are classic for osteosarcoma, further imaging of the primary tumor by MRI or CT is required to evaluate the extent of the tumor for surgery-planning purposes. The imaging should include the entire tumor-bearing bone in order to assess for skip metastases (i.e., isolated tumor foci within the same bone as the primary tumor). Although both CT and MRI are equally accurate for local staging of tumors, an MRI is preferable for evaluating soft-tissue extension and joint and marrow involvement.[344]

Because patients with metastatic disease at presentation have a significantly worse outcome than do patients with localized disease, a thorough search for sites of metastases is imperative. Staging workup for osteosarcoma includes a technetium-99 bone scan to evaluate the involvement of other bones and a chest CT to detect pulmonary metastases. Studies evaluating the utility of FDG PET/CT in osteosarcoma staging show that FDG PET/CT may be more sensitive and accurate than a bone scan in detecting bone metastases.[345,346] Furthermore, the results of several studies suggest that changes in the metabolic tumor activity as assessed by PET/CT during therapy correlate with the percentage of tumor necrosis at the time of definitive surgery.[347,348] A histologic confirmation is indicated in pulmonary lesion(s) that cannot be unequivocally defined as being a metastatic disease. Finding one or more pulmonary (or pleural) nodules of at least 1-cm diameter *or* three or more nodules of at least 0.5-cm diameter generally indicates definite pulmonary metastases and may not require a biopsy. Fewer or smaller lesions may or may not represent metastatic disease; therefore, confirmation by resection may be indicated.[344] There are no laboratory studies that are diagnostic or prognostic

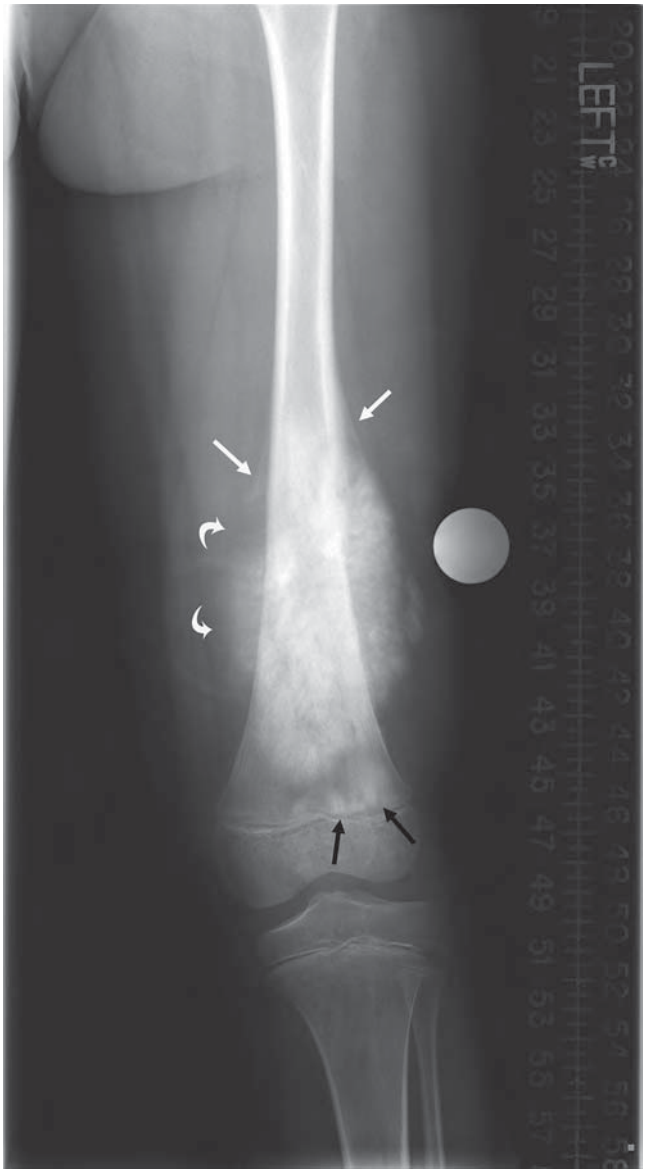

Figure 99.5 Radiographic appearance of osteosarcoma involving the distal femoral diaphysis and metaphysis, with Codman triangles at the proximal end of the tumor *(white arrows)* and an associated sunburst periosteal reaction *(curved arrows)*. Skip metastases are suggested by the presence of round sclerotic foci abutting the growth plate *(black arrows)*.

for osteosarcoma.[349] General laboratory tests such as a complete blood count; electrolyte counts including calcium, magnesium, and phosphorus; liver and renal function tests; and alkaline phosphatase and lactate dehydrogenase (LDH) measurements should be performed to obtain baseline values.[349]

Pathology

None of the radiographic features described previously are pathognomonic for osteosarcoma; therefore, a tissue biopsy is required to make a definitive diagnosis. The differential diagnosis for a bone lesion with aggressive features (e.g., the presence of a Codman triangle, associated soft-tissue mass, permeative appearance) on imaging studies includes Ewing sarcoma, lymphoma, and metastatic tumor. It may also occasionally include benign bone lesions, such as osteochondroma and giant cell tumor, and nonneoplastic conditions, such as osteomyelitis, eosinophilic granuloma, and aneurysmal bone cyst. The biopsy should be performed by an orthopedic surgeon experienced in the management of malignant bone tumors, ideally by the same surgeon who will perform the definitive surgery. Proper planning of the biopsy with careful consideration of the future definitive surgery is important so as not to jeopardize the subsequent treatment, particularly in the case of a limb-salvage procedure.[350]

Osteosarcoma is thought to be derived from primitive bone-forming mesenchymal cells.[135] The histologic diagnosis is based on the presence of a malignant sarcomatous stroma associated with the production of *tumor* osteoid or immature bone.[351] Several types of osteosarcomas have been identified based on histologic, clinical, and radiographic features including conventional osteosarcoma (80%), which is the type most frequently encountered in children and adolescents. Conventional osteosarcomas are further subdivided into osteoblastic (50% of conventional OS), fibroblastic (25%), and chondroblastic (25%) variants depending on the presence of the predominant type of matrix.[352] The main feature that distinguishes these tumors from the malignant fibrosarcomas and chondrosarcomas, which also arise from primitive mesenchymal cells, is the production of osteoid.[351]

Other variants of osteosarcoma include parosteal, telangiectatic, small-cell, periosteal, low-grade central, and high-grade surface.[353,354] All variants are considered to be high-grade tumors except for low-grade central and parosteal osteosarcomas, which are considered to be low-grade tumors, and periosteal osteosarcoma, which is thought to be intermediate grade.[354,355] Small-cell osteosarcoma can be histologically confused with other small, round cell tumors, especially Ewing sarcoma.[356] The tumors may stain positive for CD99 and may contain the Ewing sarcoma breakpoint region 1 (EWSR1) gene rearrangement.[357] Two other distinct variants of osteosarcoma have been described, osteosarcoma of the jaw and extraosseous osteosarcoma. Osteosarcoma of the jaw tends to occur in older patients, has an indolent course, and is more often associated with local recurrences than with distant metastases. Extraosseous osteosarcoma is rarely encountered and usually occurs after exposure to radiation.[358]

In contrast to other pediatric sarcomas, osteosarcomas do not have any specific translocations or other molecular genetic abnormalities that can serve as diagnostic or tumor-specific markers of disease. Cytogenetically, osteosarcoma tumors have complex numerical and structural chromosomal abnormalities with significant cell-to-cell variation and heterogeneity, highlighting the complexity and instability of the genetic makeup of osteosarcoma.[359–363]

Treatment

The mainstays of therapy for osteosarcoma are surgery and chemotherapy. The outcome of patients with nonmetastatic osteosarcoma has improved dramatically during the past 3 to 4 decades from an EFS rate of 10% to 20% to one of 65% to 70%, mostly because of the addition of adjuvant chemotherapy as well as improvements in surgical and diagnostic imaging techniques.[343,364–366] The care of patients with osteosarcoma requires a team approach involving oncologists, orthopedic surgeons, pathologists, oncology nurses, physical therapists, social workers, and child life specialists.

Chemotherapy

Localized Disease. Because of current combinations of surgery and chemotherapy, long-term disease-free survival and OS rates for osteosarcoma are greater than 60%. Based on the results of cooperative group trials over the past years (Table 99.12), the current standard three-drug chemotherapy regimen for patients with localized disease includes doxorubicin, cisplatin, and high-dose methotrexate (MAP). Patients receive neoadjuvant chemotherapy with these drugs for approximately 10 weeks and then undergo

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TABLE 99.12

Treatment Results in Selected Cooperative Group Studies of Localized Osteosarcoma

Study (Reference)	Study Duration	Number of Patients	Chemotherapy	Event-Free Survival	Comment
INT 0133[407]	1993–1997	172 168 167 170	MAP MAP + MTP-PE MAPI MAPI + MTP-PE	6-yr 64% 6-yr 63% 6-yr 58% 6-yr 71%	No significant difference in EFS between groups
P9754[663]	1999–2002	111 54 56	MA (600 mg/m^2) P MA (600 mg/m^2) PI MAPIE	2-yr 69%	No significant difference in EFS between groups
POG-8651[369]	1986–1993	55 45	MAP/surgery wk 0 MAP/surgery wk 10	5-yr 69% 5-yr 61%	No significant difference in EFS between groups
ISG/SSG I[371]	1997–2000	182	MAPI (15 g/m^2)	5-yr 64%	Outcome similar to studies using standard dose ifosfamide
COSS 86[664]	1986–1988	56 72 41	MAPI + ia (HR) MAPI (HR) MAP (LR)	10-yr 63% 10-yr 70% 10-yr 66%	No difference in EFS between groups
EOI[665]	1993–2002	250 254	AP q3wk AP q2wk	5-yr 39% 5-yr 41%	Improved histologic response in interval compressed arm, but no difference in EFS
EURAMOS1[374]	2005–2011	358 357 N/A N/A	MAP (GR) MAP + INF (GR) MAP (PR) MAPIE (PR)	3-yr 74% 3-yr 77% N/A N/A	No significant difference in EFS between groups

M, methotrexate; A, doxorubicin; P, cisplatin; MTP-PE, muramyl tripeptide-phosphatidyl ethanolamine; I, ifosfamide; E, etoposide; ISG/SSG, Italian Sarcoma Group and Scandinavian Sarcoma Group; ia, intra-arterial cisplatin; COSS, Cooperative German-Austrian-Swiss Osteosarcoma Study Group; HR, high risk; LR, low risk; EOI, European Osteosarcoma Intergroup; GR, good responder; INF, interferon; EURAMOS1, European and American Osteosarcoma Study; PR, poor responder; N/A, not available.

definitive surgery, which is followed by adjuvant chemotherapy for approximately 20 weeks. The exceptions to this therapy scheme in localized disease are low-grade central and parosteal osteosarcomas and some periosteal osteosarcomas without high-grade features; these variants are traditionally treated with surgery alone.[354]

The benefit of adjuvant chemotherapy in the treatment of osteosarcoma was clearly demonstrated in two prospective trials that randomly assigned patients to observation or chemotherapy following surgery.[342,365,367] The concept of *neoadjuvant* or *induction* chemotherapy (i.e., chemotherapy given before definitive surgical resection) was prompted by the development of techniques for limb-sparing procedures and the need to control disease while the prosthesis was being constructed.[368] In a prospective randomized trial by the Pediatric Oncology Group (POG) of neoadjuvant chemotherapy versus primary surgery followed by adjuvant chemotherapy[369] outcomes were similar between the two groups, with 5-year EFS rates of approximately 61% and 69% ($p = 0.8$), respectively. Subsequent trials examining whether intensifying or adding other chemotherapy agents have not resulted in substantial improvements in OS for patients with osteosarcoma.[370,371]

The use of immunostimulatory agents in the treatment of osteosarcoma was tested in a joint POG and CCG randomized study (INT-0133).[5] The aim of this study was to determine whether adding the macrophage-activating agent MTP-PE (muramyl tripeptide phosphatidylethanolamine; mifamurtide) to chemotherapy would enhance survival in patients with newly diagnosed osteosarcoma. The study also evaluated whether adding ifosfamide to the standard three-drug MAP regimen improved survival. Initial results suggested that there may be some modest benefit to patients treated with MTP-PE, and with additional follow-up of patients treated on this study show that adding MTP-PE had an improved 6-year OS rate from 70% to 78%. The drug is not available in the United States for clinical use.

The most recent study, an international trial (EURAMOS 1) for patients with resectable localized or metastatic osteosarcoma, has completed accrual. All patients were initially treated with standard three-drug induction with MAP. Those whose disease was classified as having a good response (>90% tumor necrosis) after induction chemotherapy continued with the same chemotherapy but were randomized to receive pegylated interferon α-2b, an immune-modulating cytokine that some Scandinavian studies have shown to have activity against osteosarcoma.[372,373] Patients whose disease had poor histologic responses were randomized to receive high-dose ifosfamide and etoposide in addition to the standard three drugs. Preliminary results of the cohort of patients who experienced good responses to induction MAP chemotherapy showed no improvement in EFS rates of patients who received interferon α-2b versus those that received MAP alone; however, only 75% of patients who were randomized to receive interferon actually started the drug, and of those, only 55% completed all specified therapies.[374]

Metastatic Disease. The presence of metastatic disease at presentation is associated with a poor prognosis. In the absence of an available clinical trial, most patients with metastatic disease receive MAP chemotherapy with high-dose ifosfamide with or without etoposide.[369,375,376]

The ability to control all foci of macroscopic disease is essential in managing metastatic osteosarcoma. Patients with pulmonary metastatic disease have a survival rate of 30% to 50%, whereas patients with bone metastases have a worse prognosis.[377,378] Similarly, patients with multifocal osteosarcoma have a dismal prognosis.

Multiple studies have demonstrated that the removal of all sites of metastatic or recurrent disease (e.g., pulmonary lesions), even after completion of chemotherapy, can result in long-term survival.[379–381] In a large analysis of the Cooperative Osteosarcoma Study Group, which included more than 1,700 consecutively treated patients, the 10-year survival probability was 40%

for metastatic patients who had all sites of metastatic disease resected.[382] However, patients with extrapulmonary metastases (e.g., bone metastases) are less likely to be cured, particularly those with multifocal disease.[383] Ongoing strategies to improve the outcome of patients with metastatic osteosarcoma include the use of MTP-PE,[384] bisphosphonates,[385,386] and molecularly targeted agents such as those against vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors, mammalian target of rapamycin (mTOR), and Src kinase.[387]

Local Control. Osteosarcoma is relatively radioresistant; therefore, surgery alone is the mainstay of local control. The choice of limb salvage versus amputation for extremity tumors depends on the location and extent of the tumor, the ability to achieve good surgical margins, and proximity to the joints and neurovascular bundle. As long as the tumor is removed in its entirety with disease-free margins, then the type of surgery—limb salvage versus amputation—does not seem to influence outcome.[388] On the basis of the results of studies assessing quality of life and functional measures, most patients who undergo limb salvage procedures report an improved quality of life and demonstrate better functional ability than do those who have amputations.[389,390]

Pelvic Tumors and Unresectable Disease. Patients with primary tumors of the axial skeleton generally have poor outcomes because surgery does not provide adequate local control. In some pelvic and most vertebral primary tumors, complete resection often is not possible. Most pelvic osteosarcomas can be treated by hemipelvectomy; however, more centrally located pelvic tumors, especially those involving the sacrum, are unresectable. Only a few pelvic osteosarcomas can be treated by limb-sparing resection (i.e., internal hemipelvectomy). Contraindications to resection are unusually large extraosseous extensions with sacral plexus or major vascular involvement. In general, these tumors cannot be resected with negative margins and are best treated by chemotherapy and radiotherapy.

Radiation Therapy. Historically, osteosarcoma has been considered to be relatively radioresistant; therefore, radiation therapy is generally not used as a definitive primary treatment of this disease. Radiation may be used in patients with microscopic positive margins of resection in doses of 55 to 68 Gy with local control rates ranging between 67% and 78%.[391] Patients with gross residual or unresected disease have been treated to doses of 70 Gy or higher. Treatment volumes have consisted of the gross residual disease or tumor bed plus a 1- to 2-cm clinical margin. In the setting of metastatic or unresectable osteosarcoma, radiotherapy may also be of benefit by improving pain in up to 76% of cases.[392] Despite the relative rarity of patients with osteosarcomas requiring radiation, its use can be of benefit in the management of both local and metastatic sites of disease.

Recurrent Disease. Outcomes for patients with local or distant relapse are poor.[393–395] Effective therapy for these patients is challenging and is limited by the paucity of salvage chemotherapeutic agents active against this disease. Chemotherapy agents that have been used in the relapse setting include high-dose ifosfamide,[396] gemcitabine and docetaxel,[397–399] cyclophosphamide, and etoposide.[400] More recently, targeted agents such as sorafenib have been used.[401] Isolated pulmonary disease with a single unilateral lung lesion is more likely to result in long-term disease-free survival than is bilateral pulmonary disease or bony metastasis. A longer disease-free interval (>12 to 24 months) from initial therapy is associated with longer disease-free survival.[394,402,403]

Prognostic Factors

The most important prognostic factors for survival in patients with osteosarcoma are the presence of metastatic disease and the extent of tumor necrosis following induction chemotherapy, with more than 90% necrosis (i.e., grades 3 or 4) being favorable and less than 90% (i.e., grades 1 or 2) necrosis being less favorable.[370,404–407] Other prognostic variables have been less definitive in predicting outcomes. Some of these factors include age, tumor size, and tumor location.[382] Location is important because axial tumors, such as those of the pelvis, skull, or vertebrae, fare worse because of the difficulty in achieving a complete surgical resection with disease-free margins.

Ewing Sarcoma

Epidemiology

The term Ewing sarcoma was previously used to refer to the least-differentiated of a group of small, round cell tumors that share a recurring chromosomal translocation (discussed in detail in the following paragraphs). Over the years, this group of tumors became designated as the Ewing sarcoma family of tumors and includes Askin tumors (Ewing sarcoma arising in the chest wall) and the more histologically differentiated peripheral primitive neuroectodermal tumors. The 2013 World Health Organization classification proposed that the phrase *Ewing sarcoma* be used to refer to this family of tumors.[408] We will follow this recommendation in this section.

Ewing sarcoma is the second most common primary bone tumor in pediatric patients after osteosarcoma, accounting for approximately 2% of childhood malignancies. About 200 cases of Ewing sarcoma are diagnosed in the United States per year.[183] Most of these tumors arise in the 2nd decade of life. There is a slight male predominance, and African and Asian children are rarely affected by this cancer.[183] Ewing sarcoma can arise in bone and, less commonly (about 30%), in the soft tissue (i.e., extraosseous) anywhere in the body.[409]

Biology and Molecular Genetics

The cell-of-origin of Ewing sarcoma is unknown but is presumed to arise from a mesenchymal stem cell.[410–413] These tumors characteristically have recurrent chromosomal translocations that, in almost all cases, involve the *EWSR1* gene on chromosome 22 and a member of the ETS family of transcription factors.[414,415] Of Ewing sarcoma tumors, 85% harbor t(11;22) (q24;q12), resulting in the fusion of the *EWSR1* and *FLI1* genes. Another 10% to 15% of tumors are associated with t(21;22) (q22;q12), which generates the *EWSR1-ERG* fusion gene. Less commonly, EWS, or the related protein FUS, is fused to another ETS family transcription factor (i.e., ETV1, EIAF, FEV, or ETV4), resulting in the same Ewing phenotype.[416]

Heterogeneity in the *EWSR1-FLI1* fusion gene is based on the location of the chromosomal breakpoint. The most common rearrangement, designated type 1, consists of the first seven exons of *EWSR1* fused to exons six to nine of *FLI1*. This fusion gene accounts for over half of all cases. The type 2 rearrangement, accounting for about 25% of cases, fuses *EWSR1* to exon 5 of *FLI1*. There is no prognostic significance based on the type of fusion protein.[417,418] As a chimeric transcription factor, EWS-FLI1 is believed to regulate a number of critical downstream target genes that have been implicated in tumor biology, including members of the Sonic Hedgehog and GLI1 pathways.[419,420]

Evidence shows that directly inhibiting EWS-FLI1's oncogenic activity by altering its interaction with critical binding partners such as RNA helicase A (RHA) significantly alters tumor biology. A small-molecule inhibitor that disrupts the EWS-FLI1/RHA interaction decreases xenograft growth and increases tumor cell apoptosis.[421] Additional evidence suggests that several intracellular signal transduction pathways are significantly altered in these tumors, including insulin-like growth factor receptor 1 (IGF-1R)

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and the phosphatidylinositol-3 kinase (PI3K)–mTOR pathway.[422] Drugs targeting these pathways are being explored as possible new therapeutic agents for Ewing sarcoma, including anti–IGF-1R antibodies and mTOR inhibitors.[423–426]

Clinical Presentation and Natural History

Patients with osseous Ewing sarcoma typically present with localized pain and swelling in the affected bone and may have other nonspecific symptoms such as fever, decreased appetite, and weight loss, which are usually seen in advanced disease.[427,428] In contrast to osteosarcoma, Ewing sarcoma is equally distributed between extremity and axial sites. The lower extremity—primarily, the femur—is the most common site of disease, followed by the pelvis and the chest wall.[429] Ewing sarcoma can also affect nonosseous structures, and this usually occurs in the soft tissues, but it can also arise in the GI tract, the kidneys, the adrenal gland, the lung, and other rare sites. The presenting symptom in these cases is site specific.

Metastatic disease is present in approximately 25% of patients at the initial diagnosis.[430] The most frequent site of metastases is the lungs, followed by the bones, and bone marrow.[429] Other sites of metastases, such as the lymph nodes, the liver, or the brain, are relatively rare unless in end-stage disease.

There is no specific blood or urine test to diagnose Ewing sarcoma. Abnormal laboratory findings at the time of diagnosis may include elevated LDH and alkaline phosphatase levels. LDH is useful as a gauge of tumor burden and usually falls with effective therapy and rises with disease recurrence.[431]

Diagnostic Staging Evaluation

An evaluation of suspected Ewing sarcoma includes a radiographic examination of the primary tumor site and documentation of the presence or absence of distant metastases. Plain radiographs and MRI or CT scans should be initially obtained to characterize and define the local extent of the primary tumor. An MRI provides the most precise definition of the extent of the tumor and its relation to nearby nerves and vessels. Lesions that originate in the long bones characteristically involve the diaphyses, with extension toward the metaphyses. On plain films, a lytic or mixed lytic–sclerotic lesion is usually identified in the bone. A multilamellar periosteal reaction (i.e., onion skin appearance [Fig. 99.6]) and lifting of the periosteum (i.e., Codman triangle), or less frequently, radiating bone spicules, may be present. The lesion is usually poorly marginated and has a permeative and destructive pattern.

CT scans of the chest should be obtained to evaluate for pulmonary metastases. A biopsy of solitary pulmonary nodules should be strongly considered before classifying the disease as being metastatic. A technetium-99m whole-body radionuclide bone scan should be obtained to detect bone metastases. FDG PET/CT is highly sensitive in screening for bone metastases Ewing sarcoma[432–434] and may be a useful predictor of outcome, similar to histologic response, after induction chemotherapy.[435] Bilateral bone marrow sampling is required to complete the staging of all patients regardless of primary site or tumor size. Microscopically detectable bone marrow metastases occur in less than 10% of patients and are associated with a poor prognosis.[436]

Pathology

A biopsy of the tumor and an histopathologic examination are required for diagnosis. It is critical for the surgeon performing the diagnostic biopsy to place the incision appropriately to avoid complicating future resection. Under light microscopy, Ewing sarcoma falls under the category of small, round, blue cell tumors that includes neuroblastomas, rhabdomyosarcomas, lymphoblastic lymphomas, and, less commonly, histiocytosis and small-cell osteosarcomas. Ewing cells have a high nuclear-to-cytoplasmic ratio and appear homogenous, with uniform round nuclei that contain evenly distributed chromatin and little mitotic activity.[415] The cytoplasm is typically scant and weakly eosinophilic; however, intracellular accumulation of glycogen may confer a positive periodic acid–Schiff test. Some tumors may demonstrate neuroectodermal differentiation. These tumors typically have Homer-Wright pseudorosettes on light microscopy and positive immunohistochemical staining for synaptophysin, neuron-specific enolase, S100, and CD57.[415] More than 95% of Ewing sarcomas express CD99 (encoded by the *MIC2* gene) on their cell membranes.[429] Although CD99 staining of the cell membranes in a honeycomb pattern is suggestive of Ewing sarcoma, it is not pathognomonic and may be seen in a variety of tumors, including synovial sarcoma, mesenchymal chondrosarcoma, and B-cell and T-cell leukemia/lymphoma.[437–440]

The rapid identification of *EWSR1* gene rearrangements by performing reverse transcription–polymerase chain reaction assays or fluorescence in situ hybridization assays on fresh, frozen, or paraffin-embedded specimens is useful for expeditiously discriminating between Ewing sarcoma and other morphologically similar small round cell tumors.[414]

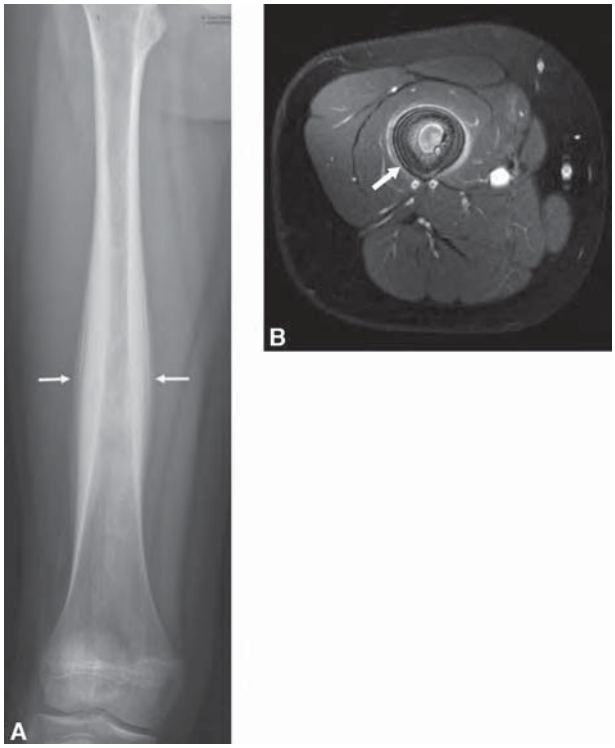

Figure 99.6 Multilamellar periosteal reaction (*arrows*) in a plain radiograph **(A)** and MRI axial T2-weighted image **(B)** of the femur of a young girl who presented with intermittent leg pain. Diagnostic considerations included Ewing sarcoma, osteomyelitis, osteosarcoma, and Langerhans cell histiocytosis. Open biopsy of the lesion revealed Ewing sarcoma.

Treatment

Chemotherapy. Ewing sarcoma is a very chemotherapy-sensitive tumor, and the outcome of patients with this disease has greatly improved as a result of intensifying systemic multiagent chemotherapy, particularly that of anthracycline and alkylating agents, as well as improvements in surgical and radiation techniques and supportive care (e.g., use of hematopoietic growth factors). Using current treatment strategies, over 70% of patients who present with localized disease are cured of their disease. However, the prognosis

of patients with metastatic or recurrent disease remains poor, with a long-term survival rate of less than 30%.[441]

Localized Disease. Results of the major treatment trials for localized Ewing sarcoma are summarized in Table 99.13.[411,442–454] Early cooperative group studies using adjuvant chemotherapy documented the efficacy of a four-drug regimen with vincristine (V), actinomycin D (A), cyclophosphamide (C), and doxorubicin (D) in combination with local control measures. By using these strategies, survival rates were improved from less than 20% to more than 40%.[448,449] The next generation of studies confirmed the benefit of the addition of ifosfamide and etoposide in the treatment of Ewing sarcoma. A randomized phase III study conducted by the first American Intergroup Ewing trial (INT-0091/POG-8850/CCG-7881) demonstrated a superior outcome in patients with localized disease treated with VD(A)C alternating with ifosfamide (I) and etoposide (E) compared to VD(A)C alone (5-year EFS, 69% ± 3% versus 54 % ± 4% respectively, $p = 0.005$).[447] Similar improvements in survival with the addition of ifosfamide have been reported in studies by the National Cancer Institute and multiple European cooperative groups.[444,455,456]

Dose intensification of the VDC-IE regimen in a second intergroup POG-CCG study (CCG-7942/POG-9354) of nonmetastatic Ewing sarcoma showed that a dose-intensified 30-week treatment schedule did not improve EFS beyond that of the standard 48-week schedule.[446] However, a strategy of interval compression delivering the five-drug standard therapy (VDC-IE) in 30 weeks (chemotherapy every 14 days versus every 21 days) led to improved survival rates, with a median 5-year EFS rate of 73% for the compressed cohort and 65% for the patients treated every 21 days (see Table 99.13).[454]

In Europe, the collaborative study EURO-Ewing 99 is using risk-based stratification to assign patients into three risk groups based on tumor volume, presence, and pattern of metastatic disease, and histologic response to six cycles of four-drug induction chemotherapy (VIDE).[429,457] After induction therapy, the study includes two randomized comparisons: patients with localized disease having a good response (i.e., less than 10% viable tumor after VIDE) continue therapy with either VAC or VAI; patients with either large tumors (i.e., more than 200 mL) or tumors having a poor response (i.e., more than 10% viable tumor after VIDE) receive VAI or busulfan–melphalan megatherapy. This study is nearing completion of accrual (see Table 99.13).

Metastatic Disease. The treatment for patients who present with metastatic Ewing sarcoma is challenging. In contrast to the improvement in survival for patients with nonmetastatic disease, no comparable benefit from the addition of IE has been demonstrated for patients who present with metastatic disease. In the first CCG-POG intergroup study (INT-0091), 120 patients with metastatic disease were evaluated using treatment regimens similar to those previously described for nonmetastatic patients. The addition of ifosfamide and etoposide in the metastatic group provided no survival advantage for those who did or did not receive IE.[458] Results of studies testing dose-intensification strategies, including the use of total body irradiation and myeloablative chemotherapy followed by autologous stem cell rescue, have been disappointing.[436,459–463] The EURO-Ewing 99 study described previously had an additional arm (R3) for patients with newly diagnosed primary disseminated disease. Two-hundred and eighty-one patients were treated with six cycles of VIDE, one cycle of VAI, and then surgery and/or radiation followed by high-dose busulfan–melphalan and autologous stem cell transplant. The 3-year EFS rate of these patients was 27% ± 3%.[461] Poor prognostic factors included age of 14 years or older, tumor volume of 200 mL or more, increased number of bone metastases, and bone marrow and bone involvement.

Recurrent Disease. Although most patients with Ewing sarcoma can experience a complete response to multimodal therapy, about 30% of patients with localized disease and more than 70% of those with metastatic disease at initial presentation experience recurrent disease. The median time to relapse in most studies is approximately 16 to 21 months from diagnosis.[464–466] The overall prognosis for these patients is dismal, with less than 20% survival. However, patients with early relapse (i.e., less than 2 years from diagnosis) fare worse than do those with late relapse (i.e., less than 10% survival).[464–466] In terms of long-term follow-up surveillance and counseling, it is important to recognize that recurrences of Ewing sarcoma can occur very late, more than 5 years from diagnosis.[467]

There is no established salvage regimen for patients with recurrent disease. Combination chemotherapy regimens of cyclophosphamide and topotecan[468–470]; irinotecan and temozolomide[471,472]; ifosfamide, carboplatin, and etoposide[473–475]; and gemcitabine and docetaxel[476] have demonstrated antitumor activity in the relapse setting. Myeloablative chemotherapy and, in some cases, total body irradiation have been attempted in an effort to improve the prognosis for patients with high-risk or relapsed disease, with only modest improvement in outcome and at the cost of significant toxicity.[477,478]

Local Control. Local control with surgical resection or high-dose radiotherapy in addition to chemotherapy is imperative for curative therapy in Ewing sarcoma. The inclusion of 12 to 15 weeks of systemic chemotherapy before the introduction of local control measures has become standard practice, regardless of tumor size, location, or stage, unless the tumor causes an immediate threat to survival, such as spinal cord compression or cardiopulmonary compromise. As of 2013, there has been no randomized trial to determine whether surgery or radiotherapy is superior in achieving local control. For decades, radiotherapy was the standard local treatment modality, but surgery is now the preferred modality if a complete resection is feasible.[479] In prospective but nonrandomized trials, the improved survival in the surgical resection group has generally been attributed to the allocation of larger tumors with a correspondingly poorer prognosis to the radiation group. In some situations, particularly in patients who are at high risk for local treatment failure after resection, a combination of surgery and radiation is warranted. Decisions about local control should take into account resectability, functional outcome, long-term morbidity, the risk of late-onset secondary malignancies in tissues exposed to high-dose radiotherapy, and patient preference. Factors that influence the success of local control include the initial location of the primary tumor, with central disease having a poorer prognosis, and initial tumor response to chemotherapy as shown by the percentage of tumor necrosis.[237]

Surgery. In general, patients with resectable primary tumors receive induction chemotherapy followed by definitive surgery alone. For extremity tumors, limb salvage is preferred; however, if limb salvage or irradiation are not feasible, then amputation is warranted. Central pelvic or spinal lesions are frequently treated with radiation alone because surgery with negative margins is often not feasible. Chest wall lesions often present as large tumors extending into the thoracic cavity. Preoperative chemotherapy can greatly reduce the size, vascularity, and friability of the tumor, facilitating resection and decreasing the risk of intraoperative tumor rupture.[480–482] Because surgical outcome is improved in patients receiving preoperative chemotherapy, they are less likely to require postoperative chest wall radiotherapy, which has well-established risks of cardiac and pulmonary damage and radiation-induced second malignancies.[483]

Radiation Therapy. Radiation therapy is delivered in either the adjuvant or definitive setting based on the finding of involved surgical margins or unresectable disease. The selection of patients for radiation therapy should be made by a multidisciplinary team involving orthopedic oncologists, pediatric oncologists, and pediatric

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TABLE 99.13

Treatment Results in Selected Clinical Studies of Localized Ewing Sarcoma

Study (Reference)	Study Duration	Schedule	Patients	5-Year Event-Free Survival (%)[a]	p Value[a]	Comments
IESS Studies						
IESS-I[449]	1973–1978	VAC VAC + WLI VACD	342	24 44 60	VAC vs VAC + WLI, 0.001 VAC vs VACD, 0.001 VAC + WLI vs VACD, 0.05	Value of D Benefit of WLI?
IESS-II[443]	1978–1982	VACD-HD VACD-MD	214	68 48	0.03	Value of aggressive cytoreduction
INT-0091 (POG-8850/ CCG-7881)[447]	1988–1993	VACD VACD + IE	200 198	54 69	0.005	Value of addition of IE
CCG-7942/POG-9354[446]	1995–1998	VDC + IE 48 wks VDC + IE 30 wks	492	72 70	0.57	No benefit from dose escalation of alkylating agents
AEWS0031[454]	2001–2005	VDC + IE every 3 wks VDC + IE every 2 wks	568	65 73	0.48	Value of interval compressed therapy
ROI, Bologna, Italy						
REN-3[442]	1991–1997	VDC + VIA + IE	157	71		Surgery in 78% of patients
SFOP, France						
EW-88[450]	1988–1991	VD + VD/VA	141	58		Histologic response better predictor of outcome than tumor volume
EW-93[453]	1993–1999	SR: VACD IR: VACD + IE HR: VD + IE + BuMel/ ASCT	214	70 54 48		No advantage of IE in the IR group; potential benefit of BuMel/ASCT for HR group
UKCCSG/MRC Studies						
ET-1[445]	1978–1986	VACD	120	41: extremity, 52; axial, 38; pelvic, 13		Tumor site as the most important prognostic factor
ET-2[444]	1987–1993	VAID	201	62: extremity, 73; axial, 55; pelvic, 41		Importance of the administration of high-dose alkylating agents (I)
CESS Studies						
CESS-81[448]	1981–1985	VACD	93	55: <100 mL, 80; ≥100 mL 31 (both 3 yr) Viable tumor <10, 79; >.10, 31 (both 3 yr)		Tumor volume (< or ≥100 mL) and histologic response are prognostic factors
CESS-86[451]	1986–1991	SR: VACD HR: VAID	301	52 (10 yr) 51 (10 yr)		Benefit of intensive treatment with I for high-risk patients (≥100 mL or central axis)
EICESS Studies (CESS + UKCCSG)						
EICESS-92[666]	1992–1999	SR: VAID vs VACD HR: VAID vs EVAID	155 326	68 vs 67 51 vs 61	0.8406 0.2141	Randomized comparisons not significant; suggestion that addition of etoposide increased survival in HR group

IESS, Intergroup Ewing Sarcoma Study; V, vincristine; A, actinomycin D; C, cyclophosphamide; D, doxorubicin; WLI, whole-lung irradiation; HD, high dose; MD, moderate dose; I, ifosfamide; E, etoposide; SR, standard risk; IR, intermediate risk; HR, high risk; ASCT, autologous stem cell transplant; ROI, Rizzoli Orthopaedic Institute; SFOP, French Society of Paediatric Oncology; CESS, Cooperative Ewing Sarcoma Studies; UKCCSG, United Kingdom Children's Cancer Study Group; MRC, Medical Research Council; EICESS, European Intergroup Cooperative Ewing Sarcoma Studies.

[a] *P* values are given only for trials comparing randomized treatment arms. Values are for 5-year survival unless otherwise noted.

radiation oncologists in order to best select a local control approach that maximizes the potential for high rates of local control with minimal toxicity. Modern radiation treatment techniques are now used that rely on CT simulation, target delineation, and treatment techniques, including conformal radiation therapy, intensity modulated radiation therapy, and proton beam radiation therapy.

The dose of radiation prescribed is dependent on the volume of the disease. Patients undergoing surgical resection with microscopically involved surgical margins (either after induction chemotherapy or at diagnosis) should receive adjuvant radiation therapy to the tumor bed to a dose of 50.4 Gy at 1.8 Gy per treatment fraction daily. Patients with gross disease present at the time of radiation therapy should be treated to doses of 55.8 Gy at 1.8 Gy per treatment fraction. Patients with no tumor present at the surgical margin but a poor histologic response to preoperative chemotherapy may also benefit from adjuvant radiation therapy.[484] Other primary site prognostic factors that negatively impact local or overall disease control include the size of the tumor (≥8 cm) and the pelvic site of the disease.[485–487] Patients with these prognostic factors that place them at high risk for local failure are candidates for more aggressive surgical resections and radiation dose escalation on clinical trials.

Ewing sarcoma was historically treated with large radiation portals and POG prospectively evaluated whole-bone (i.e., conventional) irradiation compared with tailored treatment fields, and a published analysis of this trial supports the efficacy of a more limited treatment volume as defined by prechemotherapy tumor extent.[488] Modern treatment volumes for patients requiring radiation include all areas of gross disease, including areas the tumor initially infiltrated at diagnosis and the initially involved bone. Patients undergoing surgical resection, but still requiring adjuvant radiation should have the tumor bed or surgical cavity targeted. These target volumes should be expanded by 1 to 1.5 cm to account for microscopic spread of disease and treated to the doses indicated previously. The timing of radiation therapy usually occurs after the initial 12 weeks of chemotherapy. The risk of secondary sarcomas arising in irradiated bone is reported as being from 5% to 10% at 20 years from diagnosis. In patients treated with doses of 60 Gy or more, a significant excess risk of secondary bone sarcomas has been reported compared to the risk in those patients treated with doses below 48 Gy.[489]

Ewing sarcoma is one of few diseases in which radiation therapy is used curatively for metastatic disease. Whole-lung irradiation has proven effective for the consolidation of lung metastases after chemotherapy. Multiple trials have demonstrated superior EFS and OS rates when whole-lung radiation is given.[479] Paulussen et al.[490] analyzed patients in three cooperative Ewing sarcoma studies and found that the EFS rate was significantly higher for patients who received whole-lung irradiation. Whole-lung doses between 15 and 18 Gy are used with minimal acute toxicity to treat patients after initial chemotherapy.

Prognostic Factors

The most important prognostic factor for outcome still remains the presence of metastatic disease at diagnosis. Patients with isolated pulmonary metastases do better (about 30% disease-free survival) than those with bone or bone marrow metastases (about 20% disease-free survival), but all fare worse than patients with nonmetastatic disease.[447,491,492] Large tumor size and volume and high serum LDH levels at diagnosis correlate with an adverse outcome in some studies.[430,431,487] Children with nonmetastatic pelvic primary sites also have a poorer prognosis than children with primary tumors of the extremities, although this difference may be related to the larger size and more difficult resectability of pelvic tumors.[491]

Although not a prognostic factor assessable at the time of diagnosis, the histologic response to chemotherapy appears to be a strong predictor of treatment outcome. Poor histologic response (<90% tumor necrosis) correlates with a poor prognosis, whereas complete or near complete tumor necrosis correlates with good outcome, with a 5-year EFS rate of 84% to 95%.[493,494]

As understanding of the molecular pathogenesis of Ewing sarcoma continues to increase, other prognostic markers, such as those derived from genetic expression analyses of primary and metastatic tumors, may identify genetic signatures that allow for the stratification of disease into low- and high-risk subgroups.[495,496] These studies may also provide clues as to which patients may benefit from various targeted therapies.

RHABDOMYOSARCOMA

Epidemiology and Genetics

Rhabdomyosarcoma is the most common soft tissue sarcoma in children, accounting for 3% to 4% of all cases of childhood cancer and for approximately 350 new cancer cases each year in patients younger than 20 years of age.[1,182,183] Rhabdomyosarcoma is more common in males and Caucasians, and two-thirds of cases occur in patients under the age of 10 years with a median age at diagnosis of 5 years.[497] Although most cases of rhabdomyosarcoma are sporadic, there are several genetic and environmental factors that have been associated with a heightened risk of developing the disease, including:

- Germ-line P53 mutations[498]
- Costello syndrome[499]
- Beckwith-Wiedemann syndrome[500]
- Neurofibromatosis type I[501]
- Germ-line DICER1 mutations[502]
- Parental use of cocaine and marijuana[503]
- Birthing order and accelerated in utero growth[504,505]

Although rhabdomyosarcoma can arise in multiple sites of the body, the most common sites in decreasing order of frequency include the head and neck (including the orbit and parameningeal areas [35%]), the genitourinary tract (including the bladder, prostate, vagina, vulva, uterus, and paratesticular area [26%]), and extremities (19%).[10] Only about 20% of children with rhabdomyosarcoma present with disseminated disease, which most commonly involves the lung.[506]

Pathology and Molecular Biology

The International Classification of Rhabdomyosarcoma recognizes three main categories of rhabdomyosarcoma:[507]

1. Superior prognosis (both are variants of embryonal rhabdomyosarcoma):
   a. Botryoid
   b. Spindle cell
2. Intermediate prognosis
   a. Embryonal rhabdomyosarcoma
3. Poorer prognosis
   a. Alveolar rhabdomyosarcoma
   b. Undifferentiated sarcoma

In contrast, the World Health Organization recognizes four variants of rhabdomyosarcoma and these subtypes, including embryonal, alveolar, pleomorphic, and spindle cell/sclerosing rhabdomyosarcoma.[508] The presence of focal and diffuse anaplasia can be documented in about 13% of cases of rhabdomyosarcoma, and its presence may be of prognostic significance in patients with intermediate-risk embryonal rhabdomyosarcoma.[509] In children and adolescents, the two most common types of rhabdomyosarcoma are the embryonal (65%) and alveolar (25%) subtypes and each of these entities have unique clinical and biologic features that will be described in more detail.[510,511]

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Embryonal tumors have a more favorable clinical outcome, often affect younger male patients, and most commonly arise in the head, neck, and genitourinary regions (Fig. 99.7).[497,507,512] The botryoid variant of rhabdomyosarcoma resembles a bunch of grapes and characteristically arises in hollow organs such as the bladder, the biliary tract, the vagina, and occasionally, the conjunctiva and ear.[507,513] In children, spindle cell tumors most commonly arise in the paratesticular area, whereas the sclerosing variant has been reported to more commonly affect the extremities and the head and neck region.[508,514] Embryonal tumors are characterized by a loss of heterozygosity at the imprinted 11p15 locus, resulting in a loss of the maternal allele and duplication of the paternal allele that encodes the IGF-2 growth factor and is believed to participate in rhabdomyosarcoma tumorigenesis. This loss of imprinting also suggest that inactivation of a tumor suppressor gene at the maternal locus may promote tumorigenesis.[515,516] Embryonal tumors are characterized by a high background mutation rate and overall number of single-nucleotide variants (see Fig. 99.7) as well a multiple chromosomal gains and losses most often involving chromosome 8 gains (74% of cases). Gains of chromosomes 2, 7, 11, 12, 13q21, and 20 as well as losses of 1p36, 6, 9q22,14q21, and 17 have been reported.[517,518] Activating RAS pathway mutations involving KRAS, NRAS, and HRAS have been documented in 42% of cases of embryonal rhabdomyosarcoma.[519] Mutations involving the *FGFR4*, *ALK*, *BRAF*, *CTNNB1*, *PIK3CA*, and *PTPN11* genes have been described less frequently.[520,521] Embryonal tumors have also been reported to have *MDM2* amplification,[517] loss of *PTEN*, *BCL2L1* amplification, homozygous deletions of *CDKN2B*, increased GLI expression, *NF1* deletions, and *ALK* copy number gains.[519,521] In contrast to patients with embryonal tumors, children with alveolar tumors have a worse clinical outcome and their tumors more commonly arise in the trunk and extremities (see Fig. 99.7).[512,522] Genomically, these tumors are characterized by a t(2;13) (q35;q14) or t(1;13) (p36;q14), in which the *PAX3* gene on the long arm of chromosome 2 or the *PAX7* gene in chromosome 1 is fused with the *FOXO1* gene on the long arm of chromosome 13 (see Fig. 99.7).[523] In addition, *ALK* gene copy number gains are seen in the majority of cases of alveolar rhabdomyosarcoma, and copy number gains and overexpression of MYCN have been associated with an adverse clinical outcome in this disease.[521,524] Approximately 18% of alveolar rhabdomyosarcomas lack *FOXO1* rearrangements[510] and are termed fusion-negative alveolar rhabdomyosarcomas. These patients clinically behave more like patients with embryonal tumors, suggesting that the presence of a PAX/FOXO1 fusion is a key determinant of clinical behavior and should be incorporated into the risk stratification of the disease.[517,525]

Diagnosis and Staging

Given the heterogeneous location of this tumor, rhabdomyosarcoma can cause a variety of signs and symptoms. Head and neck tumors, which comprise about one-third of all cases of rhabdomyosarcoma, can present with proptosis, ophthalmoplegia, nasal drainage, and obstruction, headache, cranial nerve palsies, dysphonia, dysphagia, and palpable adenopathy.[526] Patients with genitourinary tumors, which account for 25% of cases of rhabdomyosarcoma, can present with hematuria, dysuria, hydronephrosis palpable abdominal mass, vaginal discharge, and palpable painless masses.[526] Tumors in the extremities, which account for about 20% of cases of rhabdomyosarcoma, can present with swelling, palpable adenopathy, and pain.[526] Tumors on the trunk, pelvis, and abdomen can present with nerve root compression, palpable mass or adenopathy, jaundice (biliary tract tumors), and perirectal pain and swelling.[526,527]

A staging evaluation should include a complete blood count, serum chemistries, bone or PET scan, bilateral bone marrow aspirates and biopsies, CT of the chest, CT or MRI of the primary tumor, and CT or MRI of abdomen and pelvis for abdominal, pelvic, and lower extremity tumors. A recent report from the COG

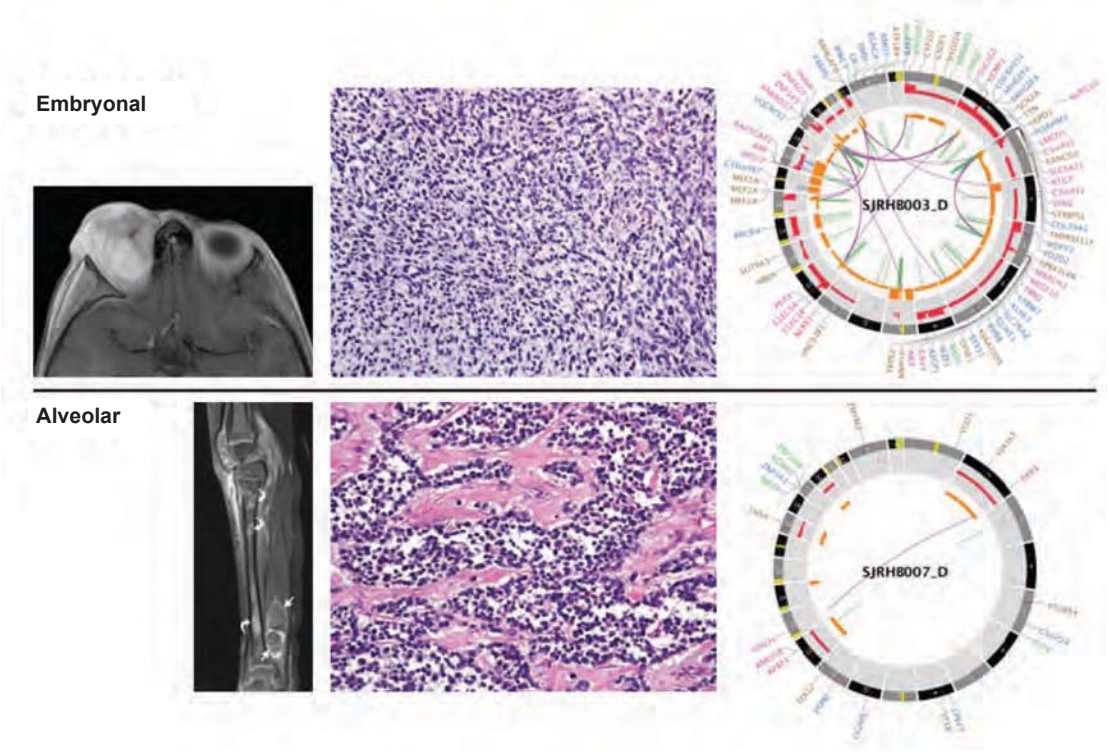

Figure 99.7 Clinical, histologic, and genomic features of embryonal and alveolar rhabdomyosarcoma.

suggests that up to one-third of patients with rhabdomyosarcoma, specifically those with embryonal noninvasive node-negative disease can be spared an extensive metastatic evaluation that includes a bone marrow examination and a bone scan.[528] Studies investigating the role of PET scan in initial staging and as a predictor of clinical outcome in pediatric rhabdomyosarcoma are ongoing.[529,530] Routine cranial imaging is not indicated unless there is evidence of spinal involvement and cerebrospinal fluid examination should be reserved for patients with parameningeal primaries.[531] Following a biopsy, a routine evaluation of regional nodes should be reserved for patients with extremity tumors and those with paratesticular tumors who are older than 10 years of age.[532] Sentinel node biopsy is increasingly being used to assess nodal involvement in patients with extremity tumors.[533]

Patients with rhabdomyosarcoma are staged using a surgicopathologic grouping system as well as a pretreatment staging system as shown in Tables 99.14 and 99.15.[534,535] About 16% of patients have group I disease (completely resected), 20% have group II disease (microscopic residual), 48% have group III disease (incompletely resected), and 16% present with group IV disease (metastatic).[536] In addition, all patients should be staged using the pretreatment tumor, regional nodes, metastasis (TNM) staging system, which stratifies patients into four different categories based on the site of the primary tumor, tumor size, presence or absence of nodal and distant disease, and invasiveness. Using both systems in addition to histologic subtype, pediatric rhabdomyosarcoma can be classified into three distinct risk groups (Table 99.16). Patients with low-risk disease, which account for about one-third of cases can be further subdivided into two subsets as shown in Table 99.16.[537] Patients with intermediate risk account for about 50% of cases, and those with high-risk disease account for about 16% of cases (see Table 99.16).

Prognostic Factors

Clinical group, stage, histologic subtype, age, and treatment (see Table 99.16) are the most important predictors of outcome in childhood rhabdomyosarcoma.[538] Patients with low-risk disease (embryonal tumors stage 1 through 3 group I, II and stage 1 group III) are expected to have a 5-year OS rate in excess of 95%.[539] Patients with intermediate-risk disease (stage 2, 3 group III embryonal and stage 1 through 3 group I through III alveolar) have an estimated 4-year EFS of about 70% and the outcome of these patients has not changed significantly over time.[540] Certain subsets of patients with intermediate-risk embryonal tumors such as those with T2 tumors, extremity primaries, and age <1 year and ≥10 years have a poorer clinical outcome.[538] Similarly, patients with intermediate-risk alveolar tumors who present with stage 3 group III N1 disease have a particularly poor prognosis.[538] Patients with high-risk disease

TABLE 99.14

Intergroup Rhabdomyosarcoma Study (IRS) Clinical Grouping Classification

Group I: Localized disease, completely resected	
	(Regional nodes not involved: Lymph node biopsy or dissection is required except for head and neck lesions) (a) Confined to muscle or organ of origin. (b) Contiguous involvement; infiltration outside the muscle or organ of origin, as through fascial planes. NOTATION: This includes both gross inspection and microscopic confirmation of complete resection. Any nodes that may be inadvertently taken with the specimen must be negative. If the latter should be involved microscopically, then the patient is placed in the Group IIb or IIc (see the following).
Group II: Total gross resection with evidence of regional spread	
	(a) Grossly resected tumor with microscopic residual disease. (Surgeon believes that he has removed all of the tumor, but the pathologist finds tumor at the margin of resection and additional resection to achieve clean margin is not feasible.) No evidence of gross residual tumor. No evidence of regional node involvement. Once radiotherapy and/or chemotherapy have been started, reexploration and removal of the area of microscopic residual does not change the patient's group. (b) Regional disease with involved nodes, completely resected with no microscopic residual. NOTATION: Complete resection with microscopic confirmation of no residual disease makes this different from Groups IIa and IIc. Additionally, in contrast to Group IIa, regional nodes (which are completely resected, however) are involved, but the most distal node is histologically negative. (c) Regional disease with involved nodes, grossly resected, but with evidence of microscopic residual and/or histologic involvement of the most distal regional node (from the primary site) in the dissection. NOTATION: The presence of microscopic residual disease makes this group different from 2b, and nodal involvement makes this group different from Group 2a.
Group III: Incomplete resection with gross residual disease	
	(a) After biopsy only (b) After gross or major resection of the primary (>50%)
Group IV: Distant metastatic disease present at onset	
	(Lung, liver, bones, bone marrow, brain, and distant muscle and nodes) NOTATION: The previous excludes regional nodes and adjacent organ infiltration which places the patient in a more favorable grouping (as noted under Group II).

Note: The presence of positive cytology in cerebrospinal fluid (CSF), pleural or abdominal fluids, as well as implants on pleural or peritoneal surfaces are regarded as indications for placing the patient in Group IV.

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TABLE 99.15

Tumor, Regional Nodes, Metastasis Pretreatment Staging Classification for Rhabdomyosarcoma

Stage	Sites	Tumor	Size	Node	Metastasis
1	Orbit Head and neck (excluding parameningeal) GU, nonbladder/nonprostate biliary tract	T_1 or T_2	a or b	N_0 or N_1 or N_x	M_0
2	Bladder/prostate, extremity, cranial parameningeal, other (includes trunk, retroperitoneum, etc.) (excludes biliary tract)	T_1 or T_2	a	N_0 or N_x	M_0
3	Bladder/prostate, extremity, cranial parameningeal, other (includes trunk, retroperitoneum, etc.) (excludes biliary tract)	T_1 or T_2	a b	N_1 N_0 or N_1 or N_x	M_0 M_0
4	All	T_1 or T_2	a or b	N_0 or N_1	M_1

Definitions:

Tumor	$T(site)_1$	Confirmed to anatomic site of origin (a) ≤5 cm in diameter in size (b) >5 cm in diameter in size
	$T(site)_2$	Extension and/or fixative to surrounding tissue (a) ≤5 cm in diameter in size (b) >5 cm in diameter in size
Regional Nodes	N_0	Regional nodes not clinically involved
	N_1	Regional nodes clinically involved by neoplasm
	N_x	Clinical status of regional nodes unknown (especially sites that preclude lymph node evaluation)
Metastasis	M_0	No distant metastasis
	M_1	Metastasis present

GU, Genitourinary.

continue to fare poorly with less than 30% of patients expected to be disease-free at 3 years following diagnosis.[541] Children with high-risk disease who are between 1 and 9 years of age, present with a favorable primary tumor, have less than three metastatic sites, and no evidence of bone or bone marrow disease have an expected 5-year failure-free survival of about 50%.[541] Recent studies have confirmed that the presence of PAX/FOXO1 fusions are the main determinant of clinical outcome and that previously identified metagene sets derived from gene-expression profiles were largely dominated by the effect of fusion status.[525] Several reports suggest that the presence of a PAX7/FOXO1 rearrangement is associated with a better clinical outcome, but these findings need to be confirmed in larger prospective studies.[525,542]

Management

Principles of Therapy

As previously mentioned, the management of rhabdomyosarcoma requires a multidisciplinary team, and current therapy is assigned according to three distinct risk groups (low, intermediate, and high) that incorporate information regarding stage, clinical group, and histology of the tumor (see Table 99.16)[537].

Local Therapy

Surgical considerations for the treatment of rhabdomyosarcoma are site specific, and given the high degree of chemosensitivity of these tumors, extensive surgeries in certain sites such as the orbit, the bladder, the vagina, and the biliary tract are unwarranted.[527] When feasible, reexcision of positive margins in patients with extremity and trunk primaries is associated with improved survival.[543] Second-look surgeries in selected patients may help identify and resect the residual viable tumor and convert patients with a partial or minor radiographic response into a complete response either reducing or eliminating the need for radiotherapy.[8,537,544] It is estimated that about 60% of patients with residual radiographic abnormalities will have evidence of a viable tumor at the time of second-look surgery, and this finding is associated with an inferior failure-free survival, particularly if microscopic or gross residual disease is left behind.[8] In addition, the results of second-look procedures can

TABLE 99.16

Risk Subgroups of Rhabdomyosarcoma

Risk	Proportion of Patients	Stage	Group	Histology	5-Year Event-Free Survival	Therapy
Low subset 1	30%	1,2	I–II III orbit	Embryonal	90%	VA ± C ± RT
Low subset 2	30%	1 3	III nonorbit I,II	Embryonal	87%	VA ± C ± RT
Intermediate	55%	2,3 1,2,3	III I–III	Embryonal Alveolar	65%–73%	VAC ± other agent + RT
High	15%	4	IV	Embryonal and alveolar	<30%	VAC ± other agents + RT

V, vincristine; A, actinomycin D; C, cyclophosphamide; RT, Radiotherapy.

help tailor the dose or eliminate the use of radiotherapy in selected cases.[8,537,542] The use of debulking procedures has a very limited role in the management of rhabdomyosarcoma, although one report suggests that patients with debulking may improve the survival of patients with embryonal retroperitoneal tumors.[6,545]

Radiotherapy should be administered to all children with the exception of those with group I embryonal tumors. A recent report also suggests that patients with stage 1, 2 group I alveolar tumors can be spared the use of radiotherapy.[546] The use of conformal radiotherapy or hyperfractionated radiotherapy does not improve local control.[547,548] The recommended doses for node-negative microscopic residual disease are 36 Gy and 41.4 Gy for those with microscopic disease and pathologically proven but grossly negative nodal disease. For a gross residual tumor, the recommended dose is 45 Gy for orbital and 50.4 Gy for nonorbital primary sites. The selection of reduced doses of radiation for patients with orbital sites of involvement is supported by the D9602 trial where the use of reduced doses of radiotherapy without an alkylating agent in patients with embryonal group IIA and orbital group III tumors did not compromise local control rates (~15% local failure).[549] The treatment volumes for patients requiring radiation include all areas of gross disease, including areas the tumor initially infiltrated at diagnosis. Patients undergoing surgical resection, but still necessitating adjuvant radiation should have the tumor bed or surgical cavity targeted. These target volumes should be expanded by 1 cm to account for the microscopic spread of disease and treated to the doses indicated previously. The timing of radiation therapy usually occurs after the initial 12 or 18 weeks of chemotherapy based on whether that patient has localized or metastatic disease. Several attempts have been made to eliminate the need for radiation therapy in very young patients due to the risks of late toxicity from radiation. Although a delay of radiation therapy in very young patients may be considered if the patient's clinical response is acceptable (both maximizing the age of the patient at the time of radiation and minimizing the volume of the radiation targets), the elimination of radiation altogether based on a complete chemotherapeutic response should not be considered outside of a clinical trial. In the D9602 trial, patients with group III vaginal tumors experienced unacceptably high local failure rates in excess of 60% when they followed a response-based approach that attempted to eliminate radiation therapy without the use of higher dose cyclophosphamide.[550]

Systemic Therapy

Chemotherapy

All children with rhabdomyosarcoma should receive chemotherapy. The most common regimen used in North America consists of vincristine, actinomycin D, and cyclophosphamide (VAC); ifosfamide is preferentially used in the European trials.[512,551] Other agents such as topotecan, melphalan, methotrexate, ifosfamide, etoposide, irinotecan, and doxorubicin have also proven active, but their addition to VAC regimens failed to improve outcomes in intermediate and high risk patients.[552] See Table 99.16 for the current outcomes and treatment recommendations for patients with low-, intermediate-, and high-risk tumors.[537] For low-risk patients, who account for one-third of all cases of rhabdomyosarcoma, the administration of VA or VAC with or without radiotherapy is associated with survival rates in excess of 90%. Because of the excellent prognosis, the recently completed trial ARST 0331 for low-risk patients examined the feasibility of limiting the duration of therapy and the exposure to alkylating agents; preliminary results are only available in abstract form. Patients with intermediate-risk disease comprise about half of all patients with rhabdomyosarcoma and their survival depending on stage, group, and histology ranges from 59% to 83%.[540] The use of alternating drug combinations with VAC and irinotecan is currently being investigated in the D9803 COG trial. Despite marked improvements in survival of nonmetastatic patients, survival for the remaining 16% of high-risk patients who present with metastatic disease has remained unchanged during the past 30 years. End intensification with high-dose chemotherapy, dose intensification of alkylating agents, and integration of novel agents has failed to improve survival in these patients.[552,553] The Soft Tissue Sarcoma Committee of COG has relied on preclinical xenograft models to identify potentially active agents and to translate these findings into frontline window studies for children with previously untreated poor prognosis rhabdomyosarcoma, and the current high-risk trial is investigating the addition of temozolomide and IGF-1R inhibitors to standard chemotherapy.[554,555]

Less than one-third of children who develop a recurrence of rhabdomyosarcoma are expected to survive long term; however, patients who had stage 1 group I embryonal tumors at diagnosis and experienced a localized relapse >18 months from diagnosis have a higher likelihood of cure.[556,557]

HEPATOBLASTOMA

Epidemiology

Approximately two-thirds of primary liver tumors in children are malignant, and they account for 1.1% of all cancers in children less than 20 years old (see Fig. 99.1).[183]

Hepatoblastoma is the most common, accounting for two-thirds of all pediatric liver cancers, with hepatocellular carcinoma accounting for the vast majority of the remaining cases.[558] The incidence rates for hepatoblastoma have steadily increased over time, doubling from 0.8 per million from 1975 to 1979 to 1.5 per million from 1990 to 1995, and more recently, increasing at a rate of 4.3% (95% CI, 0.2% to 8.7%) annually.[558,559] In contrast, the incidence of hepatocellular carcinoma (HCC) has decreased from 0.6 per million to 0.2 per million.[1]

Hepatoblastoma accounts for over 90% of all malignant liver tumors in children under the age of 5 years, and is slightly more common in males (1.2:1) and Caucasian children.[558,560,561] The median age at presentation is 16 to 18 months.[562,563] The most common risk factors for the development of hepatoblastoma include low birth weight,[564,565] familial adenomatous polyposis coli (FAP),[566,567] BWS,[40] and hemihypertrophy.[568] There are also several case reports suggesting a predisposition for hepatoblastoma in children with trisomy 18.[569,570]

Genetic Abnormalities

Most cases of hepatoblastoma are sporadic. Only about 15% of hepatoblastoma patients have genetic syndromes, the most common of these being FAP.[571] The risk for developing hepatoblastoma in FAP families is up to 800 times higher than that of the general population[566,572] and, in two studies, up to 10% of patients with sporadic hepatoblastoma had germ-line mutations of the *APC* gene.[566,573] Thus, screening of all families of hepatoblastoma patients for FAP may be appropriate.[567,573] Other common recurring genetic changes in hepatoblastoma include gains of whole chromosomes, particularly 2, 8 and 20,[571,574] and up to 18% have unbalanced translocations involving chromosome 1.[574–576]

Prematurity has been increasingly recognized as a risk factor for the development of hepatoblastoma, and in one Japanese study, hepatoblastoma accounted for nearly 60% of all cancers seen in premature babies weighing less than 1,000 g at birth.[577] In another study, a clear correlation between a rising rate in hepatoblastoma and the percentage of low- and very low-birth-weight newborns was documented.[578] A case-controlled study is currently being conducted by the COG to better delineate the role of prematurity and other factors in the development of hepatoblastoma.

Pathology

Hepatoblastoma is an embryonal tumor that is thought to arise from a liver stem cell.[579,580] Thus, tumors have various combinations of

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epithelial, mesenchymal, embryonal, macrotrabecular, and undifferentiated cells.[581] Although there is no consensus classification system, two pathologic subtypes have been identified with very different prognoses: (1) pure fetal histology and (2) small cell undifferentiated (SCU). Pure fetal tumors are composed of sheets of cells that resemble fetal hepatocytes, have minimal mitotic activity (<2/10 high-power × 400 microscopic fields) and commonly contain clusters of hematopoietic precursors.[581] These tumors are curable with surgery alone.[582] At the other end of the spectrum are SCU tumors, accounting for less than 5% of all hepatoblastomas.[581] These tumors are often associated with low alpha-fetoprotein (AFP) at diagnosis (<100 ng/mL)[583] and may lack SMARCB1/INI1 nuclear expression,[584] suggesting that a subset of these tumors may be malignant rhabdoid tumors, rather than hepatoblastomas.

Clinical Presentation

Patients most commonly present with a palpable asymptomatic abdominal mass. Other common symptoms include nausea, vomiting, anorexia, and weight loss; jaundice is uncommon. Patients presenting with fatigue may also be anemic, and acute abdominal pain may indicate tumor rupture. Hepatoblastoma patients can present with isosexual precocity, as a consequence of elevations of β-human chorionic gonadotropin (HCG), and hemihypertrophy can be seen in up to 10% of patients.[585] The presenting physical findings in children with HCC are similar to those encountered in patients with hepatoblastoma.

Evaluation and Staging

A laboratory evaluation should include a complete blood count, tests of renal and hepatic function, and urinalysis. Thrombocytosis is not uncommon in children presenting with hepatoblastoma, presumably a result of thrombopoietin production by the tumor.[586,587] The serum levels of total bilirubin, alkaline phosphatase, and alanine aminotransferase and aspartate aminotransferase are not generally useful for the differential diagnosis of malignant hepatic tumors in children. Serum levels of AFP are elevated in approximately 90% of patients with hepatoblastoma and in 67% (29 out of 43) of children with HCC in the last COG trial[588] and 70% (28 out of 40) of children with HCC in SIOPEL1.[563,589] A very low or high value (<100 ng/L or >1,000,000 ng/L) at diagnosis as well as a low early decline (<1 log) prior to definitive surgery has been associated with poor prognosis in hepatoblastoma.[590,591] If the diagnosis of HCC is established, testing for hepatitis B surface antigen, hepatitis B antibody, serum iron, total iron-binding capacity, serum ferritin, and α_1-antitrypsin should be performed. The initial radiographic evaluation often is with an abdominal ultrasound. However, a CT and/or an MRI are required for a definitive evaluation of the mass. A multiphase CT may ultimately be obtained to further define the anatomic relationship of the tumor to adjacent blood vessels—information that may be critical in surgical planning. In addition, this type of study may further suggest the most likely tumor histology. PET/CT may also be a useful modality.[592] The evaluation should also include a CT of the chest to search for metastases.[593]

The staging system used in therapeutic studies of hepatic tumors conducted by the COG segregates patients according to the resectability of the primary tumor and the presence or absence of metastatic disease.[594,595]

The European SIOP Liver Tumor Study Group (SIOPEL) uses a pretreatment extent of disease (PRETEXT) staging system (Fig. 99.8).[596,597]

This preoperative classification scheme identifies four PRETEXT categories reflecting the number of sections of the liver free of tumor and describes extension of disease into portal and hepatic veins, the vena cava, or intra-abdominal extrahepatic sites. A recent study comparing both staging systems suggests that the PRETEXT and COG systems can accurately predict survival in patients who might benefit from upfront surgical resection and reduced therapy as well as patients with unresectable disease who are at increased risk of death.[595]

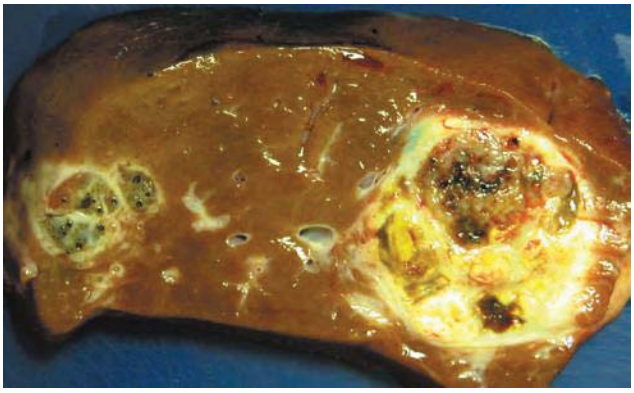

Figure 99.8 Liver resection of a patient with pretreatment extent of disease (PRETEXT) stage IV disease who underwent a successful liver transplant. The resected specimen shows multiple areas of viable tumor.

Prognostic Factors

The initial extent of disease (Staging; COG or SIOPEL)[595,598] and tumor resectability[599] as well as AFP values at diagnosis (less than 100 ng/L[583,600] or >1,000,000 ng/mL[591]), histology (pure fetal[582] or small cell undifferentiated histology[601]) are the main predictors of outcome in hepatoblastomas.[595] The presence of vascular involvement by the tumor is an additional adverse factor that has been used by the SIOPEL to classify patients as high risk. However, in a multivariate analysis, this did not hold up, perhaps because these patients often also have higher PRETEXT stage and/or metastatic disease.[600] In children with HCC, tumor resectability is essentially the only prognostic factor.[588,589] In adult patients, other important prognostic factors include cirrhosis, tumor size, the presence of metastases, satellite lesions, vascular invasion, or invasion of the tumor capsule.[602,603]

Treatment

Surgery

Complete tumor resection is essential for a cure.[599,604–607] Unfortunately, fewer than half of patients with hepatoblastoma[594] and less than 20% of children with HCC have resectable disease at the time of diagnosis.[588,589,608] Among the factors that render a liver tumor unresectable include invasion or close proximity to major vessels or involvement of both lobes.[604] Treatment with chemotherapy before definitive surgery has allowed for complete tumor resection in more than two-thirds of children whose hepatoblastomas were initially deemed unresectable.[594,609,610] In contrast, HCC is much less responsive to chemotherapy, limiting the effectiveness of preoperative pharmacologic intervention. In the SIOPEL report, all but two patients received preoperative chemotherapy and 36% (14 out of 40) eventually had gross total resection.[589] When feasible, aggressive attempts at the initial resection of HCC should be pursued.

Liver transplantation has been used by several centers for children with unresectable hepatoblastoma, with 10-year survival rates in excess of 80% for those who underwent this procedure as "primary" surgical therapy.[611–613]

This approach has also been used for patients with HCC.[589,614–619] Chemoembolization and/or radiofrequency ablation of hepatic malignancies has been increasingly used in adults with HCC.[620] Their overall role in the treatment of pediatric HCC remains to be defined.[621–623]

Radiation Therapy

Traditionally, radiation therapy has had a limited role in the treatment of hepatoblastoma or HCC given the low tolerance that the normal liver has for radiation. In the era of image-guided radiotherapy, radiation may be delivered to liver tumors with less hepatotoxicity.[624,625] Not much information is available in children regarding the use of radiotherapy in hepatoblastoma or HCC. In one report, doses of 25 to 45 Gy were associated with six of eight children with incompletely resected hepatoblastomas surviving after multimodality treatment.[626]

Chemotherapy

Before the identification of active chemotherapy, more than half of all children with hepatoblastoma were not amenable to surgical resection because of either extensive intrahepatic disease or the presence of extrahepatic metastases. Chemotherapy has enabled the successful surgical resection of many of these patients, resulting in cure rates approaching 80% in patients who have successful complete resections.[627–629] Thus, the role of chemotherapy in the treatment of hepatoblastoma is well established.[594,627,629–632] The most active agent is cisplatin.[627] Other active agents include doxorubicin, vincristine, 5-fluorouricil, carboplatin, etoposide and ifosfamide,[594,632–636] and more recently, irinotecan.[637,638] A randomized study using cisplatin-based chemotherapy demonstrated that the combination of doxorubicin and cisplatin was equivalent to the combination of cisplatin, vincristine, and 5-fluorouracil (CFV), but the latter triplet was associated with less myelosuppression, toxic deaths, and a decreased need for prolonged hyperalimentation.[594] Thus, the latter regimen has become the standard for treating patients in North America. In Europe, SIOPEL preferentially used a cisplatin–doxorubicin-containing regimen (Table 99.17).[598]

A recent SIOPEL prospective randomized trial suggests, however, that patients with localized hepatoblastoma who have three or less liver sectors involved can be successfully treated with single-agent cisplatin.[627] A significantly worse outcome was recently reported by investigators of the COG when carboplatin and cisplatin were alternated in newly diagnosed patients with advanced-stage disease (see Table 99.17), suggesting that platinum intensification in this manner was ineffective.[639] The primary role of cisplatin in the treatment of hepatoblastoma was further corroborated by the recent SIOPEL report in which dose-intensive single-agent cisplatin (given every 2 weeks) was as effective as the combination of cisplatin and doxorubicin in children younger than 16 years of age with standard-risk hepatoblastoma.[627]

The 5-year EFS for patients with hepatoblastoma using cisplatin-containing regimens is about 70%, but survival is closely dependent on the tumor stage at diagnosis: 91±4% for stage I, 100% for stage II, 64±5% for stage III, and 25±7% for stage IV.[594] Similar 5-yr EFS results based on PRETEXT staging have been published by SIOPEL investigators: 100%, 83%, 56%, and 46%, respectively, for patients with PRETEXT stages I through IV.[609] Patients with metastatic disease continue to fare poorly regardless

TABLE 99.17

Results of Intergroup Studies (Children's Oncology Group and SIOP Liver Tumor Study Group) for the Treatment of Hepatoblastoma

Study (Reference)	Number of Patients	Treatment	Outcome	Comments
POG/CCG[594]	173	CDDP–V–5-FU vs CDDP–Dox	5-yr S 69% 5-yr S 72% (p = 0.88)	Regimen without doxorubicin less toxic with similar survival
COG P 9645[639]	53 56	CDDP–V–5-FU vs CBDCA–CDDP	1-yr EFS 57% 1-yr EFS 37% (p = 0.017)	Intensified platinum regimens increase the probability of adverse outcomes in children with hepatoblastoma
SIOPEL I[609]	154	CDDP–Dox	5-yr S 75%	138 received preoperative chemotherapy only, and of these 72% had a complete resection
SIOPEL 2[610]	67 58	Standard risk (PRETEXT I, II, III) CDDP only HR–CDDP–CBDCA–Dox	3-yr S 91 ± 7% 3-yr S 53 ± 13%	97% of SR had complete tumor resections Results led to randomized comparison of CDDP vs. PLADO for SR (SIOPEL 3)
SIOPEL 3[627]	126 129	CDDP only CDDP–Dox	3-yr S 95% 3-yr S 93%	Dox can be safely omitted from treatment of SR patients
SIOPEL-3HR[628]	151	High risk (PRETEXT IV, P+, V+, E+, metastases, AFP <100 ng/mL) CDDP/Dox/CBDCA	3-yrs EFS 65%; 3-yr OS 69%	70% (106/151) achieved complete resection of tumor lesions, including metastases
SIOPEL-4[629]	62	High risk (PRETEXT IV, P+, V+, E+, metastases, AFP <100 ng/mL, or tumor rupture) CDDP/Dox/CBDCA	3-yr EFS 76%; 3-yr OS 83%	Increased dose-intensity of CDDP; GTR of all tumor achieved in 46/62 (74%) high-risk patients
HB 89[634]	72	Ifos–CDDP–Dox	"Long-term" EFS 75%	GTR in 92%
HB 94[591]	69	Ifos–CDDP–Dox	3-yr S 77%	GTR 86% (54/63) Long-term EFS of 95% in those with GTR
JPLT-1[667]	134	CDDP–pirarubicin	3-yr S 77.8%	GTR 72%
JPLT-2[668]	212	CDDP–pirarubicin Ifos–pirarubicin–etop-CBDCA	3-yr S 82.4%	GTR 82%; 3-yr S 88%. Macroscopic disease /unresectable 3-yr S 56% (p <0.001)

CDDP, cisplatin; V, vincristine; 5-FU, 5-fluorouracil; S, survival; Dox, doxorubicin; CBDCA, carboplatin; HR, high risk; PLADO, cisplatin + doxorubicin; SR, standard risk; GTR, gross total resection; Ifos, ifosfamide; etop, etoposide.

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of the regimen used, and their survival is less than 30%. Nearly one-third of patients who relapse following therapy with CFV can be salvaged with further surgery and a doxorubicin-based regimen.[640]

Currently, the COG study for patients with newly diagnosed hepatoblastoma (AHEP0731) is assigning treatment based on an assessment of risk. The factors incorporated in the risk group assignment include: COG stage, histology (pure fetal or SCU) and AFP. Very low-risk patients are defined as those with stage I tumors, pure fetal histology (PFH), and AFPs >100 ng/mL and are treated with surgery alone; those with low-risk disease are those with grossly resected tumors (stages I or II), AFPs >100 ng/mL, and who do not have SCU histology. These patients are treated with two courses of CFV chemotherapy. Intermediate-risk patients are defined as those with stage III tumors or those with stage I/II tumors with any SCU elements, as long as AFP is greater than 100 ng/mL. These patients receive six cycles of CFV with doxorubicin added to each cycle. Finally high-risk patients, defined as those with metastatic disease or AFP <100 ng/mL, regardless of stage, will receive an initial two courses of irinotecan/vincristine, followed by courses of CFV with doxorubicin.

In conclusion, although hepatoblastoma is a rare childhood cancer, cooperative group trials have enabled significant improvements in treatment. Because of the success of these trials and the rarity of these tumors, additional progress will require further international collaboration. Planning is ongoing for an international trial (Pediatric Hepatoblastoma International Therapeutic Trial [PHITT]), including all of the worldwide pediatric liver tumor cooperative groups from the United States, Europe, and Japan to evaluate what is the best chemotherapy for these children.

GERM CELL TUMORS

Epidemiology

Germ cell tumors account for 3.5% of cases of cancer in children under 15 years of age but for 16% of all cancers in those who are 15 to 19 years of age.[183] In pediatrics, germ cell tumors have a bimodal age distribution with peaks during infancy and puberty.[641] In boys under 4 years, gonadal and extragonadal tumors are evenly represented and teratomas and yolk sac carcinomas predominate, whereas most of the tumors in patients older than 10 years are located in the testis and are of nonseminomatous histology (teratoma, embryonal carcinoma, mixed). In girls, the majority of tumors in patients younger than 4 years of age are extragonadal, whereas germinomas and teratomas affecting the ovary are most commonly seen in patients over 10 years of age.[641] Sacrococcygeal tumors account for 40% of all childhood germ cell tumors and 78% of all extragonadal tumors[642]; less common extragonadal sites include the mediastinum, the retroperitoneum, the vagina, and the pineal region. Predisposing conditions for the development of pediatric germ cell tumors include cryptorchidism, Turner syndrome, Klinefelter syndrome, and androgen insensitivity syndromes.[642,643]

Pathology

Pediatric germ cell tumors comprise a diverse group of histologies reflecting the pluripotent nature of the primordial germ cells. There are several subtypes of germ cell tumors, the most common being teratomas (37%), yolk sac tumors (27%), germinomas (18%), mixed (12%), embryonal carcinomas (2%), and choriocarcinomas (2%).[644]

Laboratory Markers

AFP and the β subunit of HCG (β-HCG) are oncofetoproteins, which are elevated in the serum of patients with a variety of germ cell tumors. AFP reaches peak concentrations at 12 to 14 weeks and declines to levels of less than 10 ng/L by 1 year of age. The half-life of AFP is 5 to 7 days. AFP is elevated in patients with yolk sac and embryonal carcinomas.[642]

β-HCG is a glycoprotein secreted by the placenta, and its half-life is only 26 to 36 hours. Elevated levels in patients with germ cell tumors implies the presence of syncytiotrophoblastic cells, which are seen in germinomas, embryonal carcinomas, and choriocarcinomas.[642]

Clinical Presentation, Staging, and Treatment

The following section briefly summarizes the clinical presentation and treatment recommendations for germ cell tumors in specific anatomic areas. In general, pediatric patients with germ cell tumors can be divided into three risk categories that determine the type of therapy they receive (Table 99.18). A recent study suggests

TABLE 99.18

Staging of Pediatric Germ Cell Tumors

Stage	Extent of Disease
Testicular Germ Cell Tumors	
I	Limited to testis (testes), completely resected by high inguinal orchiectomy; no clinical, radiographic, or histologic evidence of disease beyond the testes. Patients with normal or unknown tumor markers at diagnosis must have a negative ipsilateral retroperitoneal node sampling to confirm stage I disease if radiographic studies demonstrate lymph nodes >2 cm.
II	Transscrotal biopsy; microscopic disease in scrotum or high in spermatic cord (≤5 cm from proximal end). Failure of tumor markers to normalize or decrease with an appropriate half-life.
III	Retroperitoneal lymph node involvement, but no visceral or extra-abdominal involvement. Lymph nodes >4 cm by CT or >2 cm, and <4 cm with biopsy proof.
IV	Distant metastases, including liver.
Ovarian Germ Cell Tumors	
I	Limited to ovary (peritoneal evaluation should be negative). No clinical, radiographic, or histologic evidence of disease beyond the ovaries. (Note: The presence of gliomatosis peritonei does not result in changing stage I disease to a higher stage).
II	Microscopic residual; peritoneal evaluation negative. (Note: The presence of gliomatosis peritonei does not result in changing stage II disease to a higher stage). Failure of tumor markers to normalize or decrease with an appropriate half-life.
III	Lymph node involvement (metastatic nodule); gross residual or biopsy only; contiguous visceral involvement (omentum, intestine, bladder); peritoneal evaluation positive for malignancy.
IV	Distant metastases, including liver.
Extragonadal Germ Cell Tumors	
I	Complete resection at any site, coccygectomy for sacrococcygeal site, negative tumor margins.
II	Microscopic residual; lymph nodes negative.
III	Lymph node involvement with metastatic disease. Gross residual or biopsy only; retroperitoneal nodes negative or positive.
IV	Distant metastases, including liver.

that the addition of other prognostic factors that include age, LDH and AFP levels, and the presence of a mediastinal primary tumor may help refine the risk stratification of patients with pediatric germ cell tumors.[645]

In general, following surgical resection, patients with mature and immature teratomas as well as patients with stage I gonadal tumors (see the following section) can be observed and chemotherapy can be reserved to salvage patients who recur. For patients with more advanced stage disease, the use of cisplatin-based regimens has dramatically improved the outcome of patients with germ cell tumors, and with current regimens, over 80% of children with disseminated disease and over 90% of those with localized disease can be cured.[646–648] The administration of higher doses of cisplatin may benefit subsets of patients with stage III/IV extragonadal tumors; however, this treatment is associated with significant ototoxicity.[648] Attempts to reduce the rates of ototoxicity by adding amifostine to high-dose platinum were unsuccessful in one COG trial.[649] In another trial, the addition of high-dose cyclophosphamide to standard-dose platinum proved to be feasible, but its benefit could not be adequately assessed given the low numbers of patients enrolled.[650]

Extragonadal Germ Cell Tumors

Sacrococcygeal teratoma occur in 1 in 35,000 live births, are the most common germ cell tumors in infants and newborns, and more commonly affect girls.[651] Approximately 80% of these tumors are diagnosed within the first month of life.

Four types of sacrococcygeal teratomas have been defined on the basis of the abdominopelvic extent and the presence or absence of external extension (Fig. 99.9).[651] The frequency of malignancy is closely associated with the type of teratoma and the age at presentation; girls and boys older than 2 months of age have a malignancy rate of 48% and 67%, respectively.[652] Surgery is the mainstay of therapy for sacrococcygeal tumors and should include the removal of the coccyx to minimize local recurrence. Surgical sequelae that affect bowel and bladder control are reported in 11% to 41% of survivors.[651] Children with benign sacrococcygeal tumors have an excellent outcome with surgery alone, but recurrences that often contain a yolk sac component are seen in 11% of patients with mature teratomas and in 4% of patients with immature teratomas.[653] This finding argues in favor of close follow-up of these patients for 3 years following resection.[651] Patients with malignant yolk sac tumors located in the abdomen and pelvis are highly responsive to chemotherapy and have an excellent survival (approximately 90%) when treated with platinum-containing regimens.[654]

Mediastinal Germ Cell Tumors

These tumors more commonly affect adolescent males and are associated with Klinefelter syndrome. In the first year of life, teratomas predominate, whereas older patients have yolk sac or mixed germ cell tumors. Patients may be asymptomatic or present with respiratory symptoms such as cough and dyspnea; infants and toddlers more often present with respiratory symptoms that may also include hemoptysis and upper airway obstruction.[642] The diagnosis is established by biopsy of the primary tumor or an involved supraclavicular lymph node. Staging studies should include serum chemistries; a complete blood count; tumor marker studies; a CT of the chest, abdomen, and pelvis; an MRI of the brain if clinically symptomatic; and a bone scan if metastases are evident at diagnosis in other sites such as the lung.

The use of cisplatin-containing regimens has dramatically improved the outcome for children with malignant mediastinal germ

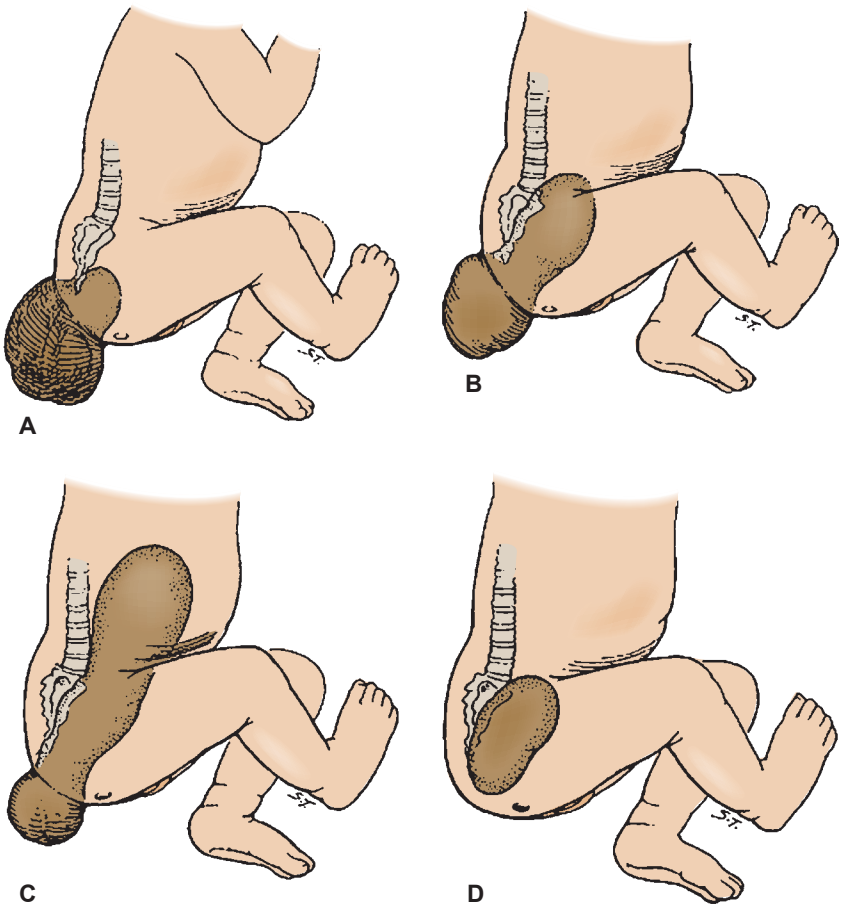

Figure 99.9 Types of sacrococcygeal teratoma. **A:** Type I predominantly external, **B:** Type II external and intrapelvic, **C:** Type III predominantly pelvic, **D:** Type IV presacral. (From Rescorla FJ, Breitfeld PP. Pediatric germ cell tumors. *Curr Probl Cancer* 1999;23(6):257, with permission from Elsevier.)

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cell tumors, but patients who are ≥12 years of age have a sixfold increased risk of death (survival <50%) compared to younger patients with tumors located in other sites.[655] The use of a high-dose platinum regimen in one trial was not associated with a significant difference in clinical outcome.[655]

Gonadal Tumors

Testicular tumors make up approximately 17% of all pediatric germ cell tumors. About 75% of testicular tumors in children are of germ cell origin; in prepubertal males, teratomas and yolk sac carcinomas are the most common histologies, whereas mixed germ cell tumors are seen in older patients.[644] Children with primary testicular tumors often present with painless testicular enlargement, and in about 20% of cases, they are associated with hydroceles or inguinal hernias.[642] The preoperative evaluation should include an ultrasound, a complete blood count, a chest radiograph, and serum chemistries, including AFP and β-HCG levels. After the diagnosis is established, the metastatic evaluation should include CT scans of the chest, abdomen, and pelvis. A bone scintigraphy should be performed in all patients with advanced stage disease, and brain imaging should be obtained when clinically indicated.

All scrotal masses should be explored through an inguinal incision. If the AFP is elevated and a malignancy is suspected, a radical orchiectomy with control of the vessels at the internal ring should be performed. If a benign lesion with a normal AFP is being considered as a diagnostic possibility, an enucleation of the mass following opening of the tunica can be performed after mobilizing the testicle and controlling the cord.[651]

Patients with nonseminomatous stage I germ cell tumors (completely resected tumors) can be treated with radical orchiectomy alone, reserving the use of chemotherapy for relapse (Table 99.19).[656,657] Children with more advanced stage disease (intermediate-risk disease; see Table 99.19) should be treated with standard courses of cisplatin, etoposide, and bleomycin and are expected to have a 6-year survival in excess of 90%.[648]

Ovarian tumors account for approximately 29% of all pediatric germ cell tumors and the incidence of this tumor increases with a peak at 18 years of age. Teratomas are the most common germ cell tumor of the ovary in children and adolescents, and dysgerminoma is the most common malignant germ cell tumor of the ovary in this patient population. Presenting symptoms include pain, abdominal distension, and acute abdomen, which may result from tumor hemorrhage, rupture, or torsion. Other less common symptoms include vaginal bleeding or precocious puberty (mostly in patients with sex-cord stromal tumors).[651] The initial work-up should include a complete blood count and serum chemistries, including AFP and β-HCG. The preoperative radiographic evaluation should include an abdominal ultrasonography and CT scans of the abdomen and pelvis. Once the tissue diagnosis is established, a metastatic evaluation that includes a CT of the chest and a bone scintigraphy (for advanced cases) should be performed. Surgical exploration should be performed through a laparotomy, and the following additional samples should be obtained: peritoneal washings, an examination of omentum with resection of adherent or abnormal areas, an exploration of retroperitoneal nodes with resection of abnormal nodes, and an inspection of the contralateral ovary with a biopsy of abnormal areas.[658] Every attempt should be made to spare uninvolved fallopian tubes and the uterus to preserve fertility. Ovarian tumors are staged using the POG/CCG staging system, which represents a simplified version of the International Federation of Gynecology and Obstetrics staging system.

Surgery alone is sufficient for a cure in patients with mature and immature teratomas, and the presence of gliomatosis elements does not affect the prognosis.[642,659,660] For patients with stage 1 seminomatous (dysgerminoma) and nonseminomatous malignant germ cell tumors, European and North American trials including a recently completed COG trial demonstrate that close observation following surgery is a reasonable strategy; although one-third of patients recur, more than 95% can be successfully salvaged and cured with chemotherapy at the time of relapse.[657,661,662] Current pediatric regimens for the treatment of stages II through IV ovarian germ cell tumors use a combination of standard-dose cisplatin, etoposide, and bleomycin with 6-year event-free survival and OS rates in excess of 86% and 93%, respectively (see Tables 99.18 and 99.19).[648]

TABLE 99.19

Treatment of Pediatric Germ Cell Tumors

Risk	Treatment	5-Year Survival (%)
Low Risk Stage 1 testicular Stage 1 ovarian	Surgery only	<95
Intermediate Risk Stage II–IV ovary Stage II–IV testes Stage I–II extragonadal	PEB × 3 or 4	<90
High Risk Stage II–IV extragonadal Stage IV ovary	PEB × 4	70–75

PEB, cisplatin, etoposide, bleomycin.

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100 Leukemias and Lymphomas of Childhood

Karen R. Rabin, Judith F. Margolin, Kala Y. Kamdar, and David G. Poplack

INTRODUCTION

The cure rates seen in pediatric oncology are some of the best in modern oncology. These are largely related to the remarkable progress in the treatment of leukemias and lymphomas, where cure rates have improved from less than 10% to 80% to 95%.[1–4] Cure rates for pediatric myeloid leukemia are lower, in the 60% to 70% range.[5,6] The improvement in outcomes for all of pediatric cancer are the result of the successful implementation of cooperative group clinical trials at the national and even international level.[7] Currently, more than two-thirds of children with cancer in the United States are treated in clinical trials.[7] Although there are many similarities in treating children and adults with these diseases, there are also differences that pertain to development and growth, drug metabolism, and psychosocial issues.

LEUKEMIAS

Incidence, Histology, and General Outcomes

Acute lymphoblastic leukemia (ALL), the most common malignancy of childhood, accounts for 75% of leukemias and is curable in 80% to 95% of cases.[8] Figure 100.1 demonstrates the improvement in survival in pediatric ALL on successive cooperative group trials from 1968 to 2005.[1] Although there are variations with age, sex, and ethnicity, the majority of ALL cases have pre–B immunophenotype and FAB L1 histology (80% of cases); 20% are of T-cell origin. The peak incidence of pediatric ALL is between ages 2 and 5 years. ALL is slightly more common in American Caucasians than in African Americans, and there is a slightly increased incidence in male patients.[9] Approximately 5,000 children are diagnosed with ALL each year in the United States, with an incidence of 29.2 per million.[9] The Surveillance, Epidemiology, and End Results (SEER) program data show that the incidence of ALL has been climbing slowly. Controversy exists concerning whether and how incidence and outcomes differ between racial and ethnic groups. Studies from the 1990s suggest that Caucasians have slightly higher survival rates than those of African ancestry and Hispanics, although this is confounded in part by the fact that those of African ancestry and Hispanics more often develop high-risk forms of ALL.[10–12] Racial and ethnic disparities in access to care and adherence to treatment may also have an adverse impact on survival.[13,14]

Acute myeloid leukemia (AML) accounts for approximately 15% to 20% of childhood leukemias, chronic myelogenous leukemia (CML) accounts for 3% to 4%, and juvenile myelomonocytic leukemia (JMML) and other rarer histologies account for less than 1%. The incidence of AML is currently estimated to be 5 to 7 cases per million in the United States.[15] Myelodysplastic syndrome (MDS) is a relatively rare diagnosis in childhood, generally progresses to AML, and is treated with similar regimens (including bone marrow transplant) as AML.[16] AML incidence is slightly higher in African Americans and Hispanics in the United States, and survival is slightly lower.[17,18] Because of intrinsic differences in drug sensitivity between ALL and AML cells, progress in treating AML has been less dramatic than that of ALL. Nevertheless, current cure rates in AML have risen to approximately 45% to 65% with the advent of more intensive conventional chemotherapy and bone marrow transplant (BMT) (Fig. 100.2).[19,20]

Etiology

Environmental Factors

There is extensive literature and a continued interest in exploring possible relationships between infectious or environmental exposures and increased risk of childhood leukemia. However, most studies show weak or no correlation of these factors with the incidence of leukemia.[21–23] Electromagnetic fields have not been found to be significant in pediatric malignancies.[24–26] There is an emerging body of evidence from population-based studies of polymorphisms of drug-metabolizing genes, such as *NQO1* and *GST* polymorphisms, that may be associated with an increase or decrease in an individual's risk for developing leukemia based on exposures to particular environmental toxins like benzene and other organic solvents, quinine-containing substances, and flavonoids.[27]

Genetics

There is significant evidence that genetic factors play a role in the etiology of pediatric leukemia. Within the leukemic blasts themselves, there are characteristic cytogenetic changes (Table 100.1), many of which have prognostic significance (see later discussion). Key genetic changes found in childhood leukemia (especially in younger patients) may occur in utero or very early in life.[28,29] Evidence includes the detection of cells bearing these changes in neonatal blood spots[30] and transmission of leukemia by twin–twin transfusion.[31] The *delayed infection* hypothesis holds that children in developed countries are spared the frequent early infections that are necessary for normal development of the immune system. An infection occurring at a later time is then hypothesized to elicit a pathologic, myelosuppressive response, providing a selective growth advantage to the preleukemic clone, leading to development of overt leukemia.[32]

In most cases, leukemia appears to result from a complex interplay between environmental and genetic factors. Recently, genome-wide association studies have identified several constitutional genetic variations with moderate but statistically significant effects on the relative risk of ALL.[33–36] Collectively, variants in *IKZF1*, *ARID5B*, *CEBPE*, and *CDKN2A* may account for up to 80% of the attributable risk of developing ALL in Europeans.[35] *ARID5B* and *GATA3* polymorphisms have been identified as associated with both ALL susceptibility and relapse hazard, and may contribute to racial disparities in outcome, because high-risk alleles occurred more frequently in subjects with a greater degree of Native American ancestry.[37]

In addition to somatic and germ-line genetic changes associated with leukemia, there are well-described cases of familial

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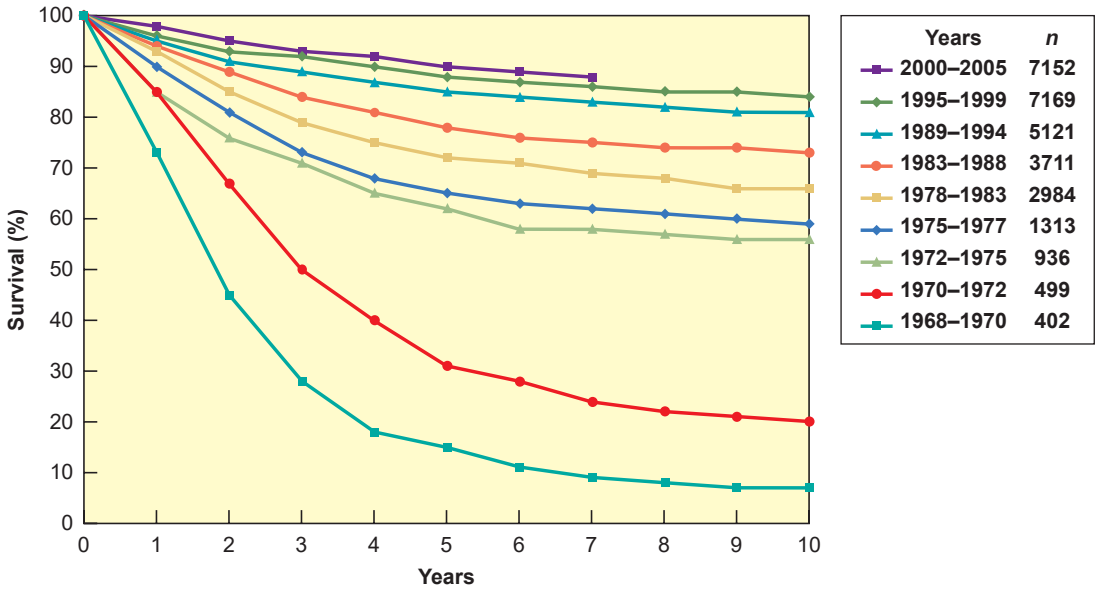

Figure 100.1 Improvement in survival of children with acute lymphoblastic leukemia. Overall survival probability by treatment era for 29,287 children with ALL who enrolled in trials from 1968 to 2005 conducted by the Children's Oncology Group (COG), the Pediatric Oncology Group (POG), and the Children's Cancer Group (CCG). The 2000 to 2005 curve includes CCG, POG, and COG. The 1995 to 1999 curve includes CCG and POG. All other curves are CCG. (Adapted from Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. *J Clin Oncol* 2012;30:1663–1669.)

leukemia, including recently described mutations in the hematopoietic transcription factor *PAX5*,[38] as well as strong associations between leukemia risk and immunodeficiency and several somatic genetic disorders (e.g., Down syndrome [DS], Bloom syndrome, ataxia telangiectasia, Shwachman-Diamond syndrome, Noonan syndrome, neurofibromatosis).[8] Recently, a new association has been described between Li-Fraumeni syndrome, a well-known cancer predisposition syndrome, and hypodiploid ALL.[39]

Patients with DS have a 10- to 20-fold increased risk of developing leukemia.[40] The occurrence of leukemia in DS patients appears to be unrelated to the other congenital abnormalities and

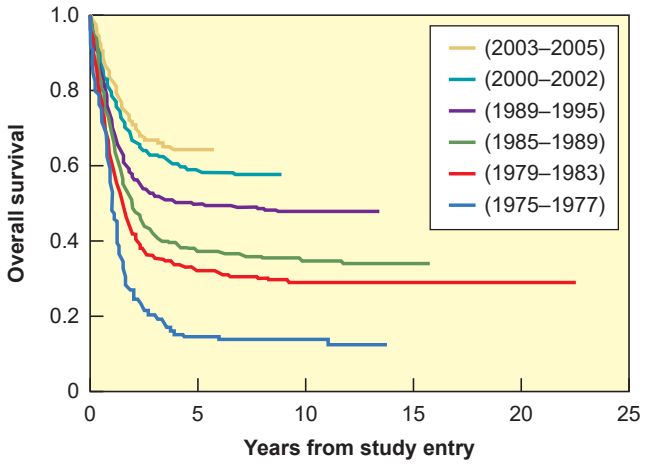

Figure 100.2 Improvement in survival of children with acute myeloid leukemia. Incremental improvements in overall survival in Children's Oncology Group and legacy trials in de novo childhood AML during the years indicated. (From Gamis AS, Alonzo TA, Perentesis JP, et al. Children's Oncology Group's 2013 blueprint for research: acute myeloid leukemia. *Pediatr Blood Cancer* 2013;60:964–971.)

TABLE 100.1

Acute Leukemia: Favorable and Unfavorable Prognostic Factors

Favorable	Unfavorable
Acute Lymphoblastic Leukemia	
Age, 1–9 years	Age, <1 or ≥10 years
White blood cell count <50,000/mcL	White blood cell count ≥50,000/mcL
High hyperdiploidy (DNA index >1.16; and especially trisomies of chromosomes 4 and 10)	Hypodiploidy (DNA index <0.81 or <44 chromosomes)
Chromosomal translocation t(12;21) or *ETV6-RUNX1*	Chromosomal alterations: t(9;22) or *BCR-ABL1*; *MLL* gene rearrangement; iAMP21 (intrachromosomal amplification of chromosome 21); *IKZF1* gene deletion or mutation; Ph-like gene expression profile
CNS-1	Extramedullary disease
Rapid response to induction chemotherapy	Slow response to induction chemotherapy
Acute Myeloid Leukemia	
Core binding factor transcription complex, t(8;21) or inv(16)	Monosomy 5, 5q deletion
Acute promyelocytic leukemia t(15;17)	Monosomy 7
Down syndrome	FLT3 internal tandem duplication
Rapid response to induction chemotherapy	Secondary AML/ myelodysplastic syndrome
	Slow response to induction chemotherapy

CNS, central nervous system.

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medical problems of DS.[41] The cytogenetic changes common in ALL occur less frequently in DS-ALL,[42–45] but two alterations are markedly enriched: (1) Janus kinase 2 (*JAK2*) activating mutations in approximately 20% of DS-ALL, and (2) rearrangements leading to overexpression of cytokine receptor-like factor 2 (*CRLF2*) in up to 50% of DS-ALL.[46–48]

In the neonatal period, DS patients have a propensity for developing a nonneoplastic entity known as *transient myeloproliferative disease* (TMD) of DS, which at presentation, can appear very similar to AML (i.e., very high white blood cell counts with peripheral myeloblasts), but usually resolves spontaneously.[49] TMD may be difficult to distinguish from AML. Although TMD itself is not a form of malignancy or leukemia, approximately 30% of the DS patients with TMD will develop AML later in childhood.[49]

When patients with DS do develop AML (especially those younger than age 2 years), they tend to develop acute megakaryoblastic leukemia (AMKL), display particular mutations in the *GATA-1* hematopoietic transcription factor, and have an excellent prognosis compared to other adult and pediatric AML subgroups. The disease-free survival (DFS) for DS patients with *GATA-1*+ AMKL is over 90%, with regimens significantly less intensive than those required for other AML cases.[50] The reasons behind the increased risk of leukemia in DS remain unknown.

Clonal Nature of Lymphoid Cancers

Lymphoid malignancies appear to have derived from an original abnormal progenitor that lost the ability to fully differentiate and formed a *clone* of leukemic blast cells. ALL blasts are characterized by early cytoplasmic and surface lymphoid antigens (detectable by flow cytometry), as well as incomplete immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements, suggesting they arise from a precursor T- or B-lymphoid progenitor. AML may arise from a multipotent or committed myeloid progenitor. Persistence of subclinical amounts of leukemia during or after therapy is called minimal residual disease (MRD).[51] MRD may be detected by flow cytometry or polymerase chain reaction (PCR)-based detection of Ig/TCR rearrangements or chromosomal translocations.[52] Use of high-throughput sequencing technologies for MRD detection is being undertaken on a research basis and may hold promise as a future clinical test with even higher sensitivity and precision.[53]

Diagnosis

The presenting signs and symptoms of a child with leukemia reflect the impact of bone marrow infiltration, the extent of extramedullary disease spread, and problems arising from changes in blood viscosity and chemistry related to the size, number, and breakdown products of leukemic blasts (tumor lysis).[12] Leukostasis from high white blood cell (WBC) counts may cause signs and symptoms ranging from mild respiratory symptoms and pulmonary infiltrates, to stroke, cranial or peripheral neuropathies, hematuria, renal failure, and ocular findings.[8] AML blasts are larger, less flexible, and contain granules that can cause inflammation and clotting. Thus, AML has a higher risk of serious systemic problems then ALL for a given high WBC (e.g., >100,000/mm^3). Gingival infiltration and orbital and periorbital soft tissue masses (chloromas) are often seen in AML.[54] AML FAB subtypes 4 and 5 have a tendency to present with chloromas.

Central nervous system (CNS) involvement is more common in ALL but is also possible with AML.[55,56] CNS involvement is most often meningeal involvement, but may manifest as cranial nerve involvement, and occasionally, frank leukemic infiltrates in the parenchyma of the brain. CNS involvement may be asymptomatic or manifest as cranial nerve palsies, seizures, or focal neurologic findings. Testicular involvement generally manifests as a painless, palpable mass. Hepatosplenomegaly is common in both ALL and AML, and may compromise respiration in small children. Lymphomatous involvement of nodes or extranodal tissue can cause superior mediastinal or superior vena cava syndromes, and bowel wall or mesenteric node infiltrations can cause intussusception or bowel perforation.

Tables 100.2 and 100.3 summarize the clinical and laboratory findings and differential diagnosis, respectively, for pediatric patients presenting with ALL. Infection and bleeding are significant risks. Fever should be managed aggressively with intravenous broad-spectrum antibiotics. Bleeding risk should be addressed by prompt transfusions of packed red blood cells, platelets, and/or plasma, as clinically indicated.

A definitive diagnosis of leukemia requires that the bone marrow has more than 20% to 25% leukemic blasts (depending on the pathologic classification system used).[57] Traditionally, the diagnosis depended on bone marrow aspirate and biopsy morphology and immunohistochemical staining patterns. The cornerstone of modern diagnosis is immunophenotyping, using flow cytometry antibody panels (see Chapter 107) to define the lineage of the leukemic clone. Cytogenetics also play a critical role.

Other diagnostic approaches that have become standard in many centers include fluorescent in situ hybridization (FISH), and PCR (to identify specific fusion proteins arising from chromosomal rearrangements). Finally, several techniques are used primarily on a research basis, such as RNA expression arrays, DNA copy number and single-nucleotide polymorphism (SNP) arrays, and whole-genome and whole-exome sequencing.[58–61] These techniques will be used increasingly for clinical decision making as well, such as to identify cryptic, actionable mutations[62] or to predict susceptibility to targeted therapy.[63,64]

TABLE 100.2

Clinical and Laboratory Features at Diagnosis in Children with Acute Lymphoblastic Leukemia

Clinical and Laboratory Features	Percentage of Patients
Symptoms and Physical Findings	
Fever	61
Bleeding (e.g., petechiae or purpura)	48
Bone pain	23
Lymphadenopathy	50
Splenomegaly	63
Hepatosplenomegaly	68
Laboratory Features	
Leukocyte count (mm^3)	
<10,000	53
10,000–49,000	30
>50,000	17
Hemoglobin (g/dL)	
<7.0	43
7.0–11.0	45
>11.0	12
Platelet count (mm^3)	
<20,000	28
20,000–99,000	47
>100,000	25
Lymphoblast morphology	
L1	84
L2	15
L3	1

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TABLE 100.3

Differential Diagnosis in Childhood Acute Lymphoblastic Leukemia

- Nonmalignant conditions
  - Juvenile rheumatoid arthritis
  - Infectious mononucleosis
  - Idiopathic thrombocytopenic purpura
  - Pertussis; parapertussis
  - Aplastic anemia
  - Acute infectious lymphocytosis
- Malignancies
  - Neuroblastoma
  - Retinoblastoma
  - Rhabdomyosarcoma
- Unusual presentations
  - Hypereosinophilic syndrome

TABLE 100.4

Definitions of Central Nervous System Disease Status at Diagnosis of Acute Lymphoblastic Leukemia Based on Cerebrospinal Fluid Findings[65]

Status	Cerebrospinal Fluid Findings
CNS-1	No lymphoblasts
CNS-2	<5 WBCs/mcL with definable blasts on cytocentrifuge examination
CNS-3	≥5 WBCs/mcL with blast cells (or cranial nerve palsy)

Management

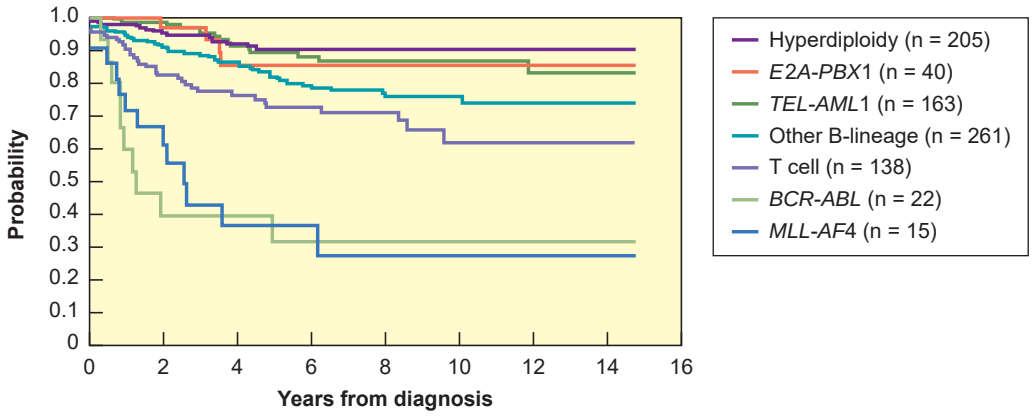
Prognostic Factors in Management of Pediatric Acute Lymphoblastic Leukemia

As outcomes with modern therapy in pediatric ALL have improved, many factors previously shown to be important for predicting a prognosis have lost statistical significance. Five factors have retained prognostic significance and constitute the basis on which patients are stratified in most treatment protocols. These factors are (1) age at presentation, (2) WBC at presentation, (3) specific cytogenetic abnormalities, (4) presence or absence of CNS involvement (Table 100.4), and (5) rapidity of initial response to chemotherapy.[8] The 1996 National Cancer Institute (NCI) consensus criteria standardized definitions of age, initial WBC, and CNS involvement to facilitate a comparison of clinical trial results between groups.[65] Cooperative groups differ in incorporation of cytogenetics and response to therapy. General principles of modern ALL protocols include treatment of low-risk disease with less-intensive chemotherapy to minimize toxicity while maintaining excellent overall survival (OS) (90% to 95%), whereas high-risk disease (OS, 40% to 85%) is treated with more intensive therapy.[8]

The age-determined risk groups are less than 1 year (infant ALL), 1.0 to 9.99 years (standard risk ALL), and ≥10 years (high-risk ALL). Infants constitute a very high-risk group. *MLL* (11q23) rearrangements are frequent, especially t(4;11)(q21;q23), and are associated with hyperleukocytosis, extramedullary disease, and common acute lymphoblastic leukemia antigen (CALLA) (CD10) negativity.[66,67] Cure rates on current infant protocols have improved modestly to approximately 50%.[68,69] Results are poorest in infants under 3 months of age with *MLL* rearrangement. Unlike for some other high-risk ALL subgroups, current evidence does not suggest a benefit of stem cell transplant.[69]

Adolescents and young adults have also traditionally demonstrated lower cure rates, although survival has improved significantly with early intensive postinduction therapy.[12,70] In B-lineage ALL, presenting with a WBC count more than 50,000/mcL, and particularly over 100,000/mcL, is associated with a high risk of relapse.

Blast cell cytogenetics and ploidy have a significant prognostic impact in ALL (Fig. 100.3). High hyperdiploidy (≥50 chromosomes or DNA index >1.16) is associated with good prognosis, particularly with trisomies of chromosomes 4 and 10.[71–73] Hypodiploidy (<45 chromosomes) and especially haploidy



Number at risk									
Hyperdiploidy	205	190	144	108	80	52	25	10	1
E2A-PBX1	40	36	27	19	14	9	6	0	0
TEL-AML1	163	144	105	83	60	46	30	10	0
Other B-lineage	261	221	161	130	92	50	28	13	3
T cell	138	112	75	60	36	22	8	3	1
BCR-ABL	22	15	7	5	3	2	0	0	0
MLL-AF4	15	9	6	4	4	2	1	1	0

Figure 100.3 Kaplan-Meier analysis of event-free survival according to biologic subtype of leukemia. (From Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet* 2008;371:1030–1043.)

(23 chromosomes) are associated with a poor prognosis.[72,74] Two other recently identified unfavorable abnormalities are *IKZF1* alterations[75] and intrachromosomal amplification of chromosome 21 (iAMP21).[76] *CRLF2* overexpression has been found to have an independent adverse prognostic impact in some studies, but not others.[77,78]

The most common translocation in pediatric ALL is t(12;21)(p12;q22), forming the *ETV6-RUNX1* (also known as *TEL-AML1*) fusion. This cryptic translocation occurs in 25% of US pediatric ALL cases, less frequently in other geographic and ethnically defined populations, and is associated with a favorable prognosis.[79,80] The Philadelphia chromosome (Ph+) refers to the t(9;22)(9q34;q11) translocation, forming the *BCR-ABL1* fusion. Ph+ ALL is less prevalent in children than adults and was historically associated with a dismal prognosis. Treatment of Ph+ ALL and CML were revolutionized by imatinib mesylate, a selective tyrosine kinase inhibitor active against the *BCR-ABL1* fusion. The addition of imatinib to conventional chemotherapy has improved survival from approximately 30% to 40% to 80%, and hematopoietic stem cell transplant (HSCT) in first remission is no longer considered as the standard of care.[81–83] Ongoing studies are investigating the efficacy of later generation tyrosine kinase inhibitors such as dasatinib.[84]

Numerous translocations occur in T-lineage ALL, but they are not generally associated with a prognosis. Activating *NOTCH1* mutations occur in over 50% of T-ALL cases, and are associated with a favorable prognosis.[85,86] Because mutated *NOTCH1* activity depends on γ-secretase activity, γ-secretase inhibitors are being investigated as a novel therapeutic approach. Early T-cell precursor (ETP) is a very unfavorable prognostic subgroup identified based on a distinctive immunophenotype, constituting approximately 10% of T-lineage ALL, which bears a gene expression signature and mutational profile similar to myeloid leukemia.[61,87]

CNS and testicular involvement are unfavorable prognostic signs, for which therapy is intensified.[56] The CNS is considered a sanctuary site because many systemic treatments do not adequately penetrate the blood–brain barrier, so CNS preventive therapy is required in all patients to prevent eventual CNS relapse. CNS prophylaxis is usually achieved through systemic and intrathecal chemotherapy. Although CNS involvement at diagnosis has generally been treated in the past with the addition of radiation to intensive chemotherapy, some advocate for reserving CNS radiation for use only in the case of CNS relapse.[88] Both testicular and CNS relapses require site-directed radiation as well as systemic reinduction due to increased risk of subsequent bone marrow relapse.[12,89]

All modern pediatric ALL treatment protocols use the prognostic factors outlined here, in varying ways, to stratify patients into different risk groups that receive treatment of different intensity. The first phase of treatment (*induction*) lasts 4 to 6 weeks and includes a glucocorticoid, vincristine (VCR), asparaginase, and for high-risk patients, an anthracycline (usually daunomycin).[12] The Berlin-Frankfurt-Munster group begins induction with a *steroid window*, and the degree of cytoreduction during this window is used in risk assessments.[90] Some groups intensify induction with additional medications. Rapid response to induction is an important prognostic variable (Fig. 100.4).[8,73] Induction failure (≥25% marrow blasts at the end of induction) is rare (2% to 5% of cases) and generally associated with a poor prognosis, although a recent large retrospective analysis reported an unexpected heterogeneity in survival, ranging from 10% to 70%, depending on age, cytogenetics, and B- versus T-lineage disease.[91]

Induction is usually followed by a *consolidation phase* to reinforce the bone marrow remission and to administer CNS prophylaxis. Subsequent intensive phases may be termed *intensification*, *delayed intensification*, or *reinduction/reconsolidation*, with the intensity depending on the risk group.[12,92,93] These intensive courses are generally delivered within the first 6 to 12 months of treatment, followed by a *maintenance* or *continuation* phase lasting 2 to 3 years, which consists of antimetabolite treatment (usually consisting of methotrexate weekly and 6-mercaptopurine daily), periodic intrathecal (IT) treatments, and periodic *pulses* of VCR and oral glucocorticoids with the frequency of these pulses varying by protocol and treatment group. To minimize and avoid treatment-related toxicities, strict supportive care guidelines are employed. Therapy-related mortality on frontline pediatric ALL protocols is generally under 2% to 3%. Overall treatment duration is usually between 2 and 3.5 years. On current Children's Oncology Group regimens, boys receive an additional year of maintenance, but many other cooperative study groups treat both genders for approximately 2 years.

CNS-directed therapy is usually present in all phases, but is most intensive during the first several months. CNS therapy uses intrathecal chemotherapy, high-dose systemic chemotherapy (principally, higher dose methotrexate or cytarabine), and/or cranial radiation.[56,94] Because it is often associated with neurocognitive deficits, endocrine, and growth abnormalities, cranial radiation has generally been reserved only for patients at highest risk of CNS relapse (i.e., approximately 5% of patients who present with initial CNS involvement or T-cell disease), but at least one treatment group currently reserves the use of cranial irradiation only for CNS-relapsed cases.[88] Through the years, the doses of

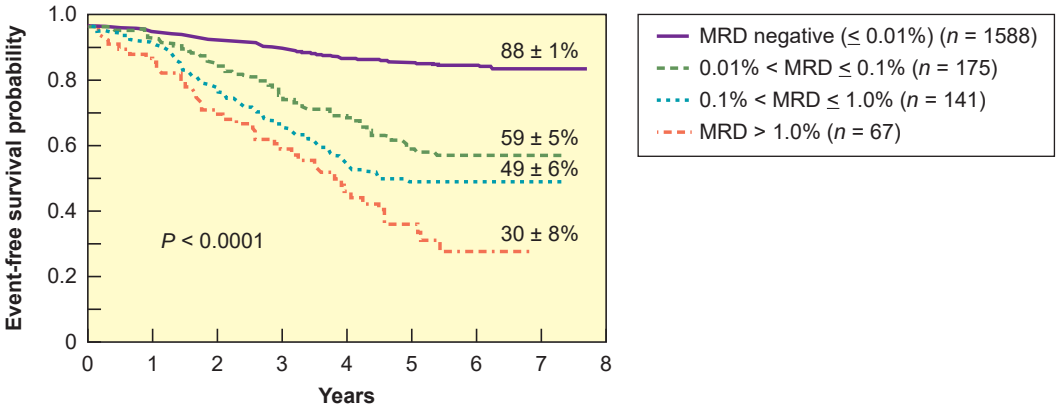


Figure 100.4 Effect of minimal residual disease (MRD) on prognosis. Event-free survival (EFS) of all patients enrolled on the Pediatric Oncology Group 9900 series therapeutic studies with satisfactory end-induction MRD. The 5-year EFS values plus or minus standard error are shown for patients with varying levels of MRD. The outcome of those with high levels of MRD is very poor, but even those with 0.01% to 0.1% MRD have only a 59% ± 5% 5-year EFS. (From Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood* 2008;111:5477–5485.)

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cranial radiation have decreased from the 24- to 36-Gy range that was used on early protocols to 18 Gy for treatment and 12 Gy on some CNS preventive therapy regimens.[56]

Therapy for relapsed ALL depends on the location of relapse (isolated extramedullary, bone marrow, or combined) and the duration of initial remission. Extramedullary relapse generally has a better prognosis than bone marrow relapse, but requires localized in addition to systemic chemotherapy.[12] For testicular relapse, local therapy involves orchiectomy, followed by irradiation of the remaining testicle and scrotal area.[12,95] Fertility will be compromised (so postpubertal males should be offered sperm banking), and hormonal supplements should be offered as needed. Prognosis after a testicular relapse remains quite good. For CNS relapse, local therapy involves intensified intrathecal therapy, varying regimens of high-dose systemic therapy, and the addition of cranial and/or craniospinal radiation.

All relapse types have a worse prognosis if they occur during therapy or within 6 months of completing therapy.[96] Interestingly, a recent study indicated that postrelapse survival does not differ according to the intensity of chemotherapy received prior to relapse.[97] Multiple medications and regimens are being tested for induction in relapsed and refractory leukemia. A common approach is to administer several intensive blocks of conventional chemotherapy agents, with or without a concomitant novel investigational agent.[98] Promising results have been recently obtained with clofarabine and clofarabine-containing regimens.[99–101] Blinatumomab, a CD19/CD3-bispecific T-cell engaging (BiTE) antibody, has shown promising response rates and prolonged survival in relapsed B-lineage ALL in adults, and is currently in a phase II international trial in pediatrics.[102,103] Promising recent results have been obtained using chimeric antigen receptor-modified T cells with specificity for CD19, termed CTL019 cells.[104] The most recent update on this trial reported that among 20 patients (including 16 pediatric) with treatment refractory CD19+ ALL, 14 patients (82%) achieved complete remission, with robust in vivo expansion of CTL019 cells, and 11 of 17 evaluable patients maintained an ongoing CR during median follow-up of 2.6 months.[105]

The decision between chemotherapy and HSCT for second complete remission has been controversial. HSCT is usually used for relapse within 6 months of the completion of initial treatment (CR1 <30 months), but the decision also depends on suitable donor availability, the difficulty (number and intensity of induction attempts needed to achieve CR2), and the overall health of the recipient.[106] Prior to HSCT, patients with relapsed ALL typically receive induction and consolidative chemotherapy, because the chances of success with HSCT are extremely low in the setting of continued active disease. Autologous BMT in ALL is no longer recommended.

Prognostic Factors and Management of Pediatric Acute Myeloid Leukemia

In contrast with ALL, there are fewer standard clinical or laboratory-based factors in AML that consistently relate to prognosis. Table 100.1 reviews prognostic factors in pediatric ALL and AML. Poor prognostic factors that predict lower remission rates and/or decreased event-free survival (EFS) include blast cytogenetics with monosomy 5 or 7, 5q deletion, FLT3/ITD, and secondary AML/MDS.[19,107–109] Adolescents and young adults have an increased risk of treatment-related mortality, but no significant difference in OS.[110] A swift response to induction chemotherapy (i.e., remission after one cycle of chemotherapy), favorable cytogenetics, and DS (with FAB M7) are predictive of better outcomes.[108] Favorable cytogenetics in AML include core binding factor alterations (the t[8;21] *AML1/ETO* fusion and inv[16]), and the t(15;17) *PML/RARα* fusion seen in acute promyelocytic leukemia (APL) (see Chapter 107). Two other recently identified favorable cytogenetic alterations (CCAAT) are mutations in *NPM1*[111] and CCAAT/enhancer-binding protein alpha (*CEBP*).[112] In addition to genetic alterations, MRD is being integrated into current AML studies as an important prognostic marker.[113] Although many findings in AML are similar between adults and children, important biologic and therapeutic differences are emerging. An example of this is that mutations of the *KIT* receptor tyrosine kinase have a similar frequency in pediatric core binding factor AML as those found in adult AML, but lack the poor prognosis seen in adults.[109]

There have been incremental improvements in AML outcomes over recent decades, with current overall survival in the 60% to 70% range (see Fig. 100.2). These improvements have come through increased intensity of therapy and improved supportive care.[114,115] The inherent drug resistance of AML cells poses challenges because the intensification of therapy required is associated with significant treatment-related toxicity, particularly infections.[19]

With the exception of DS patients with FAB M7 AML and patients with APL, most pediatric AML induction therapies include two cycles of ara-C and daunomycin, with or without thioguanine and/or etoposide. Studies have shown that the intensity of induction therapy is important for OS.[19] In the Children's Cancer Group 2891 protocol, intensively timed induction (starting the second cycle on day 14 regardless of count recovery) did not change the induction (CR1) rate, but it did have a profound effect on eventual cure rate.[116] Although fewer than 5% of pediatric AML patients present with CNS disease, as many as 20% will suffer an isolated CNS relapse.[55] Intrathecal chemotherapy (usually ara-C) has been found by several groups to effectively reduce the risk of CNS relapse.[19]

Conventional postinduction consolidative chemotherapy in pediatric AML usually involves two to three cycles of high-dose ara-C–based combinations, to which anthracycline, etoposide, or ifosfamide/cyclophosphamide may be added. There is no proven benefit to maintenance chemotherapy, so conventional treatment is rarely more than four to eight cycles.[19,117]

Gemtuzumab ozogamicin (GO), a newer agent consisting of an antibody to CD33 coupled to calicheamicin (a toxic antitumor antibiotic), has shown some promise in relapsed AML and was recently tested in a randomized, prospective trial for frontline disease by the Children's Oncology Group.[118] GO was significantly associated with improved EFS, but not OS. Other adult and pediatric trials of GO in AML have yielded mixed results, and at present, it has been voluntarily withdrawn from the commercial market and its role in AML therapy remains to be determined. Two promising novel biologic agents, the proteasome inhibitor bortezomib[119] and the kinase inhibitor sorafenib (which inhibits FLT3)[120] are currently being tested for efficacy in combination with frontline conventional chemotherapy. The indications for HSCT in pediatric AML, preparative regimens, timing (CR1 or later), and type of donor are the focus of intensive ongoing controversy and research.[116,121,122]

Pediatric APL has among the highest cure rates in pediatric AML. These patients should receive all-*trans*-retinoic acid (ATRA), which directly binds to the t(15;17) translocation, which forms a fusion protein combining the promyelocytic leukemia (*PML*) gene with the retinoic acid receptor alpha (*RARα*) gene, which is causative for this form of AML.[123] They should also receive conventional chemotherapy during both induction and consolidation phases. Patients who respond to this therapy have an 80% to 85% survival rate and should not be subjected to HSCT.[124] Similar to adult therapies for APL (see Chapter 107), arsenic trioxide, found to be useful for salvage in patients who became resistant to ATRA, is currently being tested in upfront pediatric APL regimens (both alone and in combination with ATRA and conventional chemotherapy) in an effort to improve DFS in CR1.[125] Rapid initiation of therapy is particularly important in APL due to the risk of severe bleeding complications in untreated disease. Early deaths from bleeding are often not captured on clinical trials, but population-based studies indicate that they are a significant cause of mortality.[126] Despite the remarkable successes with ATRA and arsenic trioxide with and without conventional chemotherapy, there remain patients with APL with relapsed and refractory disease, and many of these can still be cured with HSCT.[127]

DS patients with AMKL (see earlier discussion) do well with lower dose ara-C regimens and standard timing of their induction cycles. Current results in DS AMKL are the best of any AML subgroup outside of APL, with 70% to 85% EFS, with patients under 2 years showing the best results, and thus not requiring intensive chemotherapy or HSCT.[128–130]

The prognosis for pediatric patients who relapse after either conventional or HSCT therapy for AML is poor. A second CR can be obtained using similar ara-C and anthracycline-containing regimens in 20% to 70% of patients, but the likelihood of obtaining a cure is approximately half the rate for de novo AML.[131] Clofarabine alone and in combination (see ALL relapse discussion) with other chemotherapeutic agents also shows some efficacy.[100,101] HSCT in early relapse or CR2 for AML patients who were previously transplanted in CR1 is a strategy that has had some success.[131–133] Other novel therapies, including FLT3 inhibitors and hypomethylating agents, are currently being studied.[134]

Rarer Forms of Leukemia in Children

Chronic leukemias, with the exception of CML, do not occur in children. Ph+ CML is rare in childhood (<1% to 2% of all pediatric leukemia cases).[135] When it does occur, it typically presents in adolescents and is in the chronic phase. Therapy is similar to that recommended in adults, with imatinib (along with hydroxyurea if the initial counts are high, and/or the patient presents with a high degree of hepatosplenomegaly) as the mainstay of induction and maintenance therapy. The use of alternative tyrosine kinase inhibitors such as dasatinib and nilotinib are being investigated in children as well as adults.[136] Because there is no clear end point for when or whether any of these tyrosine kinase inhibitors can be safely discontinued, many pediatric oncologists continue to consider hematopoietic stem cell transplantation in remission.

JMML, a myeloproliferative disorder unique to childhood, is characterized by extreme monocytosis, hepatosplenomegaly, thrombocytopenia, and increased fetal hemoglobin.[137] Bone marrow morphology is often consistent with myelodysplasia as well as myeloproliferation, and cytogenetics frequently reveals a monosomy 7 clone. Mutations in *PTN11*, *CBL*, and other *RAS* pathway genes have also been noted.[138,139] Although the clinical course can be indolent (requiring only intermittent blood product and antibiotic support), JMML patients often progress to frank marrow failure. Neither aggressive AML-type chemotherapy nor splenectomy has been shown to significantly prolong survival, so these approaches are starting to be reserved for those patients who are symptomatic. The most definitive therapy in JMML is an allogeneic BMT.[140] Overall DFS with BMT in JMML has been in the 40% to 55% range, with relapse the major reason for failure. Novel therapies targeting the rat sarcoma (*RAS*), rapidly accelerated fibrosarcoma (*RAF*), mitogen activated protein kinase (*MEK*), mammalian target of rapamycin (mTOR), signal transducer and activator of transcription 5 (*STAT5*), and other signaling pathways are currently under investigation.[137]

Mixed phenotype acute leukemia (MPAL) is a new designation established by the World Health Organization (WHO) 2008 classification, replacing the prior diagnosis of biphenotypic acute leukemia, which was based on the European Group for the Immunological Classification of Leukemias (EGIL) and the WHO 2001 classification.[141] MPALs constitute only approximately 0.5% to 1% of acute leukemias, with combined lineage differentiation that is most often B- and myeloid, followed by T- and myeloid, and rarely B- and T- or trilineage. The WHO classification recognizes two specific subgroups, characterized by a *BCR-ABL* rearrangement and *MLL* rearrangement, as well as a subgroup for the remainder of cases, which have other nonspecific chromosomal abnormalities. There are little systematic data about this rare form of leukemia, but outcomes are generally poor. Some data suggest that ALL-directed therapy may be more effective than AML-directed therapy. Allogeneic stem cell transplant is frequently employed.

LYMPHOMAS

Lymphomas constitute approximately 10% of cancer in children, and are the third most common pediatric malignancy (behind leukemias and brain tumors).[142] Two-thirds of lymphomas in children are a heterogeneous group of lymphomas categorized as non-Hodgkin lymphomas (NHL), and the remainder are Hodgkin lymphomas (HL). Table 100.5 compares the differences in presenting and staging features between pediatric NHL and HL. The histology, biology, and management of lymphoma differs in children compared to adults. For example, the indolent, low-grade lymphomas seen in adults are rarely seen in children. Additionally, radiation and certain types of chemotherapy are minimized when possible to reduce detrimental effects on growth and development and other late effects of therapy such as second malignancies.

Non-Hodgkin Lymphoma

The Revised European–American Classification of Lymphoid Neoplasms (REAL) classification, which has served as the basis for the WHO classification of hematopoietic and lymphoid tumors, categorizes lymphomas according to phenotype and differentiation.[143]

TABLE 100.5

Comparison of Hodgkin Lymphoma and Non-Hodgkin Lymphoma in Pediatric Patients

Feature	Hodgkin Lymphoma	Non-Hodgkin Lymphoma
Age	Mostly >10 y	Any age in children
Stage at diagnosis	Mostly localized	Commonly widespread
Constitutional symptoms	Alter prognosis	Do not affect prognosis
CNS involvement	Rare	Occurrence increases with AIDS
Mediastinal involvement	Most common with nodular sclerosing Hodgkin lymphoma	Most common with lymphoblastic lymphoma
Gastrointestinal involvement	Rare	Occurs
Abdominal nodal involvement	Can be small or large, mesenteric rare	Usually enlarged, mesenteric common
Bone involvement	Rare	Occurs
Marrow involvement	Rare	Common

Adapted from Rademaker, J. Hodgkin's and non-Hodgkin's lymphomas. *Radiol Clin North Am* 2007;45:69–83.

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Pediatric NHLs appear in four major categories: (1) precursor T- and, less commonly, precursor B-lymphoblastic lymphoma (30% of pediatric NHLs), (2) Burkitt and Burkitt-like lymphoma (40% to 50%), (3) diffuse large B-cell lymphoma (15%), and (4) anaplastic large cell lymphoma (10%).[142] These subtypes are high-grade, acute diseases and, rarely, truly localized diseases. Major improvement in the survival of children with NHL has occurred during the past 20 years, correlating with the advent of systemic multiagent chemotherapy regimens, as opposed to a reliance on localized therapies such as surgery and radiation.

The Cotswold revision of the Ann Arbor NHL staging and classification system used in adult NHLs has mostly been replaced in pediatrics by the St. Jude's Research Hospital staging system outlined in Table 100.6, which incorporates common presentations of pediatric NHL including increased extranodal involvement, bone marrow and CNS involvement, and on contiguous spread of disease.[144] Surgical/pathologic staging is no longer carried out in either pediatric NHL or HL cases. Diagnosis generally relies on biopsy and radiologic scans, usually a combination of computed tomography (CT) and nuclear medicine scans and, occasionally, magnetic resonance imaging.[145] The current 5-year EFS rates for early low-stage disease are in the 90% to 95% range and from 70% to 90% for the higher stage presentations.[4,142]

Unlike ALL or HL, there is no sharp age peak for the occurrence of NHL in children. There is a marked imbalance in the male to female incidence, which approaches a 3:1 ratio.[142,146] With the exception of children with rare, inherited, or acquired immunodeficiency syndromes (e.g., Wiskott-Aldrich syndrome, common variable immune deficiency, ataxia–telangiectasia, X-linked lymphoproliferative syndrome, HIV/AIDS, or exposure to immunosuppressive drugs after solid organ or bone marrow transplants), most children who develop NHL have a history of normal health and no known risk factors. The main exception to this concerns the possible etiologic role of the Epstein-Barr virus (EBV). EBV is strongly associated with lymphomas in HIV patients, lymphoproliferative diseases found in posttransplant patients, and endemic Burkitt lymphoma, and it is found in many cases of HL (see later discussion) in otherwise healthy children.[147,148]

Lymphoblastic lymphomas share many molecular, biologic, cytogenetic, and therapeutic characteristics with ALL, and are now treated with similar chemotherapy protocols.[12,146] The distinction between ALL and lymphoblastic lymphoma is somewhat arbitrary because those patients with more than 25% lymphoblasts in their bone marrow at diagnosis (despite the existence of a large lymphomatous mass elsewhere in the body) are designated as having ALL. Morphologically, the cells are indistinguishable, and the immunophenotypes generally overlap.[146,149] In contrast to ALL however, precursor T-lymphoblastic lymphoma is more common than precursor B-lymphoblastic lymphoma, with more than 75% of lymphoblastic lymphoma cases demonstrating precursor T-cell immunophenotype.

The typical presentation of children with lymphoblastic lymphoma is that of a patient with rapidly enlarging neck and mediastinal lymphadenopathy. Particular attention needs to be paid to hydration status, kidney function, and whether the kidneys are directly involved with the disease. CT and positron-emission tomography (PET) are helpful for assessing the degree of organ involvement, but care must be taken in requiring children with mediastinal masses to lie supine (which can compress both central blood vessels and airways) or undergo sedation (which causes vasodilation and decreased blood return to the heart).[150] A histologic diagnosis should be sought in the least invasive way possible. Prebiopsy steroids or *postage stamp irradiation* (use of a small radiation field to emergently relieve airway compression) can be done, but the steroids may jeopardize obtaining the histologic diagnosis or accurate staging. CNS status should be assessed prior to systemic chemotherapy, and prophylactic intrathecal chemotherapy should be administered early.[146] Prognostic factors include the level of bone marrow involvement at diagnosis and CNS involvement at diagnosis.

Primary therapy for lymphoblastic lymphoma (of either B- or T-cell histology) consists of multiagent chemotherapy without radiation. Stage I lymphoblastic cases do very well with short treatments (three to five cycles) of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP regimen or similar), and a relatively short (24 weeks versus 2 to 3 years with ALL) maintenance phase of antimetabolite (6-mercaptopurine daily and weekly oral methotrexate).[142,146] Higher stage (stages II, III, and IV) lymphoblastic cases require more intensive regimens and a prolonged maintenance phase such as that used for ALL. The benefit of high-dose methotrexate and cranial radiation remain controversial in lymphoblastic lymphoma.[151–153] Nelarabine is a novel nucleoside analog with preferential cytotoxicity in T-lineage lymphoid malignancies that is being studied in current protocols.[154] Because activating NOTCH1 mutations are found in the majority of T-cell

TABLE 100.6

St. Jude Children's Research Hospital Staging System for Pediatric Non-Hodgkin Lymphoma

Stage	Description
I	A single tumor (extranodal) or single anatomic area (nodal), with the exclusion of mediastinum or abdomen
II	A single tumor (extranodal) with regional node involvement Two or more nodal areas on the same side of the diaphragm Two single (extranodal) tumors with or without regional node involvement on the same side of the diaphragm A primary gastrointestinal tract tumor, usually in the ileocecal area, with or without involvement of associated mesenteric nodes only[a]
III	Two single tumors (extranodal) on opposite sides of the diaphragm Two or more nodal areas above and below the diaphragm All the primary intrathoracic tumors (mediastinal, pleural, thymic) All extensive primary intra-abdominal disease[a] All paraspinal or epidural tumors, regardless of other tumor sites
IV	Any of the previous with initial central nervous system or bone marrow involvement[b]

[a] Stage II abdominal disease typically is limited to a segment (usually distal ileum) of the gut plus or minus the associated mesenteric nodes only, and the primary tumor can be completely removed grossly by segmental excision. Stage III abdominal disease typically exhibits spread to para-aortic and retroperitoneal areas by implants and plaques in mesentery or peritoneum, or by direct infiltration of structures adjacent to the primary tumor. Ascites may be present, and the complete resection of all gross tumor is not possible.

[b] If bone marrow involvement is present at diagnosis, the percentage of blasts or abnormal cells must be 25% or less to be classified as stage IV non-Hodgkin lymphoma. If there are more than 25% blasts, the patient is classified as having acute leukemia (either precursor B- or T-acute lymphoblastic leukemia or L3 acute lymphoblastic leukemia).

leukemias, gamma-secretase inhibitors that block Notch 1 signaling are another class of agents whose role in treatment regimens is being assessed.[155,156] Relapse in lymphoblastic lymphoma is, fortunately, an uncommon problem, but when it does occur it happens either during or shortly after completion of therapy. Relapse of low-stage disease can frequently be salvaged using the more intensive chemotherapy designed for high-stage disease.[157] Lymphoblastic lymphoma patients who relapse after higher stage treatment have a poor prognosis and are candidates for nelarabine, phase I agents and/or BMT if response is obtained to salvage therapy.[157] Radiation to areas of bulk disease and total-body irradiation are incorporated into the transplant regimens, but, in general, radiation does not have a role in primary treatment or reinduction at relapse.

Burkitt and Burkitt-like lymphoma are mature B-cell lymphomas. Endemic Burkitt lymphoma (usually presenting with localized head and neck masses—most frequently, the jaw) in Africa is quite common (100 cases per million children), and 95% are associated with EBV. Sporadic Burkitt lymphoma, as seen in the United States, typically presents with an abdominal mass, occurs in 1 to 2 cases per million children, and only 15% are associated with EBV.[158] The most common presentation in the United States is of a boy age 5 to 10 years, with a right lower quadrant mass and/or acute abdomen secondary to an ileocecal intussusception. If the tumor is limited to the distal ileum or cecum, it should be completely excised along with its associated mesentery, and the bowel repaired with an end-to-end anastomosis.[159] These children have low-stage disease, require less chemotherapy, and have an excellent outcome. More frequently, the abdominal involvement is much more diffuse.

Burkitt lymphoma cells have a mature B-cell immunophenotype (expressing cell surface immunoglobulin, usually IgM) and are characterized morphologically by homogeneous round-to-oval nuclei, multiple nucleoli, and intensely basophilic cytoplasm with large vacuolated areas containing fat. Burkitt-like lymphoma shares pathologic and molecular features with diffuse large B-cell lymphoma and responds to similar chemotherapy as Burkitt lymphoma. The majority of Burkitt lymphoma cases display the t(8;14)(q24;q32) translocation in which *c-myc* from chromosome 8 is translocated to the Ig heavy-chain locus on chromosome 14. In this translocation and in two less common variants, t(2;8)(p12;q24) and t(8;22)(q24;q11), the *c-myc* oncogene is overexpressed because of the influence of Ig regulatory regions (enhancers).[160]

The diagnosis of Burkitt lymphoma must be done very expeditiously as these tumors grow swiftly and patients are at high risk of intestinal obstruction (from intussusception) and metabolic problems related to tumor lysis syndrome. Tumor lysis often begins even before chemotherapy. Special attention must be paid to serum electrolyte balance (including calcium/phosphate balance), and vigorous intravenous hydration and alkalinization to improve uric acid excretion is essential. Allopurinol or recombinant urate oxidase, rasburicase, is used to block uric acid production. Occasionally, the lysis syndrome can be severe enough to cause acute renal failure, and dialysis must be used to maintain fluid and reestablish electrolyte balance.

Children with low-stage, African-endemic Burkitt lymphoma have been successfully treated with single or multiple doses of cyclophosphamide. Therapies as brief as 4 weeks have been successfully employed.[161] These strategies result in lower cure rates, but they make it feasible to treat large numbers of children with lower toxic death and complication rates in resource-poor settings.[162] Higher stage Burkitt lymphoma (stages 3 and 4) requires significantly more intensive chemotherapy, involving much higher doses of cyclophosphamide, and the addition of an anthracycline, high-dose ara-C, methotrexate, and VP-16 to the CHOP schemas. Many current high-stage Burkitt lymphoma protocols use hematopoietic growth factor support to enhance bone marrow recovery in order to allow intensive cycles to be given approximately every 3 weeks. These protocols are intensive, but they are usually of a short (6- to 8-month) duration. There have been some efforts to reduce chemotherapy and to add biologic agents like rituximab (anti-CD20 antibody) to reduce toxicity of treatment in patients who have HIV or other medical problems.[163,164] Currently, the efficacy of the addition of rituximab to advanced stage Burkitt lymphoma protocols is being investigated.[165] The cure rate for pediatric patients with higher stage (stage III and IV) Burkitt lymphoma is now 80% to 90%.[166] The management of relapsed Burkitt lymphoma is problematic. Relapses usually occur during therapy or shortly after the cessation of therapy. Low-stage patients can sometimes be effectively treated using a high-stage primary protocol. Higher stage relapsed patients may try a salvage therapy such as R-ICE (rituximab, ifosfamide, carboplatin, etoposide) or phase I therapies, and BMT should be considered if a response is achieved.[167]

The workup and staging of large cell lymphomas are similar to that described for other forms of NHL. Diffuse large B cell lymphoma (DLBCL) tends to present with large mediastinal masses, but bone, lymph node, and abdominal presentations are also seen. Bone marrow and CNS involvement are not common. Combination chemotherapy is effective against DLBCL, and radiation does not have a role in therapy for most cases. DLBCL and anaplastic large cell lymphoma (ALCL) used to be treated similarly as large cell lymphomas, the mature B-cell lymphomas (DLBCL and Burkitt lymphoma) are now grouped together on contemporary treatment protocols. However, it is not clear that DLBCL requires as much CNS-directed therapy as Burkitt lymphoma because CNS involvement is less common for DLBCL. Primary mediastinal B-cell lymphoma (PMBL) is a subtype of DLBCL that often presents with sclerosis and has a less favorable prognosis. PMBL shares some biologic features with Hodgkin lymphoma, and alternate treatment regimens are being investigated for PMBL based on differences in biology and prognosis.[168]

ALCL tends to present with involvement of lymph nodes and extranodal sites including the skin, the lung, other soft tissues, and bone. Bone marrow and CNS involvement are uncommon. The majority of ALCL cases have T-cell immunophenotype. ALCL is also known for cytogenetically presenting with the t(2;5)(p23;q35) translocation, which fuses nucleophosmin with a transmembrane tyrosine-specific protein kinase known as anaplastic lymphoma kinase. Three to five cycles of CHOP or APO (adriamycin, prednisone, Oncovin/VCR) have been used, and more recently, Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) ALCL-99 with EFS that ranges from 70% to 85%.[169] Targeted therapy with crizotinib, an inhibitor of the anaplastic lymphoma kinase (ALK), a tyrosine kinase, has produced promising responses in early studies in relapsed ALK-positive ALCL.[170] Another targeted therapy demonstrating promising activity against both ALCL and Hodgkin lymphoma is brentuximab vedotin, an anti-CD30 directed monoclonal antibody conjugated to a microtubule-disrupting agent.[171]

Hodgkin Lymphoma

The natural history and outcome of treatment in HL is similar in young children and adults, but treatment decisions (and regimens) are different in children based on the need for attention to late effects of therapy. Risk-adapted therapy seeks to maintain excellent cure rates of 80% to 90%, whereas limiting radiation and certain types of chemotherapy that may cause long-term organ damage, growth problems, infertility, and second malignancies.[3,172] HL is rare below the age of 5 years, with the majority of pediatric cases presenting in children older than 11 years. Young patients (<10 years) have a 3:1 male to female ratio. This imbalance returns to the approximately equal male to female ratio seen in adult disease as the age of presentation climbs. The age of HL incidence in industrialized countries is bimodal, with the first peak in adults 20 to 30 years of age and the second peak occurring much later in adulthood.[173]

The etiology of HL is unknown, but EBV is found in up to 40% of cases.[174] There are also familial and geographic clusters that suggest an inherited, environmental, or infectious contribution to etiology.[174] Most children (90%) present with painless adenopathy in the neck, and 60% have involvement of anterior mediastinum,

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paratracheal, or hilar lymph nodes. The Cotswold modification of the Ann Arbor Staging System (described in Chapter 103) is used for all ages. B symptoms (defined as in adults) include unexplained fever (more than 38°C [100.4°F]), drenching night sweats, and more than 10% weight loss. B symptoms are present in approximately 30% of newly diagnosed pediatric HL cases.[175] The process of clinical staging includes both site of involvement and the presence of B symptoms and is described in detail for adult patients in Chapter 102. There is no longer any role for staging laparotomy, because localized radiation is no longer used as a sole treatment modality in pediatric HL. Increasingly, PET scans are being used to assess staging and early response and to guide further treatment, as opposed to exclusive reliance on CT.[176]

The malignant cells in HL constitute a minority (estimated at 0.1% to 10%) of the cell population of the discernible tumor.[177] Inflammatory cells (histiocytes, plasma cells, lymphocytes, eosinophils, and neutrophils) make up the bulk of the tumor. The malignant cells are actually malignant lymphocytes with specific, characterized immunophenotypes. The WHO classifies four subtypes of classic HL: (1) nodular sclerosing, (2) mixed cellularity, (3) lymphocyte rich, and (4) lymphocyte depleted. Nodular lymphocyte-predominant HL (NLPHL) is another important subtype of HL.

The most common presenting histology in pediatric HL is nodular sclerosing type, which accounts for more than 50% of the cases (40% of the younger patients but 70% of the adolescents).[173] It is characterized by bands of fibrosis and a thickened lymph node capsule that are discernible even in a gross pathologic section. These nodes and masses tend to form scars that can lead to residual masses, which appear as opacified lesions on radiologic studies for years after a full clinical response. Mixed cellularity is responsible for approximately 30% of pediatric cases. It is commonly seen in children less than 10 years of age and is highly associated with EBV positivity. The lymphocyte-depleted form of HL is quite rare, except in children with HIV. The lymphocyte-rich variant is quite rare in children (approximately 5% of the cases), has a high incidence of mediastinal masses and stage III disease, but has an older average age of presentation (32 years).[173]

With the exception of NLPHL, treatment for all of the various HL histologies is the same and is based on staging. Risk-adapted therapy in pediatric HL is composed of three to six cycles of combination chemotherapy followed by involved field radiation therapy (IFRT) for higher risk patients. The composition of the chemotherapy regimens has varied during the years, but combinations of vinca alkaloids, alkylating agents (cyclophosphamide commonly used now), steroids, anthracyclines, and bleomycin have been used. Current therapies for low-risk HL attempt to reduce exposure to alkylators, anthracyclines, and bleomycin and IFRT to reduce the risk of late effects.

Therapy for higher risk HL (higher stage at presentation or slow response to therapy) currently involves additional cycles of higher dose combination chemotherapy and IFRT, with EFS rates still in the 80% to 90% range when risk-adapted therapy is given.[3] Targeted therapy such as brentuximab vedotin, discussed previously, will be studied in frontline therapy regimens. Treatment for relapsed or refractory pediatric HL is beyond the scope of this chapter, but the medications (e.g., ifosfamide and vinorelbine) and approaches (e.g., BMT) are usually similar to those used in adults (see Chapter 102). Novel therapies under investigation include antibodies targeting CD30 (brentuximab vedotin), CD25, and other antigens; the proteasome inhibitor bortezomib; and histone deacetylase inhibitors; as well as cytotoxic T-cell based therapies directed against both EBV-associated and EBV-negative lymphomas.[3]

NLPHL disease affects 10% to 15% of pediatric patients, is more common among male and younger patients, and is usually clinically localized (low stage). Stage I patients do well with surgical resection only (no chemotherapy or radiotherapy) and close follow-up.[178] For stage II/III, most would be treated with several cycles of relatively low-dose chemotherapy (i.e., avoiding the use of alkylators, topoisomerase inhibitors) and radiation therapy. Preliminary evidence suggests that this approach for NLPHL leads to an overall survival close to 100%, and should decrease the late effects of secondary malignancy and infertility.[179] Many current study protocols would forgo even low-dose IFRT for these young patients who achieve a complete response with low-dose chemotherapy, but this should still be considered an experimental approach because combination low-dose chemotherapy and IFRT has been the standard of care, and there are reports of relapse after chemotherapy-only treatments.

SUPPORTIVE CARE

There is no question that a large part of the success of modern leukemia and lymphoma treatment is related to the major improvements in supportive care that coincided with improvements in chemotherapy. These advances have included improved blood products, antibiotics, antifungals, and better intensive care unit support of critically ill pediatric patients. Erythropoietin has been used sparingly in pediatric oncology, and is not generally incorporated in frontline therapies for any of the lymphoid cancers. Leukocyte growth factors (granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and PEGylated forms, such as pegfilgrastim) are used sparingly in pediatric leukemia and lymphoma therapies for multiple reasons, including (1) concerns (mostly not substantiated in the literature) that these factors may stimulate the growth of these diseases, (2) multiple studies that show no changes in overall outcome when these factors are used, (3) financial burden, and (4) the addition of needle sticks and/or the increased infection risk from entering central lines daily. Growth factors are, however, used routinely in many relapse and higher stage lymphoma protocols to enable a dose-intensive chemotherapy schedule and avoid long periods of profound neutropenia.

LONG-TERM, PALLIATIVE, AND HOSPICE CARE IN PEDIATRIC ONCOLOGY

The survivors of current lower risk (less intensively treated) forms of ALL, NHL, and HL in childhood can now expect prolonged DFS, intact fertility, lesser cognitive and social disruption, and an easier integration into standard medical and social environments than in the past. Survivors from higher doses and larger radiation fields used in the 1960s and 1970s, as well as from regimens involving higher cumulative doses of chemotherapy, continue to have an increased risk of important long-term toxicities, including endocrine, growth, fertility, and learning disabilities, along with cardiac, renal, liver, and other end-organ toxicities.[180] Second malignancies are another serious problem, arising from carcinogenic exposures to chemotherapy and radiotherapy, as well as possibly an innate propensity to develop cancer.[181] It has been estimated that pediatric cancer survivors will soon represent as many as 1 in 250 adult Americans in the 15- to 45-year-old population.[182] Reducing late effects of therapy while preserving cure rates is a crucial goal in improving outcomes in childhood leukemia and lymphoma.[183]

Even with cure rates that reach, in many categories of disease, into the over 90% region, a substantial number of children still die of leukemia and lymphoma. A small number die after failing their first regimens, but many succumb after alternately succeeding and then failing several attempts at cure. A discussion of hospice and palliative care for these children and support for their families is beyond the scope of this chapter, but this is an area of active interest and involvement in most pediatric oncology programs. Hospice and palliative care treatments in pediatrics share some of the same concerns, goals, and methods as programs for adults. However, the unique requirements of psychosocial support in children, and the impact of a possible death of a child on a family, lead most experts to guide these patients to pediatric centers.

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101 Molecular Biology of Lymphomas

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INTRODUCTION

The term lymphoma identifies a heterogeneous group of biologically and clinically distinct neoplasms that originate from cells in the lymphoid organs and have been historically divided into two distinct categories: non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL).[1] During the past few decades, significant progress has been made in elucidating the molecular pathogenesis of lymphoid malignancies as a clonal expansion of B cells (in the majority of cases) or T cells. The molecular characterization of the most frequent genetic abnormalities associated with lymphoma has led to the identification of multiple proto-oncogenes and tumor suppressor genes, whose abnormal functioning contributes to lymphoma pathogenesis. Relatively less is known about the pathogenesis of T-cell NHL (T-NHL) and HL. This chapter will focus on the molecular pathogenesis of the most common and well-characterized types of lymphoma, including B-cell NHL (B-NHL), T-NHL, HL, and chronic lymphocytic leukemia (CLL), which also derives from mature B cells. Emphasis will be given to the mechanisms of genetic lesion and the nature of the involved genes in relationship to the normal biology of lymphocytes.

THE CELL OF ORIGIN OF LYMPHOMA

The number of B and T cells in the adult are not significantly different; however, 85% of lymphomas originate from mature B cells, whereas only 10% to 15% derive from the T-cell lineage. This bias may be explained in part by the unique DNA modification events that take place in normal B lymphocytes in order to enable the production of highly efficient neutralizing antibodies and that are mechanistically more complex than those utilized by T cells to encode T-cell receptors. The biology of these processes thus represents a key concept for the understanding of lymphomagenesis.

B-Cell Development and the Dynamics of the Germinal Center Reaction

B lymphocytes are generated from a common pluripotent stem cell in the bone marrow, where precursor B cells first assemble their immunoglobulin heavy chain locus (*IGH*) followed by the light chain loci (*IGL*) through a site-specific process of cleavage and rejoining, known as V(D)J recombination.[2] Cells that fail to express a functional (and nonautoreactive) antigen receptor are eliminated within the bone marrow, whereas B-cell precursors that have successfully rearranged their antibody genes are positively selected to migrate into peripheral lymphoid organs as mature, naïve B cells.[3] In most B cells, the subsequent maturation steps are linked to the histologic structure of the germinal center (GC), a specialized microenvironment that forms following the encounter of naïve B cells with a foreign antigen, in the context of signals delivered by CD4+ T cells and antigen-presenting cells (Fig. 101.1).[3–5]

GCs are highly dynamic structures in which B cells transit back and forth between two zones that are conserved across several species: the dark zone (DZ), which consists of rapidly proliferating centroblasts (CB) (doubling time: 6 to 12 hours), and the light zone (LZ), which consists of more quiescent cells termed centrocytes (CC), amidst a network of resident accessory cells (follicular dendritic cells [FDC] and human T follicular helper [Tfh] cells).[6–9] According to currently accepted models, the DZ is the site where GC B cells modify the variable region of their *IG* genes (IgV) by the process of somatic hypermutation (SHM), which introduces mostly single nucleotide substitutions with few deletions and duplications in order to change their affinity for the antigen.[3,5,10–12] Conversely, the LZ is the site of selection based on affinity to the antigen. A critical regulator of the GC reaction is BCL6,[13,14] a transcriptional repressor[15] that negatively modulates the expression of a broad set of genes, including those involved in B-cell receptor (BCR) and CD40 signaling,[16,17] T-cell mediated B-cell activation,[16] induction of apoptosis,[16,18] response to DNA damage (by modulation of genes involved in the sensing and execution of DNA damage responses),[19–22] various cytokine and chemokine signaling pathways (e.g., those triggered by interferon and transforming growth factor beta [TGFβ]),[16,18] and plasma cell differentiation, via suppression of the PRDM1/BLIMP1 master regulator.[23–26] This transcriptional program suggests that BCL6 is critical to establish the proliferative status of CBs and to allow the execution of antigen-specific DNA modification processes (SHM and class-switch recombination) without eliciting responses to DNA damage; furthermore, BCL6 keeps in check a variety of signaling pathways that could lead to premature activation and differentiation prior to the selection for the survival of cells producing high affinity antibodies.

CBs are then believed to cease proliferation and shuttle to the LZ, where they are rechallenged by the antigen through the interaction with CD4+ T cells and FDCs.[3,4,7,8] CCs expressing a BCR with reduced affinity for the antigen will be eliminated by apoptosis, whereas a few cells with greater affinity will be selected for survival and differentiation into memory cells and plasma cells,[4] or reenter the DZ following stimulation by a variety of different signals.[9] Iterative rounds of mutations and selections lead to affinity maturation at the population level. In the GC, CCs also undergo class-switch recombination (CSR), a DNA remodeling event that confers distinct effector functions to antibodies with identical specificities.[27] SHM and CSR represent B-cell–specific functions that modify the genome of B cells via mechanisms involving single- or double-strand breaks and which depend on the activity of the activation-induced cytidine deaminase (AID) enzyme,[28–30] a notion that will become important in the understanding of the mechanisms generating genetic alterations in B-NHL.

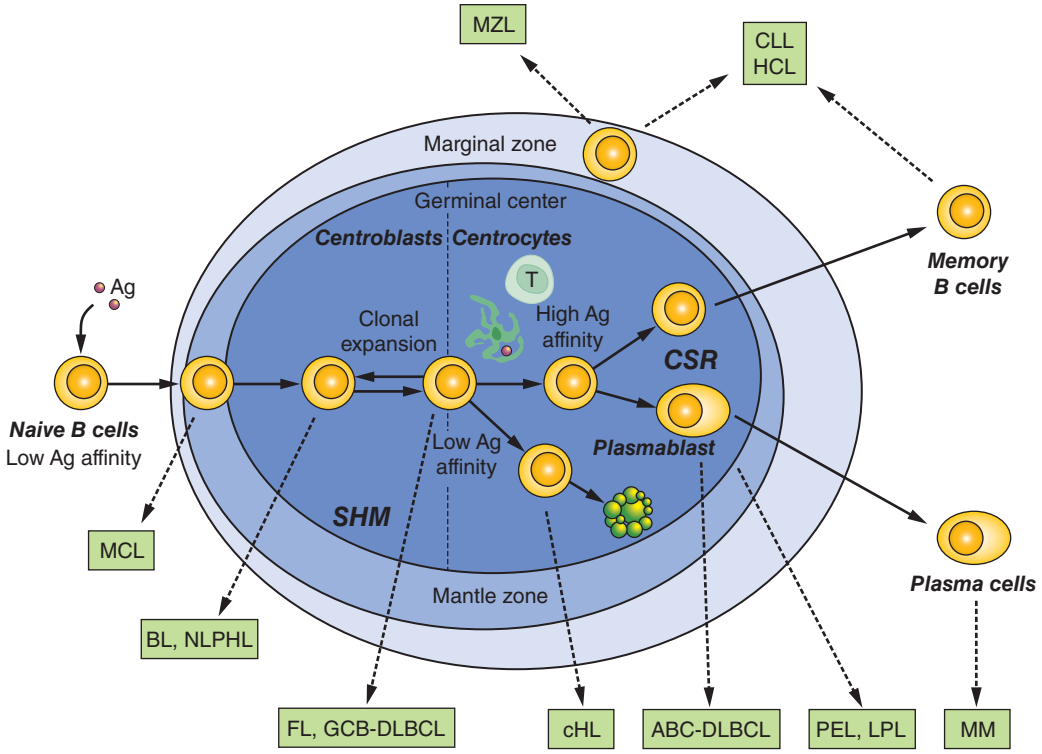

Figure 101.1 Normal B-cell development and lymphomagenesis. Schematic representation of a lymphoid follicle, constituted by the germinal center (GC), the mantle zone, and the surrounding marginal zone. B cells that have successfully rearranged their *IG* genes in the bone marrow move to peripheral lymphoid organs as naïve B cells. Upon encounter with a T-cell dependent antigen, B cells become proliferating centroblasts in the GC and eventually transition into centrocytes, which shuttle back and forth between the dark and light zone while undergoing iterative rounds of SHM and selection. Only GC B cells with high affinity for the antigen will be positively selected to exit the GC and further differentiate into plasma cells or memory B cells, whereas low-affinity clones are eliminated by apoptosis. *Dotted arrows* link various lymphoma types to their putative normal counterpart, identified based on the presence of somatically mutated IgV genes, as well as on distinctive phenotypic features. CSR, class-switch recombination; SHM, somatic hypermutation; MCL, mantle-cell lymphoma; FL, follicular lymphoma; BL, Burkitt lymphoma; GCB-DLBCL, germinal center B cell-like diffuse large B-cell lymphoma; ABC-DLBCL, activated B cell-like diffuse large B-cell lymphoma; PEL, primary effusion lymphoma; LPL, lymphoplasmacytic lymphoma; NLPHL, nodular lymphocyte predominance Hodgkin lymphoma; cHL, classical Hodgkin lymphoma; MZL, marginal zone lymphoma; HCL, hairy cell leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma.

Once these processes are completed, two critical signals for licensing GC exit are represented by engagement of the BCR by the antigen and activation of the CD40 receptor by the CD40 ligand present on CD4+ T cells. These signals induce the downregulation of BCL6 at the translational and transcriptional level, respectively, thus restoring DNA damage responses, as well as activation and differentiation capabilities.

Although oversimplified, this schematic description of the GC reaction is useful to introduce two basic concepts for the understanding of B-NHL pathogenesis. First, the activity of SHM, which introduces irreversible DNA changes in the genome, allowed for the conclusion that most B-NHL types, with the exception of most mantle-cell lymphoma (MCL), derive from GC-experienced B cells that underwent clonal expansion within the GC, because the malignant clones harbor hypermutated IgV sequences containing largely identical mutations, suggesting the derivation from a single founder cell.[31] Second, two common mechanisms of oncogenic lesions in B-NHL—namely, chromosomal translocations and aberrant somatic hypermutation (ASHM)—result from mistakes in the machinery that normally diversifies the Ig genes during B lymphocytes differentiation, further supporting the GC origin of most B-NHL (Fig. 101.2).[32] Finally, the definition of two distinct phases during GC development reflects different transient states within the same B-cell developmental step, which can be recognized in different B-NHL subtypes to some extent.

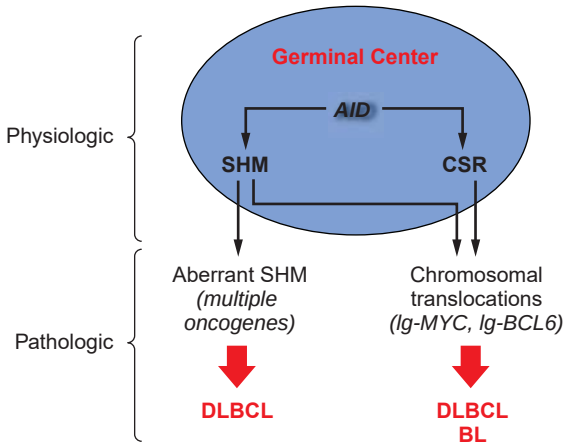

Figure 101.2 Model for the initiation of chromosomal translocations and ASHM during lymphomagenesis. B-NHL–associated genetic lesions are favored by mistakes occurring during the physiologic processes of SHM and CSR in the highly proliferative environment of the GC *(top)*. These events lead to chromosomal translocations, which in most cases juxtapose the *IG* genes to one of several proto-oncogenes (e.g., *BCL2* or *MYC*), and ASHM of multiple target genes, thus contributing to the pathogenesis of lymphoma. DLBCL, diffuse large B-cell lymphoma; BL, Burkitt lymphoma.

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T-Cell Development

The process of T-cell development proceeds through sequential stages defined according to the expression of the molecules CD4 and CD8. Committed lymphoid progenitors exit the bone marrow and migrate to the thymus as early T-cell progenitors or double negative 1 (DN1) cells, which lack the expression of both CD4 and CD8 and harbor unrearranged T-cell receptor (TCR) genes.[33] In the thymic cortex, T cells advance through the double negative stages DN2, DN3, and DN4, while undergoing specific rearrangements at the TCRβ locus in order to acquire expression of the pre-TCR.[33] Those thymocytes that have successfully recombined the pre-TCR will be selected to further differentiate into double positive cells (CD4+CD8+), which express a complete surface TCR and can then enter a process of positive and negative selection in the medulla, before exiting the thymus as single positive T cells.[33] The end result of this process is a pool of mature T cells that exhibit coordinated TCR and coreceptor specificities, as required for effective immune responses to foreign antigens. Most mature T-NHLs arise from postthymic T cells in the lymphoid organs.

GENERAL MECHANISMS OF GENETIC LESIONS IN LYMPHOMA

Analogous to other cancers, lymphoma represents a multistep process deriving from the accumulation of multiple genetic lesions affecting oncogenes and tumor suppressor genes, including chromosomal translocations, point mutations, genomic deletions, and copy number (CN) gains/amplifications.

Chromosomal Translocations

Although also found in nonlymphoid tumors, chromosomal translocations represent the genetic hallmark of malignancies derived from the hematopoietic system. These events are generated through the reciprocal and balanced recombination of two specific chromosomes and are often recurrently associated with a given tumor type, where they are clonally represented in each tumor case.

The precise molecular mechanisms underlying the generation of translocations remain partially unclear; however, significant advances have been made in our understanding of the events that are required for their initiation.[34] It has been documented that chromosomal translocations occur at least in part as a consequence of mistakes during *Ig* and TCR gene rearrangements in B and T cells, respectively, and, based on the characteristics of the chromosomal breakpoint, can be broadly divided into three groups: (1) translocations derived from mistakes of the recombination activating gene RAG-mediated V(D)J recombination process, as is the case for translocations involving *IGH* and *CCND1* in MCL or *IGH* and *BCL2* in follicular lymphoma (FL)[34–36]; (2) translocations mediated by errors in the AID dependent CSR process, such as those involving the *Ig* genes and *MYC* in sporadic Burkitt lymphoma (BL)[34]; (3) translocations occurring as by-products of the AID-mediated SHM mechanism, which also generates DNA breaks, such as those joining the *Ig* and *MYC* loci in endemic BL.[34] Conclusive experimental evidence for the involvement of antibody-associated remodeling events has been provided through in vivo studies performed in lymphoma-prone mouse models, where the removal of the AID enzyme was sufficient to abrogate the generation of *MYC-IGH* translocations in normal B cells undergoing CSR[37,38] and to prevent the development of GC-derived B-NHL.[39]

The common feature of all NHL-associated chromosomal translocations is the presence of a proto-oncogene in the proximity of the chromosomal recombination sites. In most lymphoma types, and in contrast with acute leukemias, the coding domain of the oncogene is not affected by the translocation, but its pattern of expression is altered as a consequence of the juxtaposition of heterologous regulatory sequences derived from the partner chromosome (proto-oncogene deregulation) (Fig. 101.3). This process of proto-oncogene deregulation is defined as homotopic if a proto-oncogene whose expression is tightly regulated in the normal tumor counterpart becomes constitutively expressed in the lymphoma cell, and heterotopic when the proto-oncogene is not expressed in the putative normal counterpart of the tumor cell and undergoes ectopic expression in the lymphoma. In most types of NHL-associated translocations, the heterologous regulatory sequences responsible for proto-oncogene deregulation are derived from antigen receptor loci, which are expressed at high levels in the target tissue.[34] However, in certain translocations, such as the ones involving *BCL6* in diffuse large B-cell lymphoma (DLBCL), different promoter regions from distinct chromosomal sites can be found juxtaposed to the proto-oncogene in individual tumor cases, a concept known as *promiscuous translocations*.[40–47]

Less commonly, B-NHL associated chromosomal translocations juxtapose the coding regions of the two involved genes to form a chimeric unit that encodes for a novel fusion protein, an outcome typically observed in chromosomal translocation

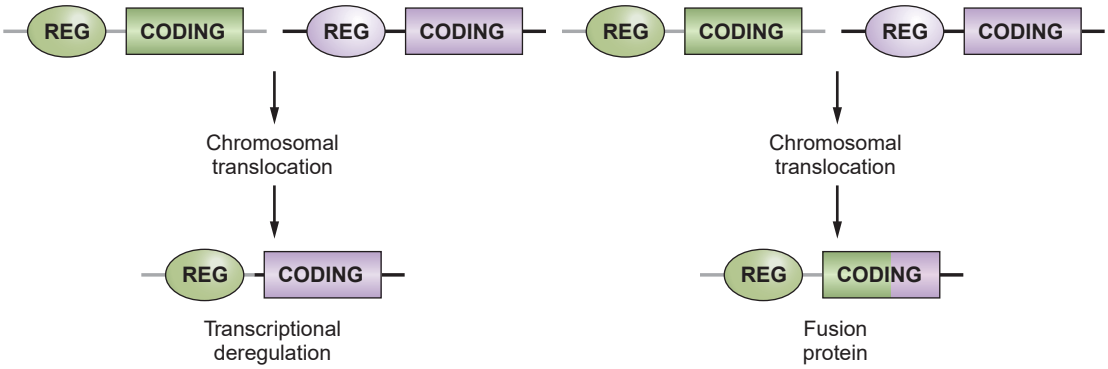

Figure 101.3 Molecular consequences of chromosomal translocations. *Top panel*: The two genes involved in prototypic chromosomal translocations are graphically represented, with their regulatory (REG) and coding sequences. Only one side of the balanced, reciprocal translocations is indicated in the figure. *Bottom panel*: Distinct outcomes of chromosomal translocations. In the case of transcriptional deregulation *(left scheme),* the normal regulatory sequences of the proto-oncogene are substituted with regulatory sequences derived from the partner chromosome, leading to deregulated expression of the proto-oncogene. In most B-NHLs, the heterologous regulatory regions derive from the *IG* loci. In the case of fusion proteins *(right scheme),* the coding sequences of the two involved genes are joined in frame into a chimeric transcriptional unit that encodes for a novel fusion protein, characterized by novel biochemical and functional properties.

associated with acute leukemia (see Fig. 101.3). Examples are the t(11;18) of mucosa-associated lymphoid tissue (MALT) lymphoma and the t(2;5) of anaplastic large-cell lymphoma (ALCL). The molecular cloning of the genetic loci involved in most recurrent translocations has led to the identification of a number of proto-oncogenes involved in lymphomagenesis.

Aberrant Somatic Hypermutation

The term aberrant somatic hypermutation defines a mechanism of genetic lesion that appears to derive from a malfunction in the physiologic SHM process, leading to the mutation of multiple non-Ig genes.[48] This phenomenon is uniquely associated with B-NHL and particularly with DLBCL, where over 10% of actively transcribed genes have been found mutated as a consequence of ASHM.

In GC B cells, SHM is tightly regulated both spatially and temporally to introduce mutations only in the rearranged *IgV* genes[49] as well as in the 5′ region of a few other genes, including *BCL6* and the *CD79* components of the B-cell receptor,[50–53] although the functional role of mutations found in these other genes remains obscure. On the contrary, multiple mutational events were found to affect numerous loci have been found mutated in over half of DLBCL cases and, at lower frequencies, in few other lymphoma types.[54–58] The identified target loci include several well known proto-oncogenes such as *PIM1*, *PAX5*, and *MYC*, one of the most frequently altered human oncogenes.[48] These mutations are typically distributed within ~2 kb from the transcription initiation site (i.e., the hypermutable domain in the Ig locus)[59] and, depending on the genomic configuration of the target gene, may affect nontranslated as well as coding regions, thus holding the potential of altering the response to factors that normally regulate their expression or changing key structural and functional properties.[48] This is the case of *MYC*, where a significant number of amino acid substitutions have proven functional consequences in activating its oncogenic potential. Nonetheless, a comprehensive characterization of the potentially extensive genetic damage caused by ASHM is still lacking, and the mechanism involved in this malfunction has not been elucidated.

Copy Number Gains and Amplifications

In addition to chromosomal translocations and ASHM, the structure of proto-oncogenes and their pattern of expression can be altered by CN gains and amplifications, leading to overexpression of an intact protein. Compared to epithelial cancer, only a few genes have been identified so far as specific targets of amplification in B-NHL, as exemplified by *REL* and *BCL2* in DLBCL[60–63] and by the genes encoding for programmed cell death 1 PD-1 ligands in primary mediastinal B-cell lymphoma (PMBCL).[64,65]

Activating Point Mutations

Somatic point mutations in the coding sequence of a target proto-oncogene may alter the biologic properties of its protein product, leading to its stabilization or constitutive activation. Over the past few years, the use of genomewide, high throughput sequencing technologies has allowed for the identification of numerous previously unsuspected targets of somatic mutations in cancer, including lymphoid malignancies. These genes will be discussed in individual disease sections. Of note, mutations of the *RAS* genes, a very frequent proto-oncogene alteration in human neoplasia, are rare in lymphomas.[66]

Inactivating Mutations and Deletions

Until recently, the *TP53* gene, possibly the most common target of genetic alteration in human cancer,[67] remained one of few bona fide tumor suppressor genes involved in the pathogenesis of NHL, although at generally low frequencies and restricted to specific disease subtypes, such as BL and DLBCL derived from the transformation of FL or CLL.[68,69] The mechanism of *TP53* inactivation in NHL is analogous to the one observed in human neoplasia in general, entailing a point mutation of one allele and a chromosomal deletion or mutation of the second allele. However, recent efforts taking advantage of genomewide technologies revealed several additional candidate tumor suppressor genes that are lost in B-NHL through specific chromosomal deletions and/or deleterious mutations. Two such genes lie on the long arm of chromosome 6 (6q), a region long known to be deleted in a large percentage of aggressive lymphomas associated with poor prognoses.[70,71] The *PRDM1/BLIMP1* gene on 6q21 is biallelically inactivated in ~25% of ABC-DLBCL cases,[72–74] and the gene encoding for the negative nuclear factor kappa B (NF-κB) regulator A20 on chromosome 6q23 is commonly lost in ABC-DLBCL, PMBCL, and subtypes of marginal zone lymphoma and HL.[75–78] Monoallelic inactivating mutations and deletions were found to affect the acetyltransferase genes *CREBBP* and *EP300* in a significant proportion of DLBCL and FL, suggesting a role as haploinsufficient tumor suppressors.[79] These two lymphoma types also harbor truncating mutations of *MLL2*,[80] which is emerging as one of the most commonly mutated genes in cancer. Among the tumor suppressors preferentially inactivated by CN losses, it is important to mention the *DLEU2/miR15-a/16.1* cluster on chromosome 13q14.3, the most frequent alteration in CLL (>50% of cases),[81] and *CDKN2A/B* (*p16/INK4a*), which is inactivated by focal homozygous deletions in transformed FL (tFL), Richter syndrome (RS), and ABC-DLBCL,[82–84] and less frequently, by epigenetic transcriptional silencing in various B-NHL.[85]

Infectious Agents

Viral and bacterial infections have both been implicated in the pathogenesis of lymphoma. At least three viruses are associated with specific NHL subtypes: the Epstein-Barr virus (EBV), the human herpesvirus-8/Kaposi sarcoma–associated herpesvirus (HHV-8/KSHV), and the human T-lymphotropic virus type 1 (HTLV-1). Other infectious agents, including HIV, hepatitis C virus (HCV), *Helicobacter pylori*, and *Chlamydophila psittaci* have an indirect role in NHL pathogenesis by either impairing the immune system and/or providing chronic antigenic stimulation.

EBV was initially identified in cases of endemic African BL,[86,87] and was subsequently also detected in a fraction of sporadic BL, HIV-related lymphomas, and primary effusion lymphomas (PEL).[88–92] Upon infection, the EBV genome is transported into the nucleus of the B lymphocyte, where it exists predominantly as an extrachromosomal circular molecule (episome).[93] The formation of circular episomes is mediated by the cohesive terminal repeats, which are represented by a variable number of tandem repeats (VNTR) sequence.[93,94] Because of this termini heterogeneity, the number of VNTR sequences enclosed in newly formed episomes may differ considerably, thus representing a clonal marker of a single infected cell.[94] Evidence for a pathogenetic role of the virus in NHL infected by EBV is at least twofold. First, it is well recognized that EBV is able to significantly alter the growth of B cells.[93] Secondly, EBV-infected lymphomas usually display a single form of fused EBV termini, suggesting that the lymphoma cell population represents the clonally expanded progeny of a single infected cell.[88,89] Nonetheless, the role of EBV in lymphomagenesis is still unclear, because the virus infects virtually all humans during their lifetime and its transforming genes are commonly not expressed in the tumor cells of BL.

HHV-8 is a gammaherpesvirus initially identified in tissues of HIV-related Kaposi sarcoma[95] and subsequently found to infect PEL cells as well as a substantial fraction of multicentric Castleman disease.[96–98] Phylogenetic analysis has shown that the closest relative of HHV-8 is herpesvirus saimiri (HVS), a gamma-2 herpesvirus of primates associated with T-cell lymphoproliferative disorders.[99] Like other gammaherpesviruses, HHV-8 is also lymphotropic, and

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infect lymphocytes both in vitro and in vivo.[95,97,98] Lymphoma cells naturally infected by HHV-8 harbor the viral genome in its episomal configuration and display a marked restriction of viral gene expression, suggesting a pattern of latent infection.[99]

The HTLV-1 RNA retrovirus was first isolated from a cell line established from an adult T-cell leukemia/lymphoma (ATLL) patient.[100] Unlike acutely transforming retroviruses, the HTLV-1 genome does not encode a viral oncogene nor does it transform T cells by cis-activation of an adjacent cellular proto-oncogene, because the provirus appears to integrate randomly within the host genome.[101–103] The pathogenetic effect of HTLV-1 was initially attributed to the viral production of a trans-regulatory protein (HTLV-1 tax) that can activate the transcription of several host genes.[104–110] However, Tax expression is suppressed in vivo, most likely to allow for an immune escape of the infected cells, questioning its role in transformation. More recently, a viral factor has been identified, which is thought to be involved in cell proliferation and viral replication and which may be responsible for HTLV-1–mediated lymphomagenesis.

An association between B-NHL and infection by HCV, a single stranded RNA virus of the Flaviviridae family, has been proposed based on the increased risk of developing lymphoproliferative disorders observed among HCV-positive patients[111] and also on the results of interventional studies demonstrating that eradication of HCV with antiviral treatment could directly induce lymphoma regression in seropositive patients affected by indolent NHL.[112] Although the underlying mechanisms remain unclear, current models suggest that chronic B-cell stimulation by antigens associated with HCV infection may induce nonmalignant B-cell expansion, which subsequently evolves into B-NHL by accumulating additional genetic lesions.

The causative link between antigen stimulation by *H. pylori* and MALT lymphoma originating in the stomach is documented by the observation that *H. pylori* can be found in the vast majority of the lymphoma specimens,[113–115] and eradication of infection with antibiotics leads to long-term complete regression in 70% of cases.[116] However, cases with t(11;18)(q21;21) respond poorly to antibiotic eradication,[117] suggesting additional players.

C. psittaci, an obligate intracellular bacterium, was recently linked to the development of ocular adnexal marginal zone B-cell lymphoma (MZL), although variations in prevalence among different geographical areas remain a major investigational issue.[118,119] In this indolent lymphoma, *C. psittaci* causes both local and systemic persistent infection, and presumably contributes to lymphomagenesis through its mitogenic activity and its ability to promote polyclonal cell proliferation and resistance to apoptosis in the infected cells in vivo. Notably, bacterial eradication with antibiotic therapy is often followed by lymphoma regression.[120]

MOLECULAR PATHOGENESIS OF B-NHL

The following section will focus on well-characterized genetic lesions that are associated with the most common types of B-NHL, classified according to the World Health Organization (WHO) classification of lymphoid neoplasia.[1] The molecular pathogenesis of HIV-related NHL will also be addressed, whereas the pathogenesis of other B-cell NHLs remains far less understood. Lymphoblastic lymphoma, which is considered the same disease as T- and B-acute lymphoblastic leukemia, will not be covered in this chapter.

Mantle Cell Lymphoma

Cell of Origin

Mantle cell lymphoma is an aggressive disease representing ~5% of all NHL diagnoses and generally regarded as incurable.[1] Based on immunophenotype, gene expression profile, and molecular features, such as the presence of unmutated IgV genes in the vast majority of cases, MCL has been historically considered as derived from naïve, pre-GC peripheral B cells located in the inner mantle zone of secondary follicles (see Fig. 101.1).[121] More recently, the observation of BCR diversity, including *IGHV* hypermutation, in a subset of tumor cases (15% to 40%) has shifted this paradigm, suggesting the existence of distinct molecular subtypes, including one influenced by the CG environment.

Genetic Lesions

MCL is typically associated with the t(11;14)(q13;q32) translocation, that juxtaposes the *IGH* gene at 14q32 to a region containing the *CCND1* gene (also known as *BCL1*) on chromosome 11q13.[122–124] The translocation consistently leads to homotopic deregulation and overexpression of cyclin D1, a member of the D-type G1 cyclins that regulates the early phases of the cell cycle and is normally not expressed in resting B cells.[125–127] By deregulating cyclin D1, t(11;14) is thought to contribute to malignant transformation by perturbing the G1-S phase transition of the cell cycle.[121] Importantly, the frequency and specificity of this genetic lesion, together with the expression of cyclin D1 in the tumor cells, provides an excellent marker for MCL diagnosis.[1]

In addition to t(11;14), up to 10% of MCLs overexpress aberrant or shorter cyclin D1 transcripts, as a consequence of secondary rearrangements, microdeletions, or point mutations in the gene 3′ untranslated region.[128–130] These alterations lead to cyclin D1 overexpression through the removal of destabilizing sequences and the consequent increase in the mRNA half-life, and are more commonly observed in cases characterized by high proliferative activity and a more aggressive clinical course. The pathogenic role of cyclin D1 deregulation in human neoplasia is suggested by the ability of the overexpressed protein to transform cells in vitro and to promote B-cell lymphomagenesis in transgenic mice, although only when combined to other oncogenic events[131,132]; however, an animal model that faithfully recapitulates the features of the human MCL is still lacking.

Other genetic alterations involved in MCL include biallelic inactivation of the *ATM* gene by genomic deletions and mutations,[133] loss of *TP53* (20% of patients, where it represents a marker of poor prognosis),[134] and inactivation of the *CDKN2A* gene by deletions, point mutations, or promoter hypermethylation (approximately half of the cases belonging to the MCL variant characterized by a blastoid cell morphology).[135] Also associated with aggressive tumors are mutations activating the Notch signaling pathway, including *NOTCH1* (12% of clinical samples) and *NOTCH2* (5% of samples); these lesions, which are mutually exclusive, mostly consist of truncating events that remove the PEST sequences required for NOTCH protein degradation and, thus, lead to protein stabilization.[136,137] Other recurrent mutations were reported in genes encoding the antiapoptotic protein BIRC3, the Toll-like receptor 2 (TLR2), the chromatin modifiers WHSC1 and MLL2, and the MEF2B transcription factor.[136] In a small number of cases, *BMI1* is amplified and/or overexpressed, possibly as an alternative mechanism to the loss of *CDKN2A*.[138,139]

Burkitt Lymphoma

Cell of Origin

BL is an aggressive lymphoma comprising three clinical variants, namely sporadic BL (sBL), endemic BL (eBL), and HIV-associated BL, often diagnosed as the initial manifestation of AIDS.[1] In all variants, the presence of highly mutated IgV sequences[140–143] and the expression of a distinct transcriptional signature[144,145] unequivocally confirm the derivation from a GC B cell.

Genetic Lesions

All BL cases, including the leukemic variants, share a virtually obligatory genetic lesion (i.e., chromosomal translocations

involving the *MYC* gene on region 8q24 and one of the *Ig* loci on the partner chromosome).[146,147] In ~80% of cases, this is represented by the *IGH* locus, leading to t(8;14)(q24;q32), whereas in the remaining 20% of cases, either *IGκ* (2p12) or *IGλ*(22q11) are involved.[146–149] Although fairly homogeneous at the microscopic level, these translocations display a high degree of molecular heterogeneity, the breakpoints being located 5′ and centromeric to *MYC* in t(8;14), but mapping 3′ to *MYC* in t(2;8) and t(8;22).[146–150] Further molecular heterogeneity derives from the exact breakpoint sites observed on chromosomes 8 and 14 in t(8;14): Translocations of eBL tend to involve sequences at an undefined distance (>1,000 kb) 5′ to *MYC* on chromosome 8 and sequences within or in proximity to the *IGHJ* region on chromosome 14.[151,152] In sBL, t(8;14) preferentially involves sequences within or immediately 5′ to *MYC* (<3 kb) on chromosome 8 and within the *IGH* switch regions on chromosome 14.[151,152]

The common consequence of t(8;14), t(2;8) and t(8;22) is the ectopic and constitutive overexpression of the MYC proto-oncogene,[153–155] which is normally absent in the majority of proliferating GC B cells,[13] in part due to BCL6-mediated transcriptional repression.[156] Oncogenic activation of MYC in BL is mediated by at least three distinct mechanisms: (1) juxtaposition of the *MYC* coding sequences to heterologous enhancers derived from the *Ig* loci;[153–155] (2) structural alterations of the gene 5′ regulatory sequences, which affect the responsiveness to cellular factors controlling its expression[157]—in particular, the *MYC* exon 1/intron 1 junction encompasses critical regulatory elements that are either decapitated by the translocation or mutated in the translocated alleles; and (3) amino acid substitutions within the gene exon 2, encoding for the protein transactivation domain.[158,159] These mutations can abolish the ability of *p107*, a nuclear protein related to *RB1*, to suppress *MYC* activity[160] or increase protein stability.[161,162]

MYC is a nuclear phosphoprotein that functions as a sequence-specific DNA-binding transcriptional regulator controlling proliferation, cell growth, differentiation, and apoptosis, all of which are implicated in carcinogenesis.[163] In addition, MYC controls DNA replication independent of its transcriptional activity, a property that may promote genomic instability by inducing replication stress.[164] Consistent with its involvement in multiple cellular processes, the MYC target gene network is estimated to include ~15% of all protein-coding genes as well as noncoding RNAs.[163,165] In vivo, MYC is found mainly in heterodimeric complexes with the related protein MAX, and such interaction is required for MYC-induced stimulation of transcription and cell proliferation.[166–172] In NHL carrying *MYC* translocations, constitutive expression of MYC induces the transcription of target genes with diverse roles in regulating cell growth by affecting DNA replication, energy metabolism, protein synthesis, and telomere elongation.[163,172,173] Furthermore, deregulated MYC expression is thought to cause genomic instability, and thus contributes to tumor progression by facilitating the occurrence of additional genetic lesions.[174] Dysregulation of MYC expression in a number of transgenic mouse models leads to the development of aggressive B-cell lymphomas with high penetrance and short latency.[162,175,176] These mouse models confirm the pathogenetic role of deregulated MYC in B cells, although the resulting tumors tend to be more immature than the human BL, most likely due to the early activation of the promoter sequences used for the expression of the MYC transgene.

More recently, the application of new genomics technologies revealed additional oncogenic mechanisms that cooperate with MYC in the development of this aggressive lymphoma. Mutations of the transcription factor 3 (TCF3) (10% to 25%) and its negative regulator ID3 (35% to 58%) are highly recurrent in all three subtypes of BL, where they promote tonic (antigen-independent) BCR signaling and sustain survival of the tumor cell by engaging the phosphoinositide 3-kinase (PI3K) pathway (Fig. 101.4).[177] In addition, TCF3 can promote cell-cycle progression by transactivating CCND3. Notably, *CCND3* is itself a target of gain-of-function mutations in 38% of sBL, where these events affect conserved residues in the carboxyl terminus of this D-type cyclin, which are implicated in the control of protein stability, leading to higher expression levels. Interestingly, *CCND3* mutations occur in only 2.6% of eBL, suggesting alternative oncogenic mechanisms in this subtype.[177] Other common genetic lesions include the loss of *TP53* by mutation and/or deletion (35% of both sBL and eBL cases),[68] inactivation of *CDKN2B* by deletion or hypermethylation (17% of samples),[85] and deletions of 6q, detected in ~30% of cases, independent of the clinical variant.[70] Finally, one contributing factor to the development of BL is monoclonal EBV infection, present in virtually all cases of eBL and in ~30% of sBL.[86,88,178,179] The consistent expression of EBER, a class of small RNA molecules, has been proposed to mediate the transforming potential of EBV in BL.[180] However, EBV infection in BL displays a peculiar latent infection phenotype characterized by negativity of both EBV-transforming antigens LMP1 and EBNA2; thus, the precise pathogenetic role of this virus has remained elusive.[181]

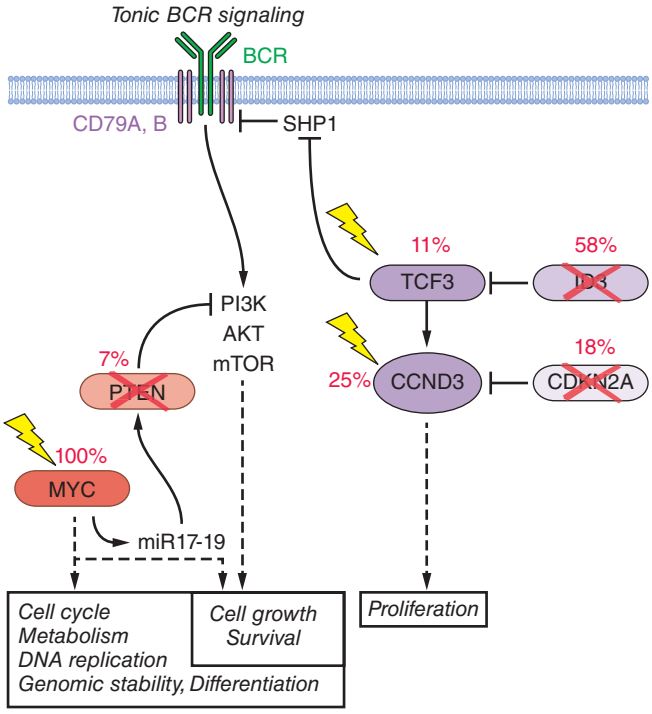

Figure 101.4 Most common genetic lesions identified in BL. *Lightning bolts* indicate activating mutations and *crosses* denote inactivating events. mTOR, mammalian target of rapamycin.

Follicular Lymphoma

FL represents the second most common type of B-NHL (~20% of diagnoses) and the most common low-grade B-NHL.[1] It is an indolent but largely incurable disease, characterized by a continuous pattern of progression and relapses that often culminates in its histologic transformation to an aggressive lymphoma with a diffuse large cell architecture and a dismal prognosis (20% to 30% of cases).[182,183]

Cell of Origin

The ontogeny of FL from a GC B cell is supported by the expression of specific GC B-cell markers such as BCL6 and CD10, together with the presence of somatically mutated Ig genes showing evidence of ongoing SHM activity.[1]

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Genetic Lesions

The genetic hallmark of FL is represented by chromosomal translocations affecting the *BCL2* gene on chromosome band 18q21, which are detected in 80% to 90% of cases independent of cytologic subtype, although less frequent in grade 3 FL.[184–187] These rearrangements join the 3′ untranslated region of *BCL2* to an *IG* J_H segment, resulting in the ectopic expression of BCL2 in GC B cells,[184,185,188–192] where its transcription is normally repressed by BCL6.[18,193] Approximately 70% of the breakpoints on chromosome 18 cluster within the major breakpoint region, whereas the remaining 5% to 25% map to the more distant minor cluster region, located ~20 kb downstream of the *BCL2* gene.[184,185,188,189] Rearrangements involving the 5′ flanking region of *BCL2* have been described in a minority of cases.[194] The *BCL2* gene encodes a 26-kd integral membrane protein that controls the cell apoptotic threshold by preventing programmed cell death and, thus, may contribute to lymphomagenesis by inducing apoptosis resistance in tumor cells independent of antigen selection. Nevertheless, additional genetic aberrations are required for malignant transformation. Most prominent among them are mutations in multiple epigenetic modifiers, including the methyltransferase *MLL2* (80% to 90% of cases),[80] the polycomb-group oncogene *EZH2* (7% of patients),[195] the acetyltransferases *CREBBP* and *EP300* (40% of cases),[79,80] and multiple core histones,[196] which may all contribute to transformation by remodeling the epigenetic landscape of the precursor tumor cell. A major role is also played by chronic antigen stimulation.[197,198]

Whole-exome sequencing and CN analysis of sequential, clonally related FL and tFL biopsies has recently provided the ability to characterize the molecular events that are specifically acquired during histologic progression to DLBCL, and thus presumably play a major role in conferring this more aggressive phenotype. tFL-specific lesions include inactivation of *CDKN2A/B* through deletion, mutation, and hypermethylation (one-third of patients),[84,136,199] rearrangements and amplifications of *MYC*,[84,200] *TP53* mutations/deletions (25% to 30% of cases),[69,201–203] loss of chromosome 6 (20%),[70] ASHM, and although larger cohorts of patients will need to be studied, biallelic loss of the immune regulator *B2M*.[84] Chromosomal translocations of the *BCL6* gene are detected in 6% to 14% of all FL cases, and were shown to have a significantly higher prevalence in the group of patients that undergo transformation into aggressive DLBCL.[204–207]

Diffuse Large B-Cell Lymphoma

DLBCL is the most common form of B-NHL, accounting for ~40% of all new diagnoses in adulthood and including cases that arise de novo, as well as cases that derive from the clinical evolution of various, less aggressive B-NHL types (i.e., FL and CLL).[1,208]

Cell of Origin

Based on gene expression profile analysis, at least three well-characterized molecular subtypes have been recognized within this diagnostic entity, which reflect the derivation from B cells at various developmental stages. Germinal center B-cell–like (GCB) DLBCL appears to derive from proliferating GC cells; ABC-DLBCL shows a transcriptional signature related to BCR-activated B cells or to B cells committed to plasmablastic differentiation; and PMBCL is postulated to arise from post-GC thymic B cells. The remaining 15% to 30% of cases remain unclassified.[209–212] Stratification according to gene expression profiles has prognostic value, because patients diagnosed with GCB-DLBCL display better overall survival compared to ABC-DLBCL,[63] but is imperfectly replicated by immunophenotyping or morphology and does not presently inform differential therapy[213,214]; thus, it is not officially incorporated into the WHO classification. A separate classification schema identified three discrete subsets defined by the expression of genes involved in oxidative phosphorylation, B-cell receptor/proliferation, and tumor microenvironment/host inflammatory response.[215]

Genetic Lesions

The heterogeneity of DLBCL is reflected in the catalog of genetic lesions that are associated with its pathogenesis, and include balanced reciprocal translocations, gene amplifications, chromosomal deletions, single point mutations, and relatively unique among all NHL, ASHM. During the past few years, the application of genomewide approaches such as whole-exome/transcriptome/genome sequencing and single nucleotide polymorphism (SNP) array analysis have provided a comprehensive picture of the DLBCL genomic landscape. One important finding of these studies is that, compared to other B-cell malignancies, DLBCL shows a significantly higher degree of genomic complexity, harboring on average 50 to more than 100 lesions per case, with great diversity across patients.[80,216,217] Although many of the identified lesions can be variably found in both molecular subtypes of the disease, consistent with a general role during transformation, others appear to be preferentially or exclusively associated with individual DLBCL subtypes, indicating that GCB and ABC-DLBCL utilize distinct oncogenic pathways (Fig. 101.5).

GCB and ABC Shared Lesions. The most prominent program disrupted in DLBCL, independent of subtype, is represented by epigenetic regulation of chromatin due to mutations in the *CREBBP/EP300* acetyltransferase genes (35% of cases) and the *MLL2* H3K4 trimethyltransferase (~30% of cases).[79,80,217] These lesions may favor tumor development by reprogramming the cancer epigenome and, in the case of *CREBBP/EP300*, by altering the balance between the activity of the BCL6 oncogene, which is typically inactivated by acetylation, and the tumor suppressor p53, which requires acetylation at specific residues for its function.[79]

Deregulated activity of the BCL6 oncoprotein due to a multitude of genetic lesions is also a major contributor to DLBCL pathogenesis, in both GCB and ABC-DLBCL. Chromosomal rearrangements of the *BCL6* gene at band 3q27 are observed in up to 35% of cases,[71,205,218] although with a twofold higher frequency in the ABC-DLBCL subtype (see Fig. 101.4).[219] These rearrangements juxtapose the intact coding domain of *BCL6* downstream and in the same transcriptional orientation to heterologous sequences derived from the partner chromosome, including *IGH* (14q23), *IGκ* (2p12), *IGλ* (22q11), and at least 20 other chromosomal sites unrelated to the *IG* loci.[40–47] The majority of these translocations result in a fusion transcript in which the promoter region and the first noncoding exon of *BCL6* are replaced by sequences derived from the partner gene.[41,220] Because the common denominator of these promoters is a broader spectrum of activity throughout B-cell development, including expression in the post-GC differentiation stage, the translocation is thought to prevent the downregulation of BCL6 expression that is normally associated with differentiation into post-GC cells. Deregulated expression of an intact BCL6 gene product is also sustained by a variety of indirect mechanisms, including gain-of-function mutations in its positive regulator MEF2B (~11% of cases),[221] inactivating mutations/deletions of *CREBBP/EP300*,[79] and mutations/deletions of *FBXO11* (~5%),[222] which encodes a ubiquitin ligase involved in the control of BCL6 protein degradation. These lesions play a critical role in lymphomagenesis by enforcing the proliferative phenotype typical of GC cells, while suppressing proper DNA damage responses; moreover, constitutive expression of BCL6 blocks terminal differentiation, as confirmed by a mouse model in which deregulated BCL6 expression causes DLBCL.[223]

DLBCL cells have also acquired the ability to escape both arms of immune surveillance, including cytotoxic T lympocytes (CTL)-mediated cytotoxicity (through genetic loss of the *B2M*/human leukocyte antigen class I [*HLA-I*] genes) and natural killer (NK) cell–mediated death (through genetic loss of the CD58 molecule).[224] Analogous effects may be achieved in PMBCL by disruption of the major histocompatibility complex class II

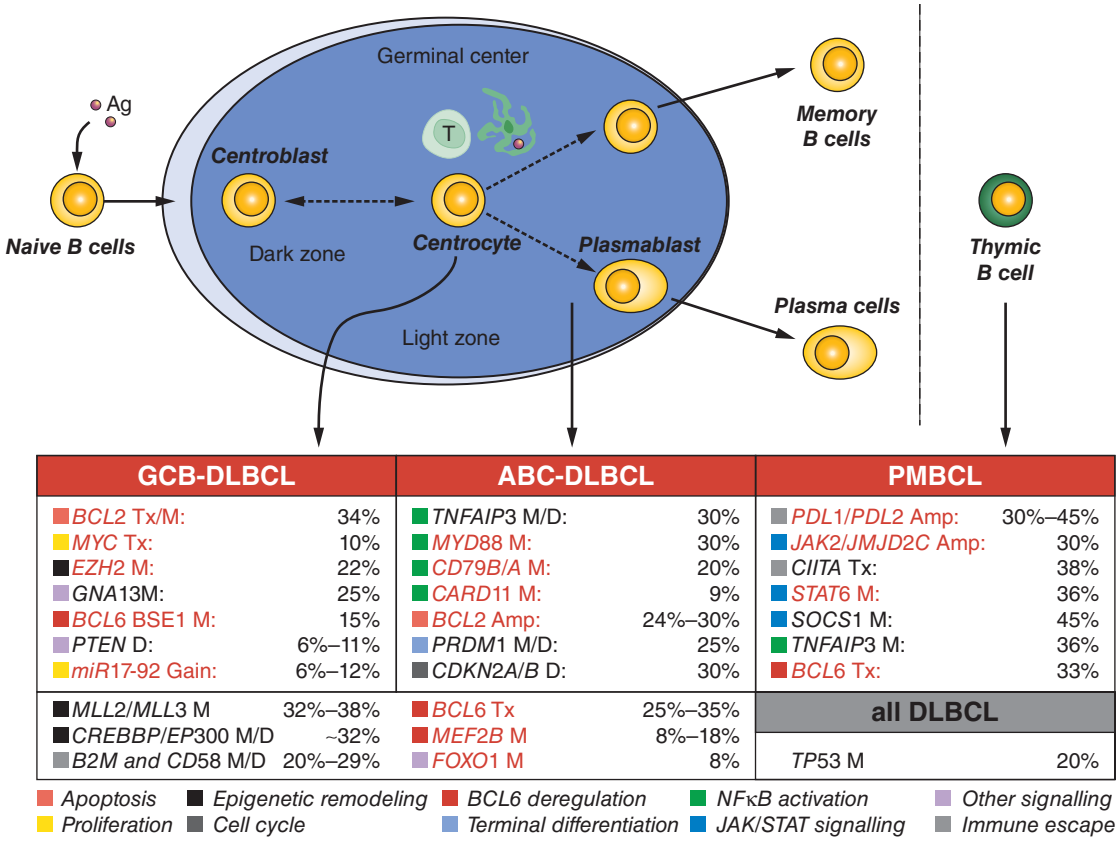

Figure 101.5 Genetic lesions associated with DLBCL. Most common genetic lesions identified in the three major DLBCL subtypes, including lesions that are shared between GCB- and ABC-DLBCL and lesions that are preferentially segregating with individual molecular subtypes. Loss-of-function alterations are in *black* and gain-of-function events are in *red*. *Color-coded squares* denote the biologic function/signaling pathway affected by the alteration.

(MHC-II) transactivator class II major histocompatibility complex transactivator (CIITA) and amplification of the genes encoding for the immunomodulatory proteins *PDL1/PDL2*.

Finally, approximately 50% of all DLBCL are associated with ASHM.[48] The number and identity of the genes that accumulate mutations in their coding and noncoding regions due to this mechanism varies in different cases and is still largely undefined. However, preferential targeting of individual genes has been observed in the two main DLBCL subtypes, with mutations of *MYC* and *BCL2* being found at significantly higher frequencies in GCB-DLBCL, and mutations of *PIM1* almost exclusively observed in ABC-DLBCL. ASHM may, therefore, contribute to the heterogeneity of DLBCL via the alteration of different cellular pathways in different cases. Mutations and deletions of the *TP53* tumor suppressor gene are detectable in ~20% of cases, including those that originate from the transformation of FL, and are more often associated with chromosomal translocations involving *BCL2*.[69]

GCB-DLBCL. Genetic lesions specific to GCB-DLBCL include the t(14;18) and t(8;14) translocations, which deregulate the BCL2 and MYC oncogenes in 34% and 10% of cases, respectively.[63,193,225,226] Also exquisitely restricted to this subtype are mutations of the *EZH2* gene,[195] which encodes a histone methyltransferase responsible for trimethylating Lys27 of histone H3 (H3K27), mutations of the S1PR2 adaptor protein GNA13, mutations affecting an autoregulatory domain within the BCL6 5′ untranslated exon 1,[219,227,228] and deletions of the tumor suppressor *PTEN*.[83,229]

Somatic mutations of the BCL6 5′ regulatory sequences are detected in up to 75% of DLBCL cases[52,230,231] and reflect the activity of the physiologic SHM mechanism that operates in normal GC B cells.[52,53] However, a functional analysis of numerous mutated *BCL6* alleles uncovered a subset of mutations that are specifically associated with GCB-DLBCL and that are not observed in normal GC cells or in other B-cell malignancies.[227] These mutations deregulate BCL6 transcription by disrupting an autoregulatory circuit through which the BCL6 protein controls its own expression levels via binding to the promoter region of the gene[227,228] or by preventing CD40-induced BCL6 downregulation in post-GC B cells.[232] Because the full extent of mutations deregulating BCL6 expression has not been characterized, the fraction of DLBCL cases carrying abnormalities in BCL6 cannot be determined.

ABC-DLBCL. Several genetic abnormalities are observed almost exclusively in ABC-DLBCL, including amplifications of the *BCL2* locus on 18q24[233,234]; mutations within the NF-κB (*CARD11*, *TNFAIP3*/A20),[75,235] B-cell receptor (*CD79B*),[236] and TLR (*MYD88*)[237] signaling pathways; inactivating mutations and deletions of *BLIMP1*[72–74]; chromosomal translocations deregulating the BCL6 oncogene; and deletion or lack of expression of the p16 tumor suppressor. Mutations of the *ATM* gene have been reported in a small subset of cases.[238]

A predominant feature of ABC-DLBCL is the constitutive activation of the NF-κB signaling pathway, initially evidenced by the selective expression of a signature enriched in NF-κB target genes, and by the requirement of NF-κB for proliferation and survival of ABC-DLBCL cell lines. This phenotype is sustained by a variety of alterations affecting positive and negative regulators of NF-κB, as well as other adaptor molecules converging on activation of NF-κB, specifically in this disease subtype. In up to 30% of cases, the *TNFAIP3* gene, encoding for the negative regulator A20, is biallelically inactivated by mutations and/or deletions, thus preventing termination of NF-κB responses.[75,76] The tumor suppressor role

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of A20 was documented by the observation that reconstitution of A20 knockout cell lines with a wild-type protein induces apoptosis and blocks proliferation, in part due to suppression of NF-κB activity.[75,76] In an additional ~10% of ABC-DLBCL, the *CARD11* gene is targeted by oncogenic mutations clustering in the protein coiled-coil domain and enhancing its ability to transactivate NF-κB target genes.[235] Less commonly, mutations were found in a variety of other genes encoding for NF-κB components, overall accounting for over half of all ABC-DLBCL[75] and suggesting that yet unidentified lesions may be responsible for the NF-κB activity in the remaining fraction of cases.

In addition to constitutive NF-κB activity, ABC-DLBCLs display evidence of chronic active BCR signaling, which is associated with somatic mutations affecting the immunoreceptor tyrosine-based activation motif (ITAM) signaling modules of *CD79B* and *CD79A* in 10% of ABC-DLBCL biopsy samples, but rarely in other DLBCLs.[236] Moreover, silencing several BCR proximal and distal subunits is toxic to ABC-DLBCL. These findings provided genetic evidence in support of the development of therapies targeting BCR signaling,[236] and indeed, kinase inhibitors that interfere with this signaling pathway are emerging as a new treatment paradigm for ABC-DLBCL.

Approximately 30% of ABC-DLBCL patients harbor a recurrent change in the intracellular Toll/interleukin-1 receptor domain of the MYD88 adaptor molecule, which has the potential to activate NF-κB as well as JAK/STAT3 transcriptional responses.[237] Although the relationship between *MYD88* mutations and TLR signaling has not been studied, MYD88 was shown to be required for the survival of ABC-DLBCLs, indicating a pathogenic role for TLR in this disease type.

A second important program that is disrupted by genetic lesions in ABC-DLBCL includes terminal B-cell differentiation. In up to 25% of ABC-DLBCL cases, the *PRDM1* gene is inactivated by biallelic truncating or missense mutations and/or genomic deletions, as well as by transcriptional repression through constitutively active, translocated BCL6 alleles.[72–74] The *PRDM1* gene encodes for a zinc finger transcriptional repressor that is expressed in a subset of GC B cells undergoing plasma cell differentiation and in all plasma cells,[239,240] and is an essential requirement for terminal B-cell differentiation.[241] Thus, BLIMP1 inactivation contributes to lymphomagenesis by blocking post-GC B-cell differentiation. Consistently, translocations deregulating the *BCL6* gene are exceedingly rare in *BLIMP1* mutated DLBCLs, suggesting that BCL6 deregulation and BLIMP1 inactivation represent alternative oncogenic mechanisms converging on the same pathway (Fig. 101.6).

PMBCL. PMBCL is a tumor observed most commonly in young female adults, which involves the mediastinum and displays a distinct gene expression profile, largely similar to HL.[211,212] A genetic hallmark of both PMBCL and HL is the amplification of chromosomal region 9q24, detected in nearly 50% of patients.[83,242] This relatively large interval encompasses multiple genes of possible pathogenetic significance, including the gene encoding for the *JAK2* tyrosine kinase and the *PDL1/PDL2* genes, which encode for inhibitors of T-cell responses[64,83,242] and have been linked to impaired antitumor immune responses in several cancers. Other lesions affecting regulators of immune responses in PMBCL include genomic breakpoints and mutations of the MHC class II transactivator gene *CIITA*, which may reduce tumor cell immunogenicity by downregulating surface HLA class II expression.[64,65,243] The ability of the previously mentioned lesions to interfere with the interaction between the lymphoma cells and the microenvironment suggests a central role for escape from immuno-surveillance mechanisms. Besides contributing to lymphomagenesis, elevated expression levels of these genes may, in part, explain the unique features of these lymphoma types, which are characterized by a significant inflammatory infiltrate. PMBCL also shares with HL the presence of genetic lesions affecting the NF-κB pathway and

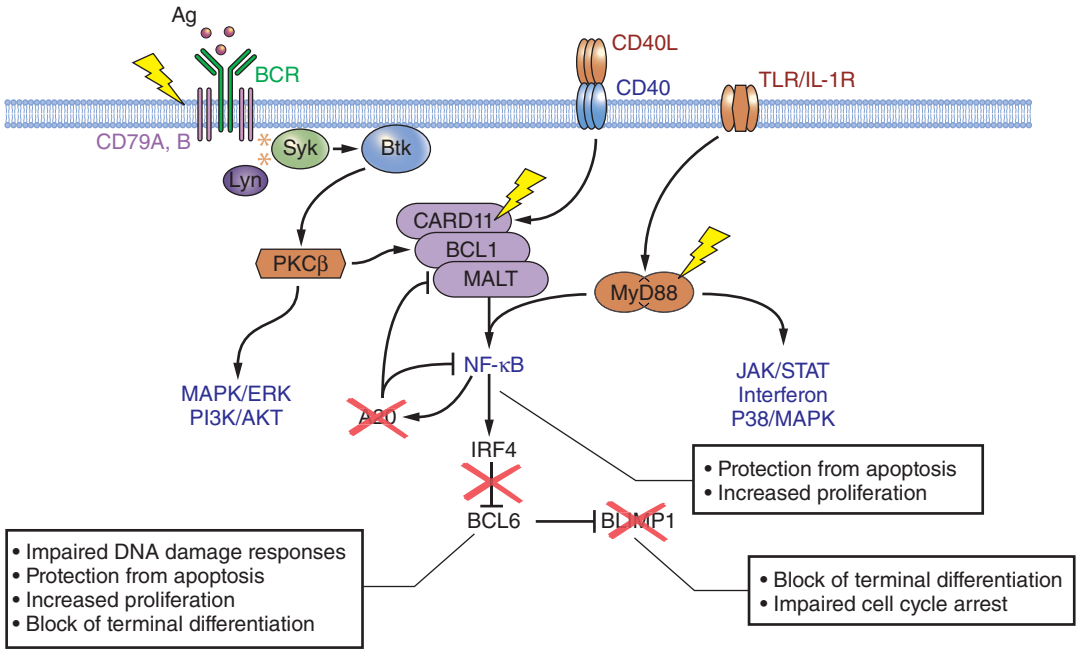

Figure 101.6 Pathway lesions in ABC-DLBCL. Schematic representation of a germinal center centrocyte, expressing a functional surface BCR, a CD40 receptor and a TLR. In normal B cells, engagement of the BCR by the antigen *(spheres)*, interaction of the CD40 receptor with the CD40L presented by T-cells, and activation of the TLR converge on activation of the NF-κB pathway, including its targets IRF4 and A20 among others. IRF4, in turn, downregulates BCL6 expression, allowing the release of BLIMP1 expression, a master plasma cell regulator required for terminal differentiation. In ABC-DLBCL, multiple genetic lesions disrupt this pathway at multiple levels in different cases (percentages as indicated); these lesions contribute to lymphomagenesis by favoring the antiapoptotic and pro-proliferative function of NF-κB, as well as chronic active BCR and JAK/STAT3 signaling, while blocking terminal B-cell differentiation through mutually exclusive deregulation of BCL6 and inactivation of BLIMP1.

the deregulated expression of receptor tyrosine kinases.[78,244–246] In particular, mutations of the transcription factor *STAT6*, amplifications/overexpression of *JAK2* (which promote STAT6 activation via interleukin 3 (IL-3)/IL-4), and inactivating mutations of its negative regulator *SOCS1* are highly recurrent in PMBCL, pointing to the JAK/STAT signaling pathway as a major disease contributor.

DLBCL Derived from CLL and FL Transformation. Recently, exome sequencing studies examining sequential biopsies of CLL/RS or FL/tFL have provided insights onto the molecular mechanisms that drive the transformation process. These analyses extended the set of genetic lesions that are specifically acquired during transformation and include *CDKN2A/B* loss, *TP53* loss, and *MYC* translocations (in both conditions), along with ASHM and *B2M* inactivation in tFL, or *NOTCH1* mutations in RS.[82,84] They also allowed for reconstruction of the evolutionary history of the dominant tumor clone during transformation, revealing that FL and tFL derive from a common ancestor mutated clone through divergent evolution, as opposed to RS, which, analogous to CLL progression,[247] arises from the predominant CLL clone through a linear pattern. Finally, comparison with de novo DLBCL showed that, despite their morphologic resemblance, the genomic landscapes of RS and tFL are largely unique in that they are characterized by distinct combinations of alterations otherwise not commonly observed in de novo DLBCL/NOS.[82,84]

Extranodal Marginal Zone Lymphoma of Mucosa-associated Lymphoid Tissue (MALT)

Cell of Origin

Mucosa-associated lymphoid tissue (MALT) lymphoma represents the third most common form of NHL,[1] and has steadily risen in incidence over the last 2 decades.[248] The presence of rearranged and somatically mutated IgV genes,[31,249] together with the architectural relationship with MALT,[1] indicate the post-GC origin of these tumors, possibly from a marginal zone memory B cell (see Fig. 101.1). A number of observations support a critical role for antigen stimulation, particularly in the pathogenesis of gastric MALT lymphoma: (1) This disease is associated with chronic infection of the gastric mucosa by *H. pylori* in virtually all cases[113–115]; (2) eradication of *H. pylori* by antibiotic treatment can lead to tumor regression in ~70% of cases[116,250]; and (3) MALT lymphoma cells express autoreactive BCR, in particular to rheumatoid factors.[251,252] Whether the development of MALT lymphoma arising in body sites other than the stomach also depends on antigen stimulation remains an open question. In this respect, it is remarkable that salivary gland and thyroid MALT lymphoma are generally a sequela of autoimmune processes, namely Sjögren syndrome and Hashimoto thyroiditis, respectively.

Genetic Lesions

Most of the structural aberrations that are selectively and recurrently associated with MALT lymphoma target the NF-κB signaling pathway, suggesting a critical role in the disease pathogenesis. The most common among these lesions is the t(11;18)(2;33) translocation, which involves the *BIRC3* gene on 11q21 and the *MALT1* gene on 18q21,[253,254] and is observed in 25% to 40% of gastric and pulmonary MALT lymphomas.[255–257] BIRC3 plays an evolutionary conserved role in regulating programmed cell death in diverse species, whereas MALT1, together with BCL10 and CARD11, is a component of the ternary complex that plays a central role in BCR and NF-κB signaling activation.[258] Notably, the wild-type proteins encoded by these two genes are incapable of activating NF-κB, in contrast to the BIRC3/MALT1 fusion protein, suggesting that the translocation confer a survival advantage to the tumor by leading to inhibition of apoptosis and constitutive NF-κB activation without the need for upstream signaling.[253,254,259] In an additional 15% to 20% of cases, *MALT1* is translocated to the *IGH* locus as a consequence of t(14;18)(q32;q21),[260,261] whereas ~5% of patients harbor abnormalities of chromosomal band 1p22, generally represented by t(1;14)(p22;q32); the latter deregulates the expression of *BCL10*, a cellular homolog of the equine herpesvirus-2 *E10* gene, which contains an amino-terminal caspase recruitment domain (CARD) homologous to that found in several apoptotic molecules.[262,263] BCL10, however, does not have pro-apoptotic activity in vivo, where it functions as a positive regulator of antigen-induced activation of NF-κB.[258,264,265] Thus, the translocation may provide both antiapoptotic and proliferative signals mediated via NF-κB transcriptional targets.

A more recently identified translocation associated, although not restricted to, MALT lymphoma is t(3;14)(p13;q32),[266,267] which leads to the deregulated expression of FOXP1, a member of the Forkhead box family of winged-helix transcription factors involved in the regulation of Rag1 and Rag2 and essential for B-cell development.[268] Finally, homozygous or hemizygous loss of *TNFAIP3* due to mutations and/or deletions has been reported in 20% of MALT lymphoma patients, typically in a mutually exclusive pattern with other alterations leading to NF-κB activation.[77] Other recurrent genetic lesions in this disease include trisomy 3,[269,270] *BCL6* alterations, and *TP53* mutations.[271–273]

Chronic Lymphocytic Leukemia

Cell of Origin

CLL is a malignancy of mature, resting B lymphocytes that originates from the oncogenic transformation of a common precursor resembling an antigen-experienced B cell.[274] This notion was conclusively demonstrated when gene expression profile studies revealed that, although CLL can express somatically mutated or unmutated IgV genes at approximately equal percentages,[275,276] all cases share a homogeneous signature more related to that of CD27+ memory and marginal zone B cells.[277,278] Moreover, an analysis of the Ig gene repertoire in these patients indicates very similar, at times almost identical, antigen receptors among different individuals.[279–284] This finding, known as *stereotypy*, strongly supports a role for the antigen in CLL pathogenesis. The histogenetic heterogeneity of CLL carries prognostic relevance, because cases with mutated Ig genes are associated with a significantly longer survival.[285,286] Intriguingly, 6% of the normal elderly population develops a monoclonal B-cell lymphocytosis (MBL) that is considered the precursor to CLL in 1% to 2% of cases.[287]

Genetic Lesions

Different from most mature B-NHL and consistent with the derivation from a post-GC or GC-independent B cell, CLL cases are largely devoid of balanced, reciprocal chromosomal translocations.[81] On the contrary, CLL is recurrently associated with several numerical abnormalities, including trisomy 12 and monoallelic or biallelic deletion/inactivation of chromosomal regions 17p, 11q, and 13q14 (Table 101.1).[81] Of these, the deletion of 13q14 represents the most frequent chromosomal aberration, being observed in up to 76% of cases as a monoallelic event, and in 24% of cases as a biallelic event. Interestingly, this same deletion is also found in subjects with MBL.[287] In all affected cases, the minimal deleted region (MDR) encompasses a long noncoding RNA (*DLEU2*) and two microRNAs expressed as a cluster, namely miR-15a and miR-16-1.[288–290] The causal involvement of 13q14–MDR-encoded tumor suppressor genes in CLL pathogenesis was demonstrated in vivo in two animal models, which developed clonal lymphoproliferative diseases with features of MBL, CLL, and DLBCL at 25% to 40% penetrance.[291] Trisomy 12 is found in approximately 16% of patients evaluated by interphase fluorescent in situ hybridization and correlates with poor survival, but no specific gene(s) have been identified.[292–294] Deletions of chromosomal region 11q22-23 (18% of cases) almost invariably encompass the

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TABLE 101.1

Most Common Genetic Lesions Associated with Non-Hodgkin Lymphoma

NHL Subtype	Genetic Abnormality	Cases Affected (%)	Involved Gene	Functional Consequences	Gene Function
Mantle cell lymphoma	t(11;14)(q13;q32)	95	*CCND1*	Transcriptional deregulation	Cell-cycle regulation
Burkitt lymphoma	t(8;14)(q24;q32) t(2;8)(p11;q24) t(8;22)(q24;q11)	80 15 5	*MYC* *MYC* *MYC*	Transcriptional deregulation Transcriptional deregulation Transcriptional deregulation	Control of proliferation and growth
Follicular lymphoma	t(14;18)(q32;q21) t(2;18)(p11;q21) t(18;22)(q21;q11)	90 Rare Rare	*BCL2* *BCL2* *BCL2*	Transcriptional deregulation Transcriptional deregulation Transcriptional deregulation	Antiapoptosis
Diffuse large B-cell lymphoma (GCB)	t(8;14)(q24;q32) t(14;18)(q32;q21) t(3;other)(q27;other) EZH2 M	10 30 15 20	*MYC* *BCL2* *BCL6* *EZH2*	Transcriptional deregulation Transcriptional deregulation Transcriptional deregulation Unknown	Proliferation and growth Antiapoptosis Master regulator of GC responses Chromatin remodeling
Diffuse large B-cell lymphoma (ABC)	t(3;other)(q27;other) TNFAIP3 M/D PRDM1 M/D CD79B M CARD11 M 18q21 amplification	25 20 20 18 9 30	*BCL6* *TNFAIP3* *PRDM1* *CD79B* *CARD11* *BCL2*	Transcriptional deregulation Loss of function Loss of function Gain of function Gain of function Increased gene dosage	Master regulator of GC responses Negative NF-κB regulator Terminal B-cell differentiation Activation of BCR signaling Positive NF-κB regulator Antiapoptosis
Primary mediastinal B-cell lymphoma	9p24.1 amplification	50	*JAK2* *PDL1, PDL2*	Increased gene dosage Increased gene dosage	JAK/STAT pathway regulation Immunomodulatory responses
Mucosa-associated lymphoid tissue (MALT) lymphoma	t(11;18)(q21;q21) t(14;18)(q32;q21) t(3;14)(p13;q32) t(1;14)(p22;q32)	30 15–20 10 5	*API2-MALT1* *MALT1* *FOXP1* *BCL10*	Fusion protein Transcriptional deregulation Transcriptional deregulation Transcriptional deregulation	Positive NF-κB regulator Positive NF-κB regulator Transcription factor Positive NF-κB regulator
Lymphoplasmacytic lymphoma	t(9;14)(p13;q32)	50	PAX5	Transcriptional deregulation	B-cell proliferation and differentiation
Anaplastic large cell lymphoma	t(2;5)(p23;q35)	60[a]	*NPM/ALK*	Fusion protein	Tyrosine kinase
Classic Hodgkin lymphoma	TNFAIP3 M/D SOCS1 M/D 2p13 amplification 9p24.1 amplification	40[b] 45 50 50	*TNFAIP3* *SOCS1* *REL* *JAK2* *PDL1, PDL2*	Loss of function Loss of function Increased gene dosage Increased gene dosage Increased gene dosage	Negative NF-κB regulator Inhibition of JAK-STAT pathway Positive NF-κB regulator Inhibition of JAK/STAT pathway Immunomodulatory responses

GCB, germinal center B-cell–like; ABC, activated B-cell-like; M, mutation; D, deletion. See also Fig. 101.5 for a more complete list of the recurrent genetic lesions in DLBCL.

[a] In the adult population; 85% in childhood.

[b] Sixty percent in Epstein-Barr Virus-negative cases.

ATM gene and may thus promote genomic instability.[295–297] These lesions can be observed in the patient germ line, and may thus account, at least in part, for the familial form of the disease. Another important target within the 11q22-23 deleted region is the *BIRC3* gene, encoding for a negative regulator of NF-κB.[298] The identification of inactivating mutations and the evidence of constitutive NF-κB activation in these cases suggests that NF-κB could serve as a therapeutic target in this poor prognostic category of patients. Deletions of 17p13, which include the *TP53* tumor suppressor and which are frequently accompanied by a mutation of the second allele,[68,299] are observed in ~7% of CLL at diagnosis but are enriched in cases that underwent transformation to RS, a highly aggressive lymphoma with poor clinical outcome.[82] More recently, gain-of-function mutations of *NOTCH1* and mutations of *SF3B1* were discovered in 5% to 10% of diagnostic CLL samples,[300–303] where they seem to predict an adverse outcome, as supported by their preferential enrichment in RS (30% of cases) and fludarabine refractory cases (25%), respectively (Fig. 101.7).[82,300–304]

HIV-Related Non-Hodgkin Lymphoma

The association between an immunodeficiency state and the development of lymphoma has been recognized in several clinical conditions, including congenital (e.g., Wiskott-Aldrich syndrome), iatrogenic (e.g., treatment with immunosuppressive agents), and viral-induced (e.g., AIDS) immunodeficiencies. Detailed investigations have been conducted on the molecular pathophysiology of HIV-related NHL, which are primarily classified into three clinico-pathologic categories: BL, DLBCL, and PEL.[1,305,306] Based

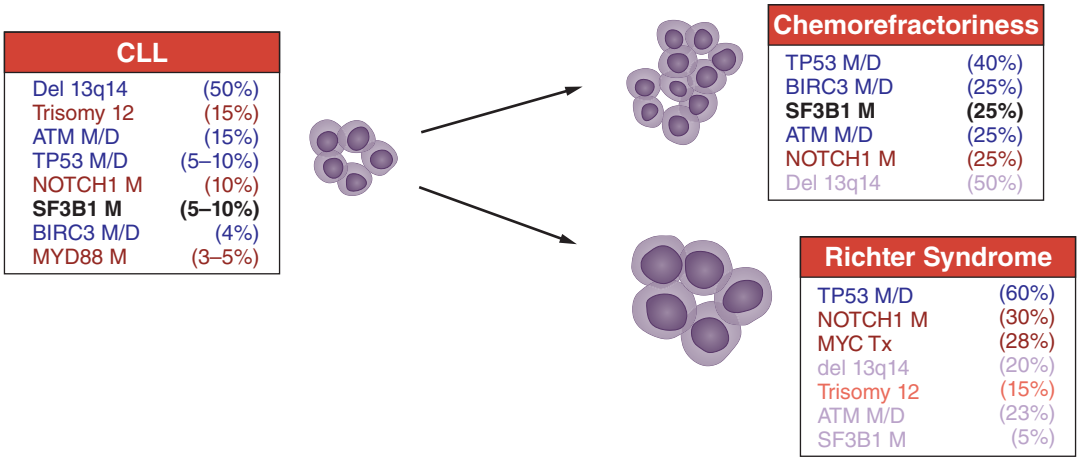

Figure 101.7 Genetic lesions associated with CLL. Frequency of common genetic alterations observed in unselected CLL cases at diagnosis, fludarabine-resistant CLL and RS. Blue, loss of function mutations; Red, gain-of-function mutations.

on the site of origin, HIV-related NHLs are generally grouped into systemic HIV-related NHL (i.e., DLBCL and BL) and HIV-related PCNSL, which is characterized by a uniform morphology consistent with a diffuse architecture of large cells.[1,305,306]

Cell of Origin

HIV-related NHLs invariably derive from B cells that have experienced the GC reaction, as indicated by the presence of somatic mutations in the *IG* and *BCL6* genes, as well as by several phenotypic and transcriptional features.[305–307] Based on the presence or absence of immunoblastic features and on the expression pattern of BCL6, CD138, and the EBV-encoded LMP1, both HIV-related DLBCL and PCNSL can be segregated into two distinct histogenetic categories. Cases displaying the $BCL6^+/CD138^-/LMP1^-$ phenotype closely resemble the phenotype of GC B cells, whereas $BCL6^-/CD138^+/LMP1^+$ cases are morphologically consistent with immunoblastic lymphoma, plasmacytoid, and reflect a post-GC stage of B-cell differentiation.[305,306] PEL consistently derives from B cells, reflecting a preterminal stage of differentiation.[97,308–310]

Genetic Lesions. The three categories of HIV-related NHL associate with distinctive molecular pathways. HIV-related BLs consistently displays activation of MYC due to chromosomal translocations that are structurally similar to those found in sBL, whereas rearrangements of *BCL6* are always absent.[307] HIV-related BLs also frequently harbor mutations in *TP53* (60%), *BCL6* 5′ noncoding sequences (60%), and, in 30% of cases, infection of the tumor clone by EBV, although the EBV-encoded antigens LMP1 and EBNA2 are not expressed.[311,312] Stimulation and selection by antigens, frequently represented by autoantigens, appear to be a prominent feature.[142,313]

Different from BL, the most frequent genetic alteration detected in HIV-related DLBCL is infection by EBV, which occurs in approximately 60% to 70% of cases and is frequently, although not always, associated with the expression of LMP1.[89,91,312] Moreover, HIV-related DLBCLs carry *BCL6* rearrangements in 20% of cases,[314] and mutations of the *BCL6* 5′ noncoding region in 70% of cases.[315]

All HIV-related PCNSLs show evidence of EBV infection.[316] However, only the subset with immunoblastic morphology expresses the LMP1 transforming protein.[317] These tumors display ASHM[57] and harbor oncogenic mutations in the *CARD11* gene (16% of cases),[318] which may explain, in part, the constitutive NF-κB activity previously recognized in this lymphoma subtype. Although some reports have suggested that HHV-8 may be related to PCNSL pathogenesis in immunocompromised patients, extensive analyses have unequivocally ruled out this hypothesis.[319,320]

The last type of HIV-related NHL that has been characterized at the molecular level is PEL, also known as body cavity–based lymphoma.[97,308,309] This entity is associated with HHV-8 infection in 100% of cases and clinically presents as effusions in the serosal cavities of the body (pleura, pericardium, peritoneum) in the absence of solid tumor masses.[97,308,309] In addition to HHV-8, PEL cases frequently show coinfection of the tumor clone by EBV.[90,92,97,308,309]

MOLECULAR PATHOGENESIS OF T-CELL NON-HODGKIN LYMPHOMA

Peripheral T-cell lymphoma (PTCL) encompasses a highly heterogeneous and relatively uncommon group of diseases representing 5% to 10% of all NHLs worldwide, with significant geographical variation in both incidence and relative prevalence.[1] PTCLs arise from mature post-thymic T cells and, according to the clinical presentation of the disease, are listed as leukemic or disseminated, predominantly extranodal, cutaneous, and predominantly nodal.[1] Although the study of T-cell neoplasms is hampered by the rarity of these diseases and the difficulty of collecting homogeneous sample series, significant advances were made over the past decades in our understanding of their biology, classification, and prognosis.

Adult T-Cell Lymphoma/Leukemia (HTLV-1 Positive)

Cell of Origin

The term ATLL identifies a spectrum of lymphoproliferative diseases associated with HTLV-I infection that is mainly restricted to southwestern Japan and the Caribbean basin.[1,321] The United States and Europe are considered low-risk areas, because less than 1% of the population are HTLV-I carriers[322] and only 2% to 4% of seropositive individuals eventually develop ATLL.[1,101,102] Clonal rearrangement of the TCR is evident in all cases, and clonal integration of the virus has been observed.[323,324]

Genetic Lesions

Compared to other mature T-cell tumors, the molecular pathogenesis of ATLL has been elucidated to a wider extent. Particularly, the role of HTLV-I has been linked to the production of a transregulatory protein (HTLV-1 tax), that markedly increases expression of all viral gene products and transcriptionally activates the expression of certain host genes, including IL-2, CD25, c-sis, c-fos, and granulocyte-macrophage colony-stimulating factor (GM-CSF).[104–106,107,108] Indeed, a property of ATLL cells is the constitutively high expression of the IL-2 receptor. The central role of

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these genes in normal T-cell activation and growth, together with the results of in vitro studies, support the notion that tax-mediated activation of these host genes represents an important mechanism by which HTLV-1 initiates T-cell transformation.[104] In addition, tax interferes with DNA damage repair functions and with mitotic checkpoints,[109,110,325] which is consistent with the fact that ATLL cells harbor a high frequency of karyotypic abnormalities.

The long period of clinical latency that precedes the development of ATLL (usually 10 to 30 years), the small percentage of infected patients that develop this malignancy, and the observation that leukemic cells from ATLL are monoclonal suggest that HTLV-1 is not sufficient to cause the full malignant phenotype.[101–103] A model for ATLL, therefore, implies an early period of tax-induced polyclonal T-cell proliferation that, in turn, would facilitate the occurrence of additional genetic events leading to the monoclonal outgrowth of a fully transformed cell. In this respect, a recurrent genetic lesion in ATLL is represented by mutations of the *TP53* tumor suppressor gene, which is inactivated in 40% of cases.[326,327]

Peripheral T-Cell Lymphoma, Not Otherwise Specified

This category represents the largest and most heterogeneous group of PTCLs, and includes all cases that lack specific features allowing classification within another entity. The majority of these cases derive from αβ CD4+ T cells and show aberrant defective expression of one or several T-cell–associated antigens.[328] Based on gene expression profiling, peripheral T cell lymphoma/not otherwise specified (PTCL/NOS) as a group appears to be most closely related to activated T cells than to resting T cells and can be segregated according to similarities with the transcriptional signature of CD4+ and CD8+ T cells. However, no correlation is observed between gene expression profiles (GEP) and immunophenotype, likely reflecting the variable detection of T-cell antigens in the disease.

Genetic Lesions

Clonal numerical and structural aberrations are found in most PTCL/NOS by conventional cytogenetics and in all cases by more sensitive approaches such as array-based methods. For a few loci, the correlation between gene CN and expression has been confirmed, suggesting a pathogenetic role. Candidate genes include *CDK6* on chromosome 7q, *MYC* on chromosome 8, and the NF-κB regulator *CARD11* at 7p22, whereas losses of 9p21 are associated with a reduced expression of *CDKN2A/B*.[329] Chromosomal translocations involving the *TCR* loci have been reported in rare cases and remain poorly understood, because the identity of the translocation partner has not been identified, with few exceptions: the *BCL3* gene, the poliovirus receptor-related 2 (PVRL2) gene found in the t(14;19)(q11;q13) translocation, and the IRF4 gene, which is cloned in two cases.[330–332] More recently, whole-exome sequencing studies revealed the presence of recurrent heterozygous mutations in the *RHOA* small GTPase gene (18% of patients), including a hot-spot Gly17Val substitution that was shown to have inhibitory effects in the Rho-signaling pathway, potentially via the sequestration of GEP proteins.[333,334] These mutations appear to segregate with a subset of Tfh-like PTCL/NOS, characterized by the expression of CD10 and PD-1, the proliferation of CD21+FDCs, and EBER positivity. In a smaller number of cases, mutations were also found in *TET2*, *DNM3TA*, *IDH2*, *TET3*, *FYN*, and *B2M*.[333,334]

Angioimmunoblastic T-Cell Lymphoma

Cell of Origin

Angioimmunoblastic T-cell lymphoma (AITL) is an aggressive disease of the elderly and accounts for about one-third of all PTCL cases in Western countries.[335] The tumor cells display a mature CD4+CD8- T-cell phenotype, with frequent aberrant loss of one or several T-cell markers and coexpression of BCL6 and CD10 in at least a fraction of cells. Gene expression profile studies have conclusively established the cellular derivation of AITL from follicular helper T cells,[336] as initially suspected based on the expression of single markers.[337]

Genetic Lesions

Until recently, the scarce number of genetic studies had failed to provide any significant clues regarding the oncogenic pathways involved in AITL. However, a major discovery emerged in 2013 from two whole-exome sequencing studies that identified highly recurrent mutations of *RHOA* in 67% of all AITL cases.[333,334] These mutations are analogous to those observed in PTCL/NOS, but are not found in other mature B- and T-cell neoplasms, strongly suggesting a role for the disruption of the RHO-signaling pathway in the pathogenesis of this disease. Additional clonal aberrations have been reported in up to 90% of AITL patients and include chromosomal imbalances as well as mutations in *TET2*, *IDH2*, and *DNMT3A*, which are common to various hematologic malignancies, whereas chromosomal translocations affecting the TCR loci are extremely rare.[329,333,334]

Cutaneous T-Cell Lymphoma

Genetic lesions are involved in a limited but significant fraction of primary CTCLs showing a molecular marker of clonality. Most notable among them are rearrangements of the *NFKB2* gene at 10q24, leading to a chimeric protein that retains the rel effector domain and can bind κB sequences in vitro,[338,339] but that lacks the ankyrin regulatory domain required for regulating the physiologic nuclear/cytoplasm distribution. The translocation may thus contribute to lymphoma development by causing constitutive activation of the NF-κB pathway.

Anaplastic Large Cell Lymphoma

Cell of Origin

ALCL is a distinct subset of T-NHL (~12% of cases), whose normal cellular counterpart has not yet been established.[1,321] The tumor is composed of large pleomorphic cells that exhibit a unique phenotype characterized by positivity for the CD30 antigen and the loss of most T-cell markers.[1,340] Based on the expression of a chimeric protein containing the cytoplasmic portion of anaplastic lymphoma kinase (ALK) (see the following), ALCL may be subdivided in two groups, displaying distinct transcriptional signatures[341]: The most common and curable is ALK-positive ALCL, and the more aggressive is ALK-negative ALCL.[1,342–344] However, the identification of a common 30-genes predictor that can discriminate ALCL from other T-NHL, independent of ALK status, suggests that these two subgroups are closely related and may derive from a common precursor.[345]

Genetic Lesions

The genetic hallmark of ALK+ALCL is a chromosomal translocation involving band 2p23 and a variety of chromosomal partners, with t(2;5)(p23;q35) accounting for 70% to 80% of the cases.[1,346] Cloning of the translocation breakpoint in t(2;5) demonstrated the involvement of the *ALK* gene on 2p23 and the nucleophosmin (*NPM1*) gene on 5q35.[347] As a consequence, the aminoterminus of NPM is linked in frame to the catalytic domain of ALK, driving transformation through multiple molecular mechanisms[347]: (1) the *ALK* gene, which is not expressed in normal T-lymphocytes, becomes inappropriately expressed in lymphoma cells, conceivably because of its juxtaposition to the promoter sequences of *NPM*, which are physiologically expressed in T cells; and (2) all translocations involving *ALK* produce proteins with constitutive tyrosine activity, due in most cases to spontaneous dimerization induced by the various fusion partners.[346] Constitutive ALK activity, in turn, results

in the activation of several downstream signaling cascades, with the JAK/STAT and PI3K/AKT pathways playing central roles.[348–351] The transforming ability of the chimeric NPM/ALK protein has been proven both in vitro and in vivo in transgenic mouse models.[352–354]

In a minority of cases, fusions other than NPM/ALK cause the abnormal subcellular localization of the corresponding chimeric ALK proteins and the constitutive activation of ALK. Among these alternative rearrangements, the most frequent involve *TPM3/TPM4*, TRK-fused genes,[355] *ATIC*,[356,357] *CLTCL1*, and *MSN*. The diversity of known ALK fusion partners was further expanded by the recent identification of a novel TRAF1/ALK fusion transcript leading to constitutive NF-κB expression.[358] No recurrent cytogenetic abnormality has been described in ALK-negative ALCL, leaving the molecular events responsible for this disease subtype largely unknown.

MOLECULAR PATHOGENESIS OF HODGKIN LYMPHOMA

HL is a B-lymphoid malignancy characterized by the presence of scattered large atypical cells—the mononucleated Hodgkin cells and the multinucleated Reed-Sternberg cells (HRS)—residing in a complex admixture of inflammatory cells.[1,359] Based on the morphology and phenotype of the neoplastic cells, as well as on the composition of the infiltrate, HL is segregated in two major subgroups: nodular lymphocyte-predominant HL (NLPHL) (~5% of cases) and classic HL (cHL), comprising the nodular sclerosis, mixed cellularity, lymphocyte-depleted, and lymphocyte-rich variants. Until recently, molecular studies of HL have been hampered by the paucity of the tumor cells in the biopsy (typically less than 1%, although occasional cases can present more than 10% HRS cells). However, the introduction of sophisticated laboratory techniques allowing for the isolation and enrichment of neoplastic cells has markedly improved our understanding of HL histogenesis.

Cell of Origin

Despite the fact that HRS of cHL cells have lost the expression of nearly all B-cell–specific genes,[360–362] both HL types represent clonal populations of B cells, as revealed by the presence of clonally rearranged and somatically mutated Ig genes.[363,364] In about 25% of cHL cases, nonsense mutations disrupt originally in-frame *IGHV* gene rearrangements (crippling mutations), thereby preventing antigen selection. These data suggest that HRS cells of cHL have escaped apoptosis through a mechanism not linked to antigen stimulation.[364]

Genetic Lesions

A number of structural alterations lead to the constitutive activation of NF-κB in cHL. Nearly half of the cases display amplification of *REL*, which is associated with increased protein expression levels[365,366]; gains or translocations of the positive NF-κB regulator BCL3 were also reported.[330] More recently, a number of inactivating mutations were found in genes coding for negative regulators of NF-κB, including *NFKBIA* (20% of cases), *NFKBIE* (15%), and *TNFAIP3* (40%), among others.[78,367–369] Notably, *TNFAIP3*-mutated cases are invariably EBV negative, suggesting that EBV infection may substitute, in part, for the pathogenic function of its protein product A20 in causing NF-κB constitutive activation.[78,369] In the past few years, genetic aberrations that modulate the tumor microenvironment have been uncovered using massively parallel DNA sequencing techniques, including genomic gains of PD-L1 (CD274) and PD-L2 (CD273) and translocations of CIITA.[64,65,243] Amplification of *JAK2*, mutations of *STAT6*, and inactivating mutations of *SOCS1*, a negative regulator of the JAK/STAT signaling pathway, are often found in NLPHL[242,246]; in an additional large fraction of cases, constitutive JAK/STAT activity is sustained by autocrine and paracrine signals.[359] *BCL6* translocations have been reported in the lymphocytic and histiocytic (L&H) cells of NLPHL, but only rarely in cHL,[370,371] and translocations of *BCL2* or mutations in positive or negative regulators of apoptosis (e.g., TP53, FAS, BAD, and ATM) are virtually absent.[369] As mentioned, an important pathogenic cofactor in cHL, but not NLPHL, is represented by monoclonal EBV infection, which occurs in approximately 40% of cHLs and up to 90% of HIV-related HLs, suggesting that infection precedes clonal expansion.[359] Of the viral proteins encoded by the EBV genome, infected HRS cells most commonly express LMP1, LMP2, and EBNA1, but not EBNA2.[359]

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102 Hodgkin's Lymphoma

Anas Younes, Antonino Carbone, Peter Johnson, Bouthaina Dabaja, Stephen Ansell, and John Kuruvilla

INTRODUCTION

Although a relatively rare type of cancer, with an estimated 8,000 new cases per year in the United States, Hodgkin's lymphoma (HL) have fascinated scientists and clinicians for more than a century.[1,2] Remarkably, before the cell of origin and the biology of HL were elucidated, it became one of the earliest human cancers to be cured with multiagent chemotherapy.[1,3] Over the past 50 years, a significant progress has been made toward our understanding of HL biology, cell of origin, pathology, and treatment options. Therefore, many seminal observations that were made during the past few decades are now considered of historical value. For example, HL histologic classification evolved through multiple systems, starting from the initial histologic classification by Jackson and Parker in 1944, to the current system which is based on the World Health Organization (WHO) classification (Fig. 102.1).[4,5]

BIOLOGY OF HODGKIN'S LYMPHOMA

Cell of Origin

Molecular studies of isolated tumor cells have demonstrated that lymphocyte-predominant (LP) cells of nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) are derived from antigen-selected germinal center (GC) B cells, whereas Reed-Sternberg (RS) cells in classic HL (cHL) appear to be derived from preapoptotic *crippled* GC B cells (Table 102.1).[6–9] Molecular features of LP cells include the presence of clonally rearranged and somatically mutated immunoglobulin (Ig)V gene cells, with signs of ongoing somatic hypermutation in a fraction of cases (see Table 102.1). These data linked the origin of LP cells in NLPHL to GC B cells. Another important feature supporting this linking was the immunohistochemical expression of BCL6 (a typical GC B-cell marker) in LP cells (Table 102.2).[10,11] Accordingly, LP cells can morphologically be observed in an environmental architecture resembling the structure of a secondary follicle, which contains a reactive GC. In fact, in the early phases of NLPHL, LP cells can be found in follicular structures in association with follicular dendritic cells (FDC) and GC type T-helper cells, in this regard resembling GC.[12]

The derivation of NLPHL from GCs is supported by the following features: (1) the expression of the BCL6 gene product and CD40 by LP cells[13,14]; (2) the occurrence of numerous CD4+/CD57+/PD1 T cells surrounding the LP cells, as seen in normal GCs and progressively transformed GCs (PTGC)[6]; (3) the presence of an FDC meshwork (CD21+/CD35+) within the tumor nodules[15]; and (4) the global gene expression profile.[16] Conversely, molecular features of RS cells in cHL demonstrate that they are probably derived from GC B cells that have acquired disadvantageous immunoglobulin variable chain gene mutations and normally would have undergone apoptosis. [6–8] In parallel to molecular investigations, biologic markers identifying distinct subsets of *mature* B cells have been used to study the cell of origin (see Table 102.2). According to the differential expression of these markers, LP and RS cells resemble *mature* B cells deriving from different stages of B-cell differentiation (i.e., GC and post-GC, respectively).

Reed-Sternberg Cells Lack Common B-Cell Markers

The loss of the B-cell phenotype in RS cells is unique among human lymphomas in the extent to which the lymphoma cells have undergone reprogramming of gene expression. As shown in gene expression profiling (GEP) studies, RS cells have lost the expression of most B-cell–typical genes and acquired expression of multiple genes that are typical for other types of cells in the immune system. Moreover, RS cell gene expression is most similar to that of Epstein-Barr virus (EBV)-transformed B cells, and cell lines derived from diffuse large-cell lymphomas showing features of in vitro activated B cells.[17]

The deregulated expression of inhibitors of B-cell molecules (inhibitor of differentiation and DNA binding 2 [ID2], activated B-cell factor 1 [ABF1], and notch 1), the downregulation of B-cell transcription factors (OCT2, BOB1, and PU.1), and the epigenetic silencing of B-cell genes (CD19 and immunoglobulin H [IgH]) all seem to be involved in the loss of the B-cell phenotype in RS cells.[17,18]

Multiple Signaling Pathways and Transcription Factors Have Deregulated Activity in Reed-Sternberg Cells

Very recently, biologic studies on HL cell lines using new technologies have shown that multiple signaling pathways and transcription factors have deregulated activity in RS cells. Involved pathways and transcription factors included nuclear factor kappa B (NF-+B), Janus kinase/signal transducers and activators of transcription (Jak-Stat), phosphoinositide 3-kinase (PI3K)–Akt, extracellular signal-regulated kinase (ERK), activating protein-1 (AP-1), notch 1, and receptor tyrosine kinases.[7,19] Functional studies have shown that in normal B GC cells, the activation of the CD40 receptor leads to NF-κB–mediated induction of the interferon regulatory factor 4/multiple myeloma oncogene 1 (IRF4/MUM1) transcription factor. CD40 engagement in HL cell lines by both soluble (s) CD40L and membrane-bound (mb) CD40L upregulates IRF4/MUM1 expression by HL cells.[20] CD40 engagement in HL cells by both sCD40L and mbCD40L enhances both clonogenic capacity and colony cell survival of HL cell lines, stimulates proliferation and rescue from apoptosis, mediates in vitro rosetting of activated CD4+ T cells to HL cells, and increases ERK phosphorylation and cell survival.[14,21]

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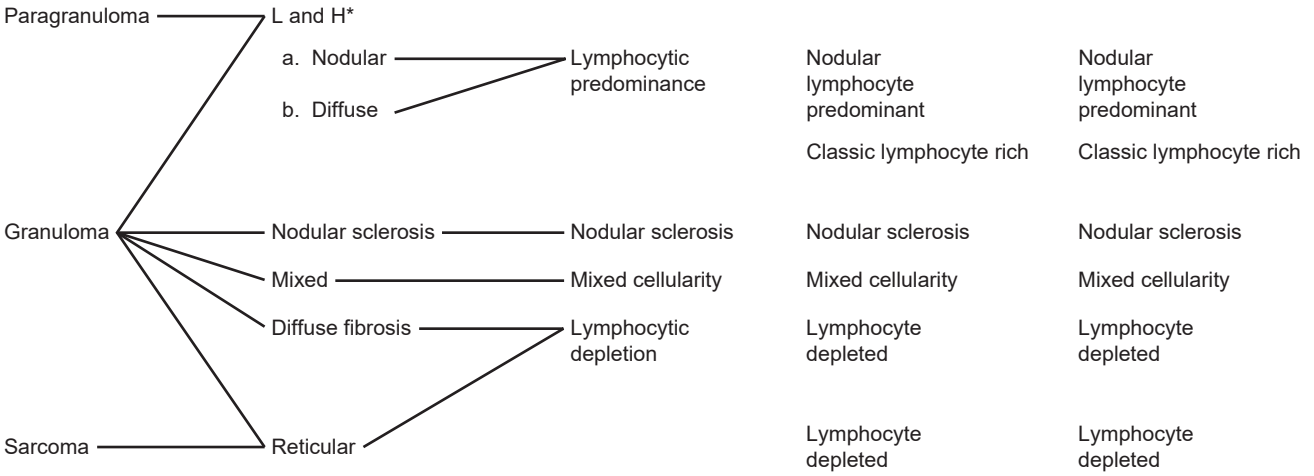

Figure 102.1 Comparison of classifications of Hodgkin's lymphoma.
*Lymphocytic and histiocytic

PATHOLOGY OF HODGKIN'S LYMPHOMA

The REAL Classification and the WHO Proposal

The most recent contribution is provided by the Revised European American Lymphoma (REAL) classification[22] and the WHO proposal, which, on the basis of a combination of phenotypic and morphologic features, subdivided HL into two distinct pathologic and biologic entities: NLPHL and cHL. cHL includes four subtypes (see Table 102.2) (see the following). LP cells of NLPHL and RS cells of cHL have different morphology, different phenotype, and different infection pattern with the EBV.[22] LP cells express CD20, CD45, and epithelial membrane antigen (EMA) antigens, whereas RS cells display CD15-positive, CD30-positive, and CD45-negative phenotypes. EBV infection is usually present only in the RS cells of cHL, which express EBV encoded latent membrane protein 1 (LMP1) (Table 102.3).[22,23]

TABLE 102.1
Cell of Origin and Cell Lineage of Hodgkin's Lymphoma

Feature[1–3]/ Expression[5,6]	RS Cells of cHL	LP Cells of NLPHL
Proposed cellular origin	Preapoptotic GC B cell	Ag-selected, mutating GC B cell
Ig gene (single-cell PCR)	Rearranged, clonal, mutated, "crippled"	Rearranged, clonal, mutated ongoing
Somatically mutated Ig VAR genes	Yes	Yes
Presence of destructive somatic mutation	Yes (25%)	No
BCR	No	Yes
B-cell specific transcription factors (OCT-2, BOB1, PU1)	Very rarely	Yes
B-lineage commitment and maintenance factor PAX-5	Yes (low level)	Yes

PCR, polymerase chain reaction; Ig, immunoglobulin; VAR, variable; BCR, B-cell receptor.

Nodular Lymphocyte-Predominant Hodgkin's Lymphoma

Morphology

NLPHL is characterized by a nodular, or a nodular and diffuse, proliferation of RS cell variants known as LP cells. LP cells are large and usually have one large multilobated nucleus and scant cytoplasm. The nucleoli are usually multiple, basophilic, and smaller than those seen in classical RS cells.

TABLE 102.2
Expression of Molecular Markers in Hodgkin's Lymphoma

Expression[5,6]	RS Cells of cHL	LP Cells of NLPHL
B-cell markers (CD20, CD79)	Rarely	Yes
GC B-cell markers (BCL6, AID)	Rarely	Yes
Plasma cell markers (MUM1, CD138)	Often	No
Molecules involved in Ag presentation (MHC class II, CD40, CD80, CD86)	Yes	Yes
Markers for non–B cells (e.g., TARC, granzyme B, perforin)	Yes (variably)	No
T-cell markers	Yes (rarely)	No

AID, activation-induced cytidine deaminase; MUM1, multiple myeloma oncogene 1; Ag, antigen; MHC, major histocompatibility complex; TARC, thymus and activation-regulated chemokine.

TABLE 102.3

Morphologic, Phenotypic and Virologic Features of Reed-Sternberg Cells of Classic Hodgkin's Lymphoma, and Lymphocyte-Predominant Cells of Nodular Lymphocyte Predominant Hodgkin's Lymphoma[25]

Features/ Expression	cHL/RS Cells	NLPHL LP Cells
Tumor cells	Diagnostic RS cells	LP or "popcorn" cells
Pattern	Diffuse, interfollicular, nodular	Nodular
Background	Lymphocytes, (T cells > B cells) histiocytes, eosinophils, plasma cells	Lymphocytes, (B cells > T cells) histiocytes
Fibrosis	Common	Rare
CD15	+	−
CD19	+ (20%–30%)	+
CD20	+ (20%–30%)	+
CD22	+ (20%–30%)	−
CD30	+	−
CD40	+	+
CD45	−	+
EMA	−	+
IRF4/MUM1	+	+
BCL6	+ (30%)	+
EBV infection	+ (30%–40%)	−

TABLE 102.4

Comparative Expression of Molecular Markers and Cell Microenvironment[10,11,15,26,27]

	NLPHL	THCRBCL
Expression		
CD15	−	−
CD30	Usually −	− or +
EMA	+	Usually +
CD20	+	+
CD79a	+	+
IRF4	+	− or +
EBV	−	Usually −
Cell population		
T cells	v or +	+
B cells/B and T cells	+	−
CD57 + rosetting T cells	+ or −	−
CD40L + rosetting T cells	−	−
IRF4/MUM1 + rosetting T cells	+	−
Histiocytes	− or +	+
DRCs meshworks	+	−

THCRBCL, T/histiocyte cell–rich B-cell lymphoma; DRCs, dendritic reticulum cells.

Phenotype

LP cells are positive for CD20, CD79a, CD75, BCL6, and CD45 and epithelial membrane antigen in nearly all cases (see Table 102.3).[11,19] CD75, formerly LN1, is superior to CD20 and CD79a in detecting LP cells. LP cells express CD75 strongly, whereas small reactive B cells in the background show weak cytoplasmic positivity in the Golgi area but no membranous staining, in accordance with their mantle cell phenotype. CD20 is expressed in LP cells equally or less than in the small reactive B cells in the background. CD79a is even worse than CD20 in detecting LP cells because preferentially stains of small reactive B cells. The OCT2, BOB1, PAX5, and PU.1 B-cell transcription factors and the activation-induced deaminase enzyme (which is involved in somatic hypermutation and class switch recombination mechanisms in Ig genes), are consistently coexpressed (see Table 102.1).[11,19]

Microenvironment

The LP cells reside within nodules consisting of spherical meshworks of FDCs that are filled with nonneoplastic inflammatory cells. Inflammatory cells include small B cells, T cells that specifically express CD3 and CD4, and histiocytes. Furthermore, the inflammatory cells of nodules of NLPHL are characterized by an increase in GC-derived CD57+, IRF4/MUM1+, and PD-1+ T cells (see Table 102.3). Tia1 and CD40L-positive CD3/CD4 positive T cells are absent. PD1 ringing is a feature commonly seen in NLPHL.[15,19,24,25]

In conclusion, LP cells of NLPHL clearly resemble GC B cells in many phenotypic and genetic aspects, and proliferate in association with a cellular microenvironment that retains key features of a normal GC environment. Although LP cells are found to be even more similar to diffuse large B-cell lymphoma (including T-cell/histiocyte-rich large B-cell lymphoma) in terms of phenotypic and gene expression aspects, the environmental characteristics discriminate between these lymphomas (Table 102.4).

Microenvironment and Histologic Patterns

Well-recognized morphologic features of NLPHL include a nodular, or a nodular and diffuse, proliferation of scattered LP tumor cells, set against a background of reactive lymphocytes reminiscent of a primary follicle. Different patterns are recognizable in NLPHL on morphologic and immunohistologic grounds. Fan and colleagues[26] identified six distinct immunoarchitectural patterns (*classical* nodular, serpiginous/interconnected nodular, nodular with prominent extranodular LP cells, T-cell–rich nodular, diffuse with a T-cell–rich background, and diffuse, B-cell–rich pattern) and two variant patterns (presence of small GCs within the nodules and the presence of prominent sclerosis) (Fig. 102.2). In the nodular pattern originally described by Fan and colleagues[26] as pattern A, rare LP cells are seen outside of the nodule. In other patterns, however, increasing numbers of LP cells extend outside of the neoplastic nodules and infiltrate the perinodular space (see Fig. 102.2).[26]

A recent study recognized an additional nodular pattern of NLPHL in which LP cells reside in an environment reminiscent of lymphoid follicles and do not invade the extranodular space (Fig. 102.3).[12,27] The recognition of this pattern primarily relies on the identification within the nodules of BCL6+ and CD20+ LP cells, surrounded by rosetting PD1+ T cells. CD23 and CD21 immunostaining usually detects meshworks of FDCs, which entrap the LP cells and the surrounding T-cell rosettes. LP tumor cells are localized within an environment reminiscent of a secondary follicle or, more frequently, within neoplastic nodules reminiscent of a primary follicle without residual GCs (see Fig. 102.3).

Regarding the relationship of these histopathologic patterns to the clinical course of the disease, the pattern A of Fan and colleagues was usually seen in those patients presenting with earlier

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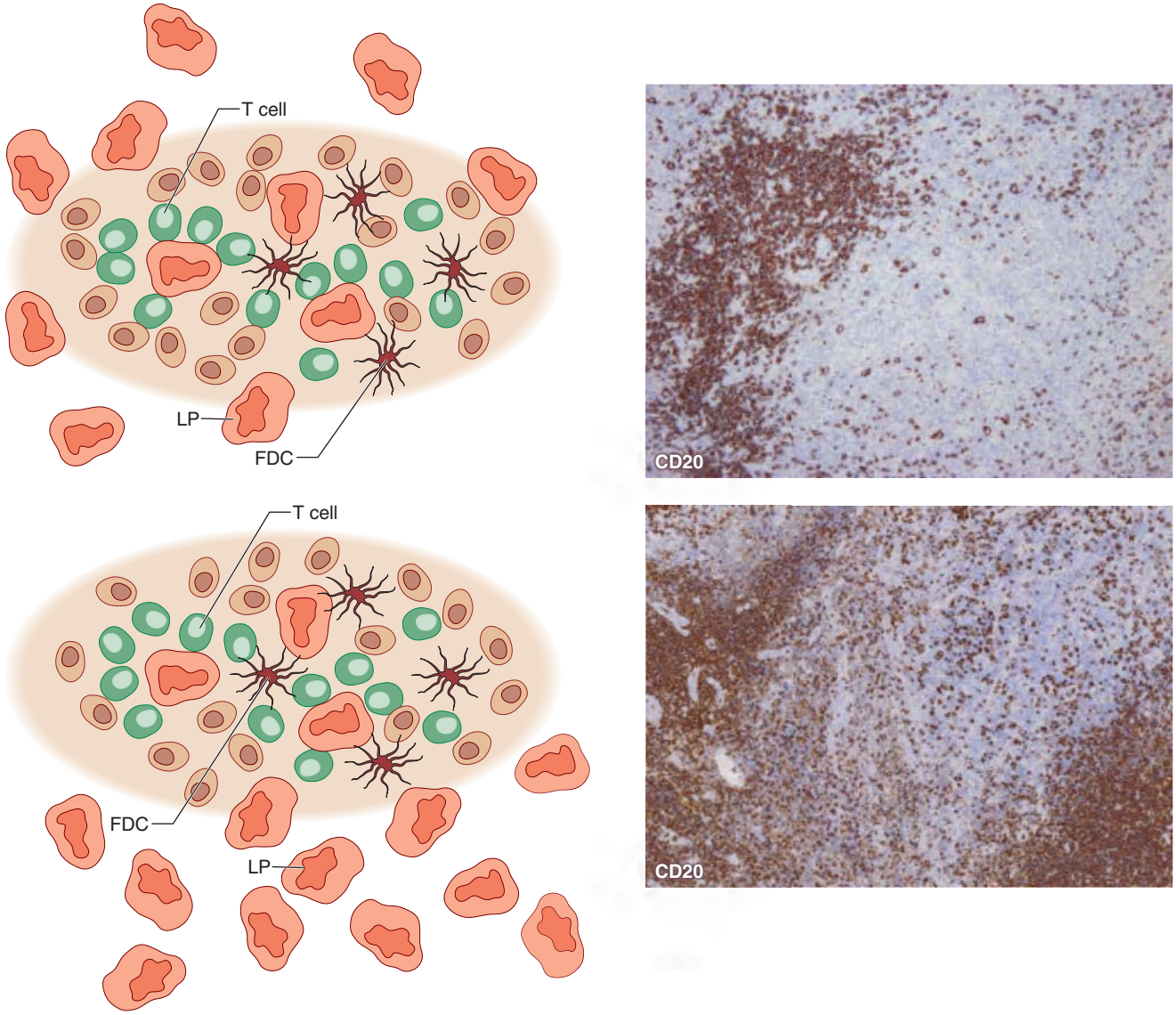

Figure 102.2 Major patterns on nodular lymphocyte predominate Hodgkin's lymphoma, as described by Fan and colleagues. Schematic representation *(to the left)*, microphotographs of CD20 immunostaining of LP cells *(to the right)*. *(Top)* Pattern A of Fan and colleagues.[26] *Classical* nodular pattern with rare extranodular LP cells. *(Top left)* In the *classical* nodular pattern, described by Fan and colleagues, the *B-cell–rich* nodules usually contain a prominent FDC meshwork that encompasses the LP cells. In these cases, the neoplastic LP cells are found to be located predominantly within the nodular structures, but rare LP cells extend outside of the nodule. *(Top right) Classical* nodular pattern. The pattern is characterized by scattered CD20+ LP cells within a nodular, reactive background dominated by small IgD+ B cells (not shown). The nodules contain a prominent CD23+ positive FDC meshwork that encompasses the LP cells (not shown). Rare LP cells can be found outside of the nodules. *(Bottom)* Pattern C of Fan and colleagues.[26] Nodular pattern with prominent extranodular LP cells. *(Bottom left)* During the progression of the disease, more LP cells extend outside of the nodules and infiltrate the perinodular space. Importantly, the presence of numerous LP cells outside the nodules may predict for progression to a diffuse pattern. The presence of many extranodular LP cells may characterize the pattern described by Fan and colleagues as "nodular with prominent extranodular LP cells". *(Bottom right)* Microphotograph of CD20 immunostaining of LP cells. This pattern shows more CD20+ LP cells *(at the center)* extending outside of the nodules. The extranodular LP cells are set in a background of reactive T cells and are not associated with FDC meshworks (not shown). Images were acquired with the Olympus dotSlide Virtual microscopy system using an Olympus BX51 microscopy equipped with PLAN APO 2×/0.08 and UPLAN SApo 40×/0.95 objectives.

clinical stage NLPHL. In general, the clinical impact of the different histopathologic patterns is still uncertain. Understanding this issue is difficult, because more than one pattern is frequently present at the same time.[28]

Classic Hodgkin's Lymphoma

Morphology

The so-called RS cell is the diagnostic key for this lymphoma because of its typical morphology: a giant cell with bi- or multinucleation and huge nucleoli. The typical morphology of binucleated and multinucleated RS cells and their mononuclear variant, the so-called Hodgkin's cell, are not specific to cHL, because they can also be observed in B-NHL (especially in diffuse large B-cell lymphoma [DLBCL] of the anaplastic variant), but they are pathognomonic for cHL in conjunction with an abundant cellular background composed of a varying spectrum of nonneoplastic inflammatory cells.[5]

Based on the characteristics of the reactive infiltrate, four histologic subtypes have been distinguished: lymphocyte-rich cHL (LRCHL), nodular sclerosis (NS) cHL, mixed cellularity (MC) cHL, and lymphocyte depletion (LD) cHL. LRCHL accounts for only a small fraction (3% to 5%) of all HLs. Most LRCHLs

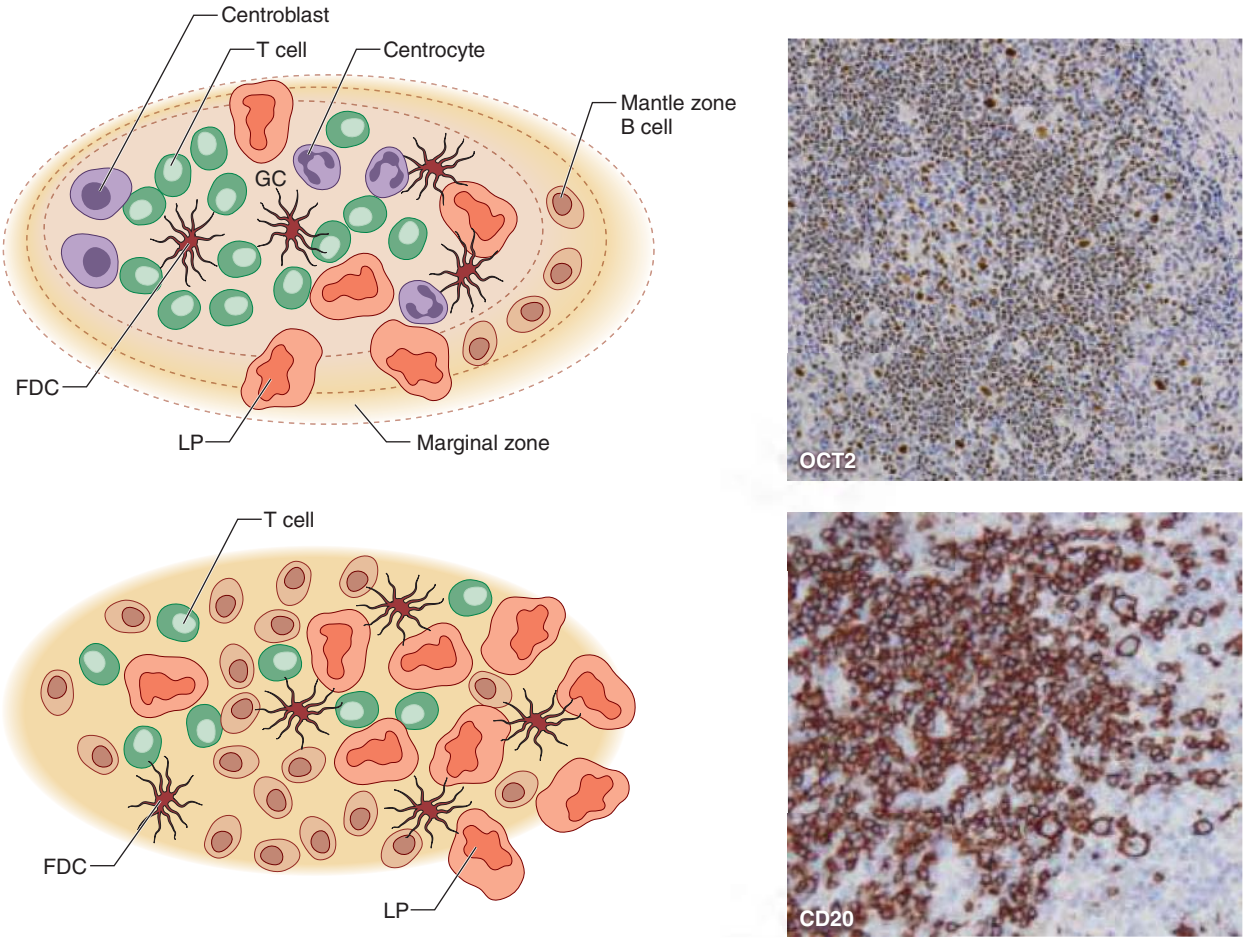

Figure 102.3 Nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) may show a nodular pattern in which tumor cells do not invade the surrounding spaces. Schematic representation and microphotographs of OCT2, BCL6, and CD20 immunostaining of LP cells. OCT-2+, BCL6+, and CD20+ LP cells, surrounded by rosetting T cells (see schematic representation, *to the left*), are localized in an environment reminiscent of lymphoid follicles with (*Top*) or without (*Bottom*) a recognizable germinal center containing reactive B cells. In this pattern, LP cells do not extend outside of the nodules. *Top*: A schematic figure and microphotographs of OCT2 immunostaining of LP cells located in a follicle with recognizable germinal center. *Bottom*: A schematic figure and microphotograph of CD20 immunostainings of LP cells located in a nodule without a recognizable germinal center. Images were acquired with the Olympus dotSlide Virtual microscopy system using an Olympus BX51 microscopy equipped with PLAN APO 2×/0.08 and UPLAN SApo 40×/0.95 objectives.

have a better prognosis than do other cHLs and are characterized histologically by a small number of RS cells expressing a cHL immunophenotype. Based on these histologic and clinical features, there is no clear consensus on whether LRCHL represents a distinct disorder or just an early presentation of cHL. On the other hand, LRCHL cases display features intermediate between those of cHL and NLPHL.[11,19]

Phenotype

Phenotypically, RS cells of cHL are consistently positive for CD30, CD15, CD40, and IRF4/MUM1 (see Table 102.3).[23]

Microenvironment

cHL is a lymphoid neoplasm, derived from B cells, composed of mononuclear Hodgkin's cells and multinucleated RS cells residing in an abundant cellular microenvironment. In cHL, microenvironmental cell types include T- and B-reactive lymphocytes, eosinophils, mast cells, histiocytes/macrophages, plasma cells, and granulocytes (Fig. 102.4).[25,29–33] In addition, a great number of fibroblast-like cells and interdigitating reticulum cells are detectable, often in association with RS cells, within the collagen bands of NS cHL. Fibrosis—considered a common morphologic feature of HL lesions—is found more frequently in cHL subtypes than in NLPHL. An abnormal network of cytokines and chemokines and/or their receptors in RS cells is involved in the attraction of many of the microenvironmental cells into the lymphoma background (see Fig. 102.4).[8,34]

Nonmalignant inflammatory/immune cellular components of the HL microenvironment express molecules involved in cancer cell growth and survival, such as CD30L or CD40L, or in immune escape, such as programmed death 1 (PD-1). For example, CD30L+ eosinophils and mast cells, and proliferation-inducing ligand (APRIL)+ neutrophils, are consistently admixed to RS cells, whereas CD40L-expressing CD4+ T lymphocytes rosette RS cells. A considerable fraction of infiltrating CD4+ T cells are regulatory T (Treg) cells. Treg cells and PD-1+ T cells also interact with RS cells, which produce the Treg attractant galectin-1 and the PD-1 ligand (PDL-1).[7,25,29] The nonmalignant cells that compose most of the cellular background of cHL are recruited and/or induced to proliferate by tumor cells. They in turn produce soluble or membrane-bound molecules involved in tumor cell growth and survival. Numerous molecules are involved directly or indirectly in the recruitment and/or proliferation of cells constituting the cHL microenvironment. Normal cells may be recruited by cytokines/chemokines produced by RS cells or by T cells and

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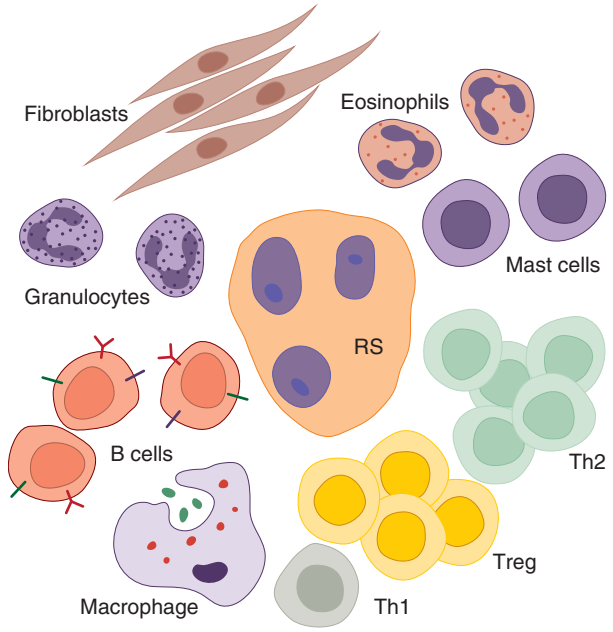

Figure 102.4 Reed-Sternberg (RS) cell and its microenvironment. An RS cell is shown within a rich, polymorphic cellular microenvironment that expresses members of the TNFR family protein and is embedded in a network of cytokines and chemokines. Treg, regulatory T cell; Th1, T-helper cell type 1); Th2, T-helper cell type 2.

fibroblasts activated by RS cells. RS cells produce molecules capable of inducing proliferation and/or differentiation of eosinophils, Treg cells, and fibroblasts.[29]

Epstein-Barr Virus Infection

Generally, in the different histologic subtypes of cHL, the immunophenotypic and genetic features of RS cells are identical, whereas their association with EBV shows differences. EBV is found in RS cells in about 40% of cHL cases in the Western world, mostly in cases of MC and LD HL, and less frequently in NS and LRCHL. Conversely, EBV is found in RS cells in nearly all cases of HL occurring in patients infected with HIV.[35] Independent studies have recently demonstrated that EBV can transform antigen receptor–deficient GC B cells, which enables their escape from apoptosis. The continued survival of the *rescued* preapoptotic B cells allows their proliferation. The EBV-encoded latent membrane protein (LMP) 2A is likely to function as the surrogate receptor through which B-cell signaling is triggered. This mechanism of EBV/LMP2A-induced the escape of antigen receptor-deficient GC B cells from apoptosis offers an intriguing model of lymphomagenesis. EBV infection might also affect the microenvironment composition by increasing the production of molecules involved in immune escape and T-cell recruitment, such as interleukin 10 (IL-10), CCL5, CCL20, and CXCL10.[36] LMP1 could have an interacting role with the microenvironment. Recent evidence indicates that EBV can manipulate the tumor microenvironment through the secretion of specific viral and cellular components into exosomes, small endocytically derived vesicles that are released from cells.[37,38] Exosomes produced by tumor cells from EBV-infected nasopharyngeal carcinoma contain LMP1, which can activate critical signaling pathways in uninfected neighboring cells, suggesting messenger functions of virus-modified exosomes.[37] Moreover, in B-cell lines, EBV-modified exosomes would activate cellular signaling mediated through integrins, actin, interferon, and NF-κB.[38] Further insights in these mechanisms are emerging from the understanding of the capability of EBV to modulate the (tumor-like) microenvironment.

DIFFERENTIAL DIAGNOSIS

Pathologically, HL subtypes should be distinguished from other B-cell lymphomas showing large and CD30 expressing tumor cells. Figure 102.5 shows B-cell lymphomas, which can be differentiated from NLPHL and cHL on immunophenotypic grounds. The figure also includes lymphomas that have overlapping features with cHL or NLPHL. Most importantly, NLPHL should be differentiated from T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL), a DLBCL subtype, and from the rare cHL variant termed lymphocyte-rich cHL.[11,19]

Nodular Lymphocyte-Predominant Hodgkin's Lymphoma

According to current criteria, the detection of one nodule showing the typical features of NLPHL in an otherwise diffuse growth pattern is sufficient to exclude the diagnosis of primary THRLBCL. NLPHL may mimic THRLBCL in a subset of cases in which T cells, rather than B cells, are predominant. This typically occurs in older lesions in which T cells have infiltrated the nodules of B cells and disrupted the nodular architecture.[11] This finding was previously termed NLPHL with diffuse areas; the current preferred term is NLPHL, THRLBCL-like. These kinds of lesions have not been associated with aggressive clinical behavior. The presence of small B-cells and CD4+/CD57+ T cells points to a NLPHL diagnosis, whereas the absence of small B-cells, and the presence of CD8+ cells and TIA1+ cells points to primary

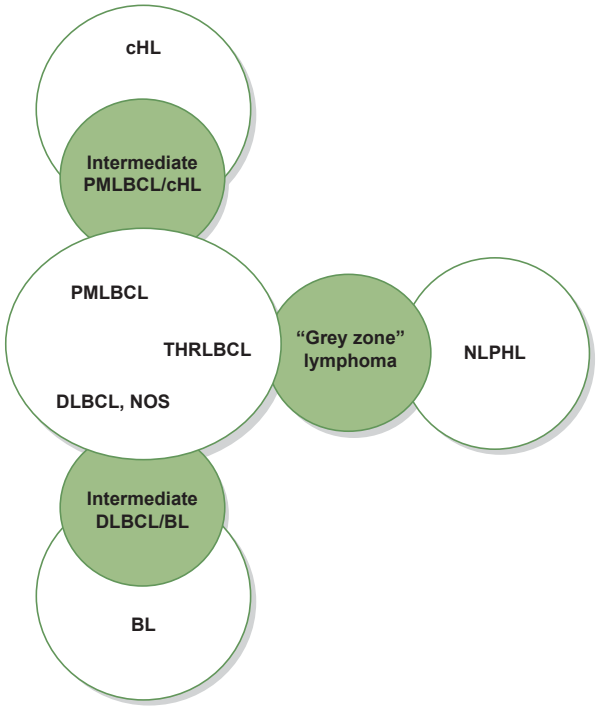

Figure 102.5 Provisional borderline categories for B-cell lymphomas that do not clearly fit into one entity. They include the intermediate PMLBCL/cHL category and a "grey zone" lymphoma between THRLBCL and NLPHL. PMLBCL, primary mediastinal large B-cell lymphoma; THRLBCL, T-cell/histiocyte-rich large B-cell lymphoma; NOS, not otherwise specified.

THRLBCL (see Table 102.4). However, there may be a morphologic and phenotypic gray area between THRLBCL and NLPHL. CD4+/CD57+/PD1 small lymphocytes resetting around typical CD20+/BCL6+ LP cells are useful for the differential diagnosis with PTGC, LRCHL, and THRLBCL. In addition, staining for OCT2, PAX5, and PU1 should be considered as an important diagnostic tool. Interestingly, IgD identifies a subgroup of cases (10% to 20%) with peculiar phenotypical and clinical features.

LRCHL is the most difficult cHL subtype to differentiate from NLPHL, and misclassification has frequently been found in retrospective studies. RS cells in LRCHL can resemble LP cells morphologically; but, immunophenotypically RS cells in LRCHL are positive for CD30 and often express CD15. CD20 can be expressed but is typically weaker and less uniform than CD30 expression. NLPHL is PAX5+, OCT2+, and PU.1+, whereas cHL, including LRCHL, is PAX5+/−, OCT2−, and PU.1−. The distinction between NLPHL and LRCHL is essential, owing to therapeutic and prognostic differences.

cHL

cHL variants should be distinguished from DLBCL subtypes or DLBCL NOS variants that express CD30 (see Fig. 102.5), despite the fact that RS cells have lost much of the B-cell–specific markers. Most or all RS cells also lack the transcription factors OCT2, BOB.1, and PU.1. Instead, RS cells display, in varying frequency, molecules not normally expressed by B cells and B-NHL, such as CD30, CD15, CD70, thymus and activation-regulated chemokine (TARC), A20, fascin, and RANTES.

Finally, cHL cases rich in neoplastic cells may resemble, in particular, large B-cell lymphoma displaying anaplastic morphology and expressing CD30 or primary mediastinal large B-cell lymphoma (PMBCL). There is also a true morphologic and biologic overlap between PMBCL and cHL cases (see Fig. 102.5).

Overlapping Features of PMLBCL with cHL: The So-Called Mediastinal Gray Zone Lymphoma

Mediastinal B-cell lymphomas are mostly represented by NS cHL and PMLBCL. Although PMLBCL and NS cHL have several distinctive pathologic features (Table 102.5),[39–42] these entities exhibit strikingly similar clinical presentations (young women with an anterior mediastinal mass) and, in some cases, show overlap in pathologic, genetic, and molecular features (see Table 102.5). A provisional category, designated *B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL* has been introduced in the WHO proposal to encompass such cases. Table 102.5 shows the main morphologic, phenotypic, and genetic features that may be useful in distinguishing PMLBCL, cHL, and the provisional intermediate category PMLBCL/cHL.

Variant sharing features of DLBCL with anaplastic morphology and cHL may also occur. This shows an expression of CD30, CD15, surface markers, and transcription factors of B cells, commonly absent from RS cells (CD45RB, CD20, CD79, and OCT2).

Molecular Features

In accordance with overlapping phenotypes between cHL and B-cell lymphomas, these lymphomas have a gene expression profile that is intermediate between DLBCL and HL, but closely resembles PMLBCL. Activation of the NF-κB pathway, known to enhance the survival of RS cells, is also a feature of PMLBCL and may represent a survival pathway shared by both neoplasms, likely through the activation of antiapoptotic genes. Activation of the PI3K/AKT pathway were recently identified as a further shared pathogenic mechanism between PMLBCL and cHL. Taken together, molecular features that are common to both lymphoma entities include a decrease of BCR pathway signaling, constitutive NF-κB activation, activation of the cytokine–JAK-STAT pathway, and aberrant activation of the PI3K/AKT pathway. The identification of molecular links between PMLBCL and cHL supports the hypothesis that there may be some pathogenetic overlap between the two entities and that these diseases may in fact represent opposite ends of a continuum.[39–42]

TABLE 102.5

Morphologic and Phenotypic Features that may be Useful in a Differential Diagnosis among Primary Mediastinal Large B-Cell Lymphoma, Classical Hodgkin's Lymphoma, and the Provisional Intermediate Category PMLBCL/cHL[23,43–48]

cHL	Typical RS cells (CD30+, CD15+) Background containing T cells, B cells, plasma cells, eosinophils, fibroblasts Abundant sclerosis
Intermediate	Large cells resembling RS cells (CD20+, B-cell transcription factors +)
PMLBCL/cHL	Admixed large cells with clear cytoplasm (CD20−, CD15−, B-cell transcription factors + weak) Large cells resembling centroblasts Background containing sparse inflammatory infiltrate with eosinophils, plasma cells, histiocytes, and T cells Sclerosis (variable) Necrosis (frequent)
PMLBCL	Large cells with clear cytoplasm, multilobated nuclei, large cells with RS-like morphology (CD30+, CD15−, CD20+, B-cell transcription factors +). Diminished background containing eosinophils, plasma cells, T cells Fine compartmentalizing sclerosis

HIV-Associated Hodgkin's Lymphoma

The Pre-Highly Active Antiretroviral Therapy Era

In the first years of the AIDS epidemic, HIV-associated HL displayed clinical, pathologic, and biologic peculiarities when compared with HL in people uninfected with HIV. First, HIV-associated HL exhibited unusually aggressive clinical behavior, which mandated the use of specific therapeutic strategies, and it was associated with a poor prognosis. Second, the pathologic spectrum of HIV-associated HL differed markedly from that of HL in people uninfected with HIV. In particular, the aggressive histologic subtypes of cHL, namely MC and LD, predominated among HIV-associated HL.[43] Tumor tissue was characterized by an unusually large proportion of RS cells infected by EBV. The fact that LMP1 was expressed in virtually all HIV-associated HL cases suggested that EBV plays an etiologic role in the pathogenesis of HIV-associated HL.

The Highly Active Antiretroviral Therapy Era

People with HIV/AIDS (PWHA) seem to be at increased risk of HL than in first years of the epidemic. HL is presently the most common non–AIDS-defining cancer. Patients infected with HIV, who are modestly immunocompromised due to the improvement in CD4 counts associated with this treatment, are more at risk for

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the development of the nodular sclerosis subtype.[44] In this regard, it has been postulated that with increasing CD4+ T cells resulting from highly active antiretroviral therapy (HAART), the appropriate cellular milieu of cHL, surrounding the RS cells, may again be available. In PWHA with improved immunity, CD4+ T cells provide adequate antiapoptotic pathways and mechanisms for immune escape by tumor cells allowing, in this way, the expansion and maintenance of full expression of the disease, as occurs in cHL among people without AIDS.[35,45–47] Alternatively, HL may arise as part of an immune reconstitution syndrome. Hypothetically, RS cell may already be present in severe immunosuppressed patients, and partial restoration may allow for the recruitment of surrounding immune cells and the manifestation of the tumor.[48,49] A recent study evaluating the effect of immune reconstitution on HL incidence among a cohort of male veterans infected with HIV ever receiving combination antiretroviral therapy (cART) highlighted that immunosuppression and poor viral control may increase HL risk, specifically during immune reconstitution in the interval post-cART initiation. These findings further suggested an immune reconstitution–type mechanism in HIV-related HL development.[50]

EARLY-STAGE HODGKIN'S LYMPHOMA

The management of early-stage Hodgkin's lymphoma exemplifies several important principles of oncology. These include the progressive improvement of cure rates through careful clinical research; the identification of prognostic features and new markers of optimal response; the refinement of treatment by the exploration of multimodality approaches; the vital importance of long-term follow-up; and a holistic analysis of the outcomes of treatment. Overall, this is one of the success stories of modern oncology, with modern treatment achieving high initial cure rates (up to 90% with the first-line of therapy) and good overall survival at around 95% after 5 years or more. Because it most often affects younger people in the 2nd to 4th decade of life, this has important implications for the goals of treatment, which must include not only the maximization of initial tumor control but also the avoidance of preventable long-term side effects.

Prognostic Features

The relatively orderly progression of cHL has long been recognized.[51] It generally develops through involvement of adjacent nodes in the same anatomical site, then in adjacent nodal areas, and it is extremely rare to find isolated deposits in two distant nodes. The same is not true for nodular lymphocyte-predominant disease, which, in this respect, more closely resembles a low-grade non-Hodgkin's lymphoma: It often presents with a single isolated node in the neck, but if it does progress, the dissemination is often to distant sites without intervening nodal involvement.

The predictable spread of cHL has allowed for the construction of a staging system based on anatomical extent, so that early-stage disease is defined by involvement of nodal groups on one side of the diaphragm only, more usually the thorax. Stage I disease is confined to a single anatomical nodal group (cervical, supraclavicular, axillary, anterior mediastinal, etc.), whereas a disease affecting more than one such group is stage II.

Beyond this division on the basis of nodal involvement, many studies have identified further prognostic features through retrospective analyses of large series of patients in clinical trials, mostly treated with extended field radiotherapy. This has allowed for the subdivision of early-stage disease into favorable and unfavorable categories. These do not represent biologically distinct processes, but act as a useful indicator of the severity of the illness and its optimum management, even though the current approaches to treatment are different to those in use when the factors were identified. Although a variety of stratification systems have been devised, common features include the presence of bulky disease (usually in the mediastinum), more advanced age (with a cutoff of 40 or 50 years of age), elevated erythrocyte sedimentation rate (ESR), systemic symptoms, and multiple or extranodal sites of involvement (Table 102.6).

Radiation Therapy

The effective treatment of HL by radiotherapy began with the work of Gilbert in the 1920s.[52] He introduced the rationale for treating both the evident sites of nodal involvement and adjacent but clinically uninvolved lymph nodes, on the basis that these were likely to contain microscopic disease. Peters[53] took the same approach at the Princess Margaret Hospital in the 1940s, publishing a landmark paper in the *American Journal of Roentgenology* in 1950, which described the cure of limited HL by high-dose, fractionated radiation. She reported 5- and 10-year survival rates of 88% and 79%, respectively, for patients with stage I disease, which transformed the outlook for an illness previously thought to have no long-term survivors.

In the early days, radiation therapy utilized fields that included the entire lymphatic system, total lymphoid irradiation (TLI), to

TABLE 102.6

Criteria Used to Stratify Early-Stage Hodgkin's Lymphoma

	EORTC	GHSG	NCIC/ECOG	NCCN 2010
Risk factors	a) Large mediastinal mass (>1/3) b) Age ≥50 years c) ESR ≥50 without B symptoms or ≥30 with B symptoms d) ≥4 nodal areas	a) Large mediastinal mass b) Extranodal disease c) ESR ≥50 without B symptoms or ≥30 with B symptoms d) ≥3 nodal areas	a) Histology other than LP/NS b) Age ≥40 years c) ESR ≥50 d) ≥4 nodal areas	a) Large mediastinal mass (>1/3) or >10 cm b) ESR ≥50 or any B symptoms c) ≥3 nodal areas d) >1 extranodal lesion
Favorable	CS I–II (supradiaphragmatic without risk factors	CS I–II without risk factors	CS I–II without risk factors	CS I–II without risk factors
Unfavorable	CS I–II (supradiaphragmatic with ≥1 risk factors	CS I or CS IIA with ≥1 risk factors CS IIB with c) or d) but without a) and b)	CS I–II with ≥1 risk factors	CS I–II with ≥1 risk factors (differentiating between bulky disease and other risk factors for treatment guidelines)

EORTC, European Organisation for Research and Treatment of Cancer; GHSG, German Hodgkin's Lymphoma Study Group; NCIC, National Cancer Institute of Canada; CS, Clinical stage.

relatively higher biologic radiation dosages compared to contemporary treatment. Extended field radiation therapy (EFRT) included all nodal sites using three radiation fields classically known as mantle, para-aortic–spleen, and inverted Y. A variation of EFRT was also used known as subtotal nodal irradiation (STNI).[54] This was effective, and in many cases curative, but was accompanied by important long-term toxicities, especially the induction of second malignancies and accelerated cardiovascular disease.[55–60] It remained the principal approach to treatment of early disease until clinical trials demonstrated that a combination strategy with chemotherapy could produce superior cure rates with much less irradiation, leading to a reduction of the irradiated field size to only the involved field (IF); the latter was based on a series of studies aimed at minimizing the toxicity of radiation therapy treatment. The German Hodgkin's Lymphoma Study Group (GHSG) HD8 showed in a randomized trial that reducing the treatment volume from EFRT to involved field radiation therapy (IFRT), when combined with chemotherapy, is equally effective. The European Organisation for Research and Treatment of Cancer (EORTC) H7 showed a similar outcome comparing IFRT to STNI.[61,62]

The developments in functional imaging, treatment planning, and image-guided radiation therapy have made it possible to better define and further decrease the radiation fields. Thus, IFRT, which is based on anatomic landmarks and encompassing adjacent uninvolved nodal stations, is no longer appropriate. Based on the fact that most recurrences occur in the original nodal sites, involved node irradiation therapy (INRT) was suggested; the field, in this case, is confined to the macroscopically involved nodes on imaging studies at diagnosis. Although this requires a significant margin around the node to allow and ensure adequate coverage, it can still result in significantly lower exposure to adjacent critical structures.[63] No formal comparison has been made to the results with IFRT, but multiple studies have shown no loss of efficacy with INRT (Fig. 102.6).[64,65]

Using INRT requires acquiring images at diagnosis in treatment positions and prior to the start of chemotherapy to minimize anatomic position variations between diagnostic and radiation treatment planning imaging. Because that is not practical in most cases, new guidelines defining involved site radiation therapy (ISRT) has been introduced by the International Lymphoma Radiation Oncology Group (ILROG). The new standard of care represents a significant reduction in the volume included in the previously used IFRT by using modern imaging and radiation planning techniques to limit the amount of normal tissue being irradiated.

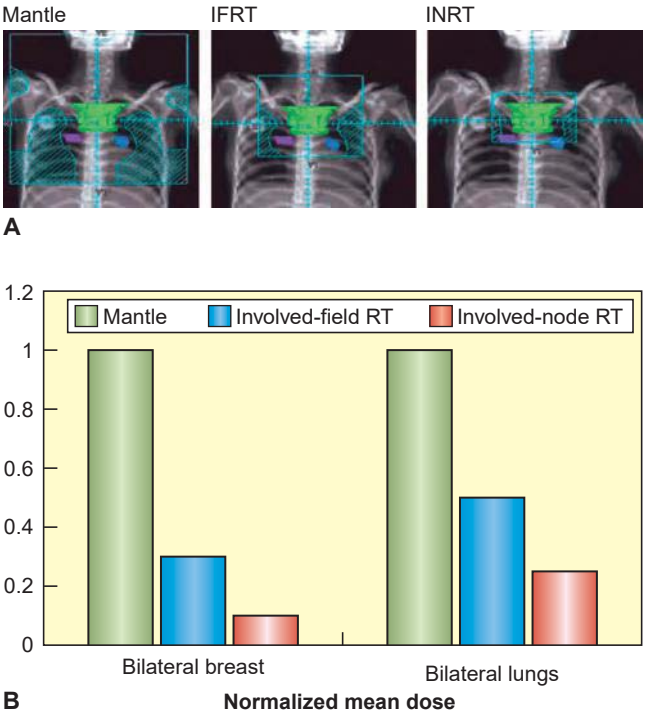

Figure 102.6 Differing radiation volumes in Hodgkin's lymphoma.

Combined Modality Therapy

The recognition that HL is highly sensitive to cytotoxic chemotherapy led to the testing of systemic treatment in early stage disease. By administering limited doses of chemotherapy, it has been shown possible to reduce both the extent and dose of radiotherapy, while still maintaining high cure rates.[66–68] The success of this approach has depended on the different treatment of favorable and unfavorable disease, with results in favorable groups excellent even after low impact chemotherapy, such as two cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) or the attenuated EBVP regimen. The EORTC H7-F study compared STNI to six cycles of EBVP followed by IFRT (36 to 40 Gy), with better results from the combined modality treatment: 10-year event-free survival was 88% versus 78%, and overall survival was 92% in both arms.[62] The GHSG HD10 study in favorable early disease compared results in a 2 × 2 randomization between two or four cycles of ABVD and 20 or 30 Gy of IFRT. All four groups had very high cure rates, with progression-free survival of 92% and overall survival of 97% at 5 years,[69] suggesting that two cycles of ABVD and 20 Gy of IFRT is sufficient treatment for carefully selected favorable disease.

A slightly different picture has emerged from studies of unfavorable early disease, where many patients present with bulky mediastinal nodes. Here, there is a threshold of treatment intensity below which the results become less favorable, with an apparent interaction between the efficacy of chemotherapy and the dose of irradiation used. Attenuated use of either modality can be compensated by the other, but if both elements are reduced too far, the freedom from treatment failure is lowered as the result of the excess of early recurrences. The EORTC H8-U trial showed the equivalence of either six or four cycles of MOPP-ABV when given before IFRT (36 to 40 Gy), or four cycles of MOPP-ABV before STNI, with 5-year event-free survivals of 84%, 88%, and 87%, respectively, and 10-year overall survival estimates of 88%, 85%, and 84%, respectively, indicating that treatment more intensive than four cycles of MOPP-ABV and IFRT was unnecessary, and that less toxic treatment might be possible.[70] More recently, the GHSG HD11 study has tested a 2 × 2 randomization between four cycles of ABVD and four cycles of the baseline bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP) regimen before either 20 Gy or 30 Gy IFRT. The least intensive arm, four cycles of ABVD and 20 Gy, showed inferior 5-year progression-free survival at 82%, compared to 87%, although overall survival was unaffected, at 94.5%.[71] This suggests that for the unfavorable early-stage group, it may hazardous to reduce treatment below a threshold of four cycles of ABVD and 30 Gy IFRT, unless some means can be found to select those patients for whom further deintensification can be attempted, such as the use of functional imaging.

Chemotherapy Alone

Recognition of the long-term toxicity of extended field irradiation has led many investigators to test approaches by which radiotherapy may be omitted altogether from the treatment of early HL.[72,73] Two large randomized trials have been performed, in pediatric and adult patients, respectively, and both demonstrated that the omission of radiotherapy slightly reduced control of the disease,

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reflected in lower progression-free survival, but had no adverse impact on overall survival.

The North American Children's Oncology Group study CCG 5942, tested the omission of low-dose IFRT (21 Gy) for those in complete remission after four cycles of COPP-ABV chemotherapy. The study was closed prematurely when an interim analysis showed a difference in the progression rates in the two arms. With a median 7.7 years follow-up, the event-free survival favored the radiotherapy group (93% versus 83%; $p = 0.004$), with most recurrences in the chemotherapy-alone group seen at the sites of original disease. There was, however, no difference in overall survival, estimated at 97% at 10 years[74].

In adults with early-stage nonbulky disease, the intergroup Eastern Oncology Cooperative Group (ECOG)/National Cancer Institute of Canada (NCIC) study tested treatment with ABVD alone to either 35 Gy STNI in favorable disease, or two cycles of ABVD followed by STNI in unfavorable cases. The first report of this study, with a median follow-up of 4.2 years, showed inferior freedom from progression in the chemotherapy-alone arms (87% versus 93%), with the unfavorable group particularly disadvantaged by the omission of radiotherapy.[75] The initial analysis showed no difference in overall survival, but with longer follow-up, a different picture emerged, with inferior 10-year survival among the patients who had received radiotherapy (87% versus 94%, respectively; $p = 0.04$). The risk of death from lymphoma was not different between the arms, but the risk of death from other causes was more than threefold higher among those treated with radiotherapy, and much of the excess was due to second cancers.[76] It is important to note, however, that this protocol involved much more extensive irradiation than is currently in use, making extrapolation of the results difficult.

In the absence of direct comparative trials between modern combined modality therapy and chemotherapy alone, a meta-analysis was performed using the intergroup study ABVD-alone group and the comparable patients from the GHSG HD10 and HD 11 studies who received ABVD and IFRT. This showed that the short-term disease control was inferior with ABVD alone, reflected in worse 8-year time to progression (93% versus 87%; hazard ratio [HR], 0.44; 95% confidence interval [CI], 0.24 to 0.78), but that overall survival was not adversely affected in these groups, with 95% alive in the long-term follow-up.[77] The impact of combined modality treatment was particularly apparent among patients who showed less than complete remission after chemotherapy, suggesting that some means of selecting those with chemosensitive disease for deescalation of therapy would be attractive, and might allow radiotherapy to be omitted without a loss of disease control.

Response-Adapted Treatment

Much interest has been generated in the possible use of functional imaging to give an early indication of chemosensitivity in HL. The technique most widely tested is 2-(18F)fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET), the application of which as an interim readout of efficacy has been enhanced by the development of a highly reproducible five-point scale for reporting the results (Table 102.7).[78] This approach appears to improve the sensitivity for the detection of residual active lymphoma when compared to conventional computed tomography,[79] but the data from prospective randomized studies using it as a guide to therapy are not yet mature enough for firm conclusions, and it is clear that there is a small but definite false-negative rate for FDG-PET, probably of the order of 5% to 10%.

Two studies have reported early results, with broadly similar outcomes (Table 102.8). The United Kingdom National Cancer Research Institute RAPID study randomized patients with nonbulky early-stage disease who had an interim PET score of 1 or 2 after three cycles of ABVD to either 30 Gy IFRT or no further therapy, and found that the 3-year progression-free survival and overall survival were not significantly different.[80] There was, however, a trend toward inferior disease control, which became significant when patients who did not receive the radiotherapy as allocated were excluded (97% versus 90.7%; HR, 2.39; $p = 0.003$). Similarly, the EORTC H10 study compared two strategies of therapy: standard treatment with ABVD and IFRT, stratified according to baseline prognostic factors, versus a nonradiotherapy approach, but using further chemotherapy, for those with negative FDG-PET scans after two cycles of ABVD.[81] The results with a short follow-up suggested inferior disease control in the experimental PET-directed arms, although the number of progressions was small and a much longer follow-up will be required to determine whether there is any detrimental effect on survival.

Taken overall, the evidence suggests that for early HL, the use of combined modality treatment produces optimum results in terms of disease control, with a very high expectation of cure from the initial therapy. There is, however, a large proportion of patients (around 90%) who will be curable with chemotherapy alone, and the number needed to treat with radiation in order to achieve 1 extra cured patient is between 15 and 30 according to these trials. Given these figures and the perceived risks of late toxicity from radiotherapy, many patients may prefer the slightly higher risk of recurrent lymphoma to the potential for longer term morbidity. This will, of course, be subject to other variables such as their age, the sites of involvement (and thus the radiotherapy fields), and their baseline risk category. In general, the results of treatment from either approach are very good, and it is reassuring that in almost all the trials carried out, a small reduction in disease control does not have any detrimental effect on overall survival, thanks to the excellent results of second-line therapy, when it is required.

TABLE 102.7

Five-Point Scale for the Interpretation of Interim FDG-PET Scanning

Score	PET/CT Result
1	No uptake above background
2	Uptake ≤ mediastinum
3	Uptake > mediastinum but ≤ liver
4	Uptake moderately increased compared to the liver at any site
5	Uptake markedly increased compared to the liver at any site
X	New areas of uptake unlikely to be related to lymphoma

ADVANCED-STAGE HODGKIN'S LYMPHOMA

In patients with advanced-stage HL (stages IIB to IV), the introduction of more effective and less toxic front-line treatment regimens during the last few decades has steadily improved the prognosis. However, complete remissions after initial therapy are not achieved in approximately 20% of patients with stage III to IV disease, eventually leading to disease progression. The current clinical challenge in patients with advanced stage disease is to increase the number of patients with durable remissions and a favorable outcome after initial treatment, while decreasing the incidence of long-term toxicities. The identification of poor prognostic features may allow for a risk-adapted approach to therapy to potentially increase the likelihood of cure and also to minimize side effects.

TABLE 102.8

Response-Adapted Clinical Trials for Early-Stage Hodgkin's Lymphoma

Trial	Eligibility	Treatment Regimens	N	Outcome	Reference
RAPID	Nonbulky Stage I/II Favorable and Unfavorable	ABVD × 3 cycles PET positive: A: ABVD + IFRT 30 Gy PET negative: B: IFRT 30 Gy C: Observation	 A: 145 B: 209 C: 211	 A: OS at 3 years = 93.9% PFS at 3 years = 85.9% B: OS at 3 years = 97% PFS at 3 years = 93.8% C: OS at 3 years = 99.5% PFS at 3 years = 90.7%	80
EORTC H10	Favorable Stage I/II	ABVD × 3 + INRT 20 Gy A: PET positive B: PET negative ABVD × 2 C: PET positive: Esc BEACOPP × 2 + INRT 30 Gy D: PET negative: ABVD × 2	 A: 33 B: 188 C: 27 D: 193	 A: Data not available B: PFS at 1 year = 100% C: Data not available D: PFS at 1 year = 94.9%	81
EORTC H10	Unfavorable Stage I/II	ABVD × 4 + INRT 30 Gy A: PET positive B: PET negative ABVD × 2 C: PET positive: Esc BEACOPP × 2, INRT 30 Gy D: PET negative: ABVD × 4	 A: 88 B: 251 C: 76 D: 268	 A: Data not available B: PFS at 1 year = 97.3% C: Data not available D: PFS at 1 year = 94.7%	81

OS: Overall survival; PFS: Progression free survival.

In general, ABVD chemotherapy remains the most widely used treatment for newly diagnosed patients with advanced-stage HL in the United States. Dose-intense regimens such as escalated BEACOPP are more commonly used in Europe, but are also considered in North America in patients with multiple poor prognostic factors. The future management of advanced-stage HL patients, however, is being shaped by PET-directed approaches and the incorporation of novel agents into these standard combinations.

Prognostic Factors in Advanced Disease

The presence of adverse prognostic factors at diagnosis is one of the methods used to select therapy in HL and the International Prognostic Score (IPS) is an established risk stratification system for advanced disease patients (Table 102.9).[82] This prognostic model was constructed using seven factors associated with a poor outcome (serum albumin less than 4 g per deciliter, hemoglobin less than 10.5 g per deciliter, male sex, age 45 years or older, stage IV disease, leukocytosis of at least 15,000 per cubic millimeter, and lymphocytopenia of less than 600 per cubic millimeter or less than 8% of the white cell count). Although the IPS is highly predictive of freedom from disease progression, it does have limitations. The IPS does not adequately define the truly high-risk patients, because only 7% of the patients in the original study were in the high-risk group and their failure-free survival (FFS) at 5 years was still quite reasonable at 42%.[82] Furthermore, treatment strategies and supportive care have changed since the development of this prognostic model, and although the IPS is still clearly predictive of outcome, its performance may not be as good as originally described.[83–85]

TABLE 102.9

International Prognostic Score (IPS)[82]

Adverse Prognostic Factors for Advanced Hodgkin's Lymphoma
≥45 years
Stage IV
Male
WBC ≥ 15,000 cells/μl
Lymphocytes < 600 cells/μl or <8% of WBC count, or both
Albumin < 4.0 g/dL
Hemoglobin < 10.5 g/dL

WBC, white blood cell count.

Therefore, efforts have been made to improve the prognostication of the IPS by the incorporation of additional clinical prognostic factors,[86,87] the inclusion of biologic parameters,[88–99] or the addition of an early disease response assessment.[100,101] Biologic factors that have been studied include molecular profiling of the tumor and the RS cells[88,93–95]; the measurement of circulating cytokines or receptors including IL-10, CCL17, or soluble CD30[89–92,98]; and the enumeration of immune cells such as macrophages in the tumor microenvironment.[96,97,99] Although many of the biologic factors have prognostic significance independent of the IPS, they have not been adopted in everyday practice due to issues of reproducibility and a lack of prospective validation. On the other hand, an early response assessment as measured by an interim PET/computed tomography (PET/CT) scan has been shown to be a very powerful prognostic tool that is independent of clinical and biologic prognostic factors, including the IPS.[100–102] Interim PET/CT scanning has been introduced into standard

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clinical practice and is also being utilized as a method to direct treatment choices.

Choice of Initial Therapy

Combination chemotherapy forms the basis of treatment for patients with advanced-stage HL (Table 102.10). Initially, the MOPP regimen (nitrogen mustard, vincristine, procarbazine, and prednisone) was developed for previously untreated patients with very advanced HL, and a long-term follow-up of patients treated with the MOPP regime has confirmed that this combination can cure advanced HL. MOPP resulted in a freedom from progression rate of 54% and an overall survival of 48 % at 20 and now 40 years.[103] Although the MOPP regimen had a significant impact on the survival of patients who may previously have died of progressive disease, at least one-third of patients relapsed after MOPP chemotherapy and long-term complications were frequently seen in patients who received the combination.

To improve patient outcomes and decrease toxicity, other chemotherapy combinations such as ABVD were developed. An initial randomized trial compared alternating cycles of ABVD and MOPP chemotherapy to a dose and scheduled modified MOPP chemotherapy, and the alternating regimen was found to be superior in respect to the complete remission rate, freedom from progression, and overall survival.[104] Subsequently, a number of randomized trials were performed using ABVD in combination with MOPP chemotherapy or using ABVD alone. MOPP, ABVD, and MOPP alternating with ABVD were compared and the complete response rate and freedom from progression was initially found to be superior in patients receiving ABVD or the alternating program, but subsequent follow-up reports of the study show no difference in disease-free or overall survival when compared to a MOPP program also given at reduced doses.[105] Two further studies compared the MOPP/ABVD hybrid regimen to MOPP alternating with ABVD, and the regimens were found to be equivalent.[106,107] When the MOPP/ABV hybrid regimen was compared to ABVD, ABVD chemotherapy was found to be superior with less toxicity.[83] The results of these trials led to ABVD chemotherapy being regarded as a standard of care for patients with advanced HL based on the clinical efficacy of the combination, the ease of administration, and the acceptable toxicity profile.

As an alternative to ABVD, the Stanford V regimen was developed as a short duration regimen combined with radiation therapy.[108] The initial single institution results with the regimen showed excellent results with a 5-year freedom from progression of 89% and an overall survival of 96%. These promising results were confirmed in a multi-institutional study.[109] The Stanford V regimen has subsequently been compared to ABVD in a number of randomized trials. Initial studies suggested that ABVD might be superior to Stanford V with a 10-year failure-free survival that was superior in ABVD treated patients; however, it has been argued that the differences in outcome may be due to the fact that radiotherapy in the Stanford V arm was administered differently from what was originally described.[110] Two subsequent randomized trials comparing ABVD to Stanford V have found no difference in response rate, failure-free survival, or overall survival between the regimens.[111,112] Overall, ABVD is felt to be superior to Stanford V in patients with advanced disease.

The GHSG also developed new regimens for patients with advanced HL, particularly standard dose and dose-escalated BEACOPP.[113] A randomized trial comparing COPP (cyclophosphamide, vincristine, procarbazine, and prednisone) alternating with ABVD to escalated or standard BEACOPP showed that patients receiving escalated BEACOPP had improved disease control and overall survival.[114] The improvement in outcome for patients treated with escalated BEACOPP was sustained with long-term follow-up.[115] Although these results were encouraging, long-term complications including acute myeloid leukemia or myelodysplastic syndrome appeared to be more frequent in patients treated with escalated BEACOPP. A similar Italian study compared six cycles of ABVD to four cycles of escalated BEACOPP followed by two cycles of standard BEACOPP and to six cycles of a multidrug intensive regimen. When the results from the ABVD arm were compared the BEACOPP arm, there was an improved progression-free survival with BEACOPP, but the overall survival was not different. Although more toxicity was seen in the BEACOPP-treated patients, poor-risk patients tended to benefit most when treated with BEACOPP.[116]

Since these initial studies, a number of randomized studies have been performed to determine the optimal number of cycles of BEACOPP needed to maintain the clinical benefit but potentially decrease toxicity, and also to define the subgroup of patients most likely to benefit from a more intensive treatment approach. In a study restricted to those younger than 60 years of age, the GHSG found that six cycles of escalated BEACOPP followed by radiotherapy to PET-positive masses was more effective in terms of freedom from treatment failure and less toxic than eight cycles of the same regimen.[117] This led the GHSG to conclude that six cycles of escalated BEACOPP is their standard for advanced HL. To determine whether the high-risk group of patients are those who benefit most from escalated BEACOPP, the EORTC 20012 trial randomized advanced-stage Hodgkin patients with an IPS ≥3 to either eight cycles of ABVD or four cycles of escalated BEACOPP followed by four cycles of standard or baseline BEACOPP.[118] At a median follow-up of 3.9 years, event-free survival, which was the primary endpoint, was similar between treatment arms. Although more relapses were observed with ABVD treatment, early discontinuations were more common in BEACOPP-treated patients. In this high-risk group of patients, however, overall survival was not significantly improved with the use of BEACOPP.

Treating physicians who favor using escalated BEACOPP as initial therapy for advanced-stage HL have pointed to the high response rate and improved event-free survival as the reason to use this combination. In contrast, those who favor using ABVD as initial therapy have cited the complication rate with escalated BEACOPP, as well as the ability to salvage relapsing patients with stem cell transplantation, as reasons to use a less intensive treatment first. To compare these approaches, a randomized comparison of ABVD and escalated BEACOPP was reported, but the analysis included second-line therapy if administered.[119] Patients with residual or progressive disease after initial ABVD or escalated BEACOPP were treated with salvage therapy, including stem cell transplantation. The authors analyzed the outcome after initial therapy, but also analyzed the outcome after salvage therapy. The freedom from first progression significantly favored patients receiving escalated BEACOPP when compared to patients treated with ABVD (85% compared to 73%; $p = 0.004$). However, after completion of all planned therapy including salvage therapy for those with residual or progressive disease, the 7-year rate of freedom from second progression was not significantly different (88% in the escalated BEACOPP group and 82% in the ABVD group; $p = 0.12$) and the 7-year overall survival rate was 89% and 84%, respectively ($p = 0.39$). Severe adverse events were more commonly seen in patients receiving escalated BEACOPP. These results have led some to suggest that initial therapy may not need to be highly aggressive in all patients due to the fact that relapsing patients may be salvaged with subsequent intensive therapy.[120] Others have pointed out that overall survival was a secondary endpoint in this study and that the study was small compared to other similar trials.[121] In an attempt to clarify whether a survival difference exists, a meta-analysis was performed that suggested that six cycles of escalated BEACOPP may well improve overall survival when compared to ABVD.[122]

Overall, it is clear that escalated BEACOPP has greater efficacy than ABVD in patients up to 60 years of age, although escalated BEACOPP-treated patients experience more toxicity, particularly if they are in the upper segment of this age range. Acute and long-term toxicity may, however, be improved by the use of six

TABLE 102.10

Frontline Regimens Commonly Used for Newly Diagnosed Patients with Hodgkin's Lymphoma

Regimen/Drug	Dose	Route	Schedule (Day)	Cycle Length (Days)	Reference
ABVD				28	105
Doxorubicin (adriamycin)	25 mg/m^2	IV	1, 15		
Bleomycin	10 units/m^2	IV	1, 15		
Vinblastine	6 mg/m^2	IV	1, 15		
Dacarbazine	375 mg/m^2	IV	1, 15		
BEACOPP (baseline)				21	113
Etoposide	100 mg/m^2, 200 mg/m^2 if PO	IV	1–3, or PO days 2–3		
Doxorubicin	25 mg/m^2	IV	1		
Cyclophosphamide	650 mg/m^2	IV	1		
Vincristine	1.4 mg/m^2 (cap at 2 mg/m^2)	IV	8		
Bleomycin	10 units/m^2	IV	8		
Procarbazine	100 mg/m^2	PO	1–7		
Prednisone	40 mg/m^2	PO	1–14		
Escalated BEACOPP				21	243
Etoposide	200 mg/m^2	IV	1–3		
Doxorubicin	35 mg/m^2	IV	1		
Cyclophosphamide	1250 mg/m^2	IV	1		
Vincristine	1.4 mg/m^2 (cap at 2 mg/m^2)	IV	8		
Bleomycin	10 units/m^2	IV	8		
Procarbazine	100 mg/m^2	PO	1–7		
Prednisone	40 mg/m^2	PO	1–14		
COPP				28	114
Cyclophosphamide	650 mg/m^2	IV	1, 8		
Vincristine	1.4 mg/m^2 (cap at 2 mg/m^2)	IV	1, 8		
Procarbazine	100 mg/m^2	PO	1–14		
Prednisone	40 mg/m^2	PO	1–14		
MOPP				28	105
Mechlorethamine	6 mg/m^2	IV	1, 8		
Vincristine	1.4 mg/m^2 (cap at 2 mg/m^2)	IV	1, 8		
Procarbazine	100 mg/m^2	PO	1–14		
Prednisone	40 mg/m^2	PO	1–14		
Stanford V				28	108
Mechlorethamine	6 mg/m^2	IV	1		
Doxorubicin	25 mg/m^2	IV	1, 15		
Vinblastine	6 mg/m^2	IV	1, 15		
Vincristine	1.4 mg/m^2 (cap at 2 mg/m^2)	IV	8, 22		
Bleomycin	5 units/m^2	IV	8, 22		
Etoposide	60 mg/m^2	IV	15		
Etoposide	120 mg/m^2 or 60 mg/m^2 IV	PO	16		
Prednisone	40 mg/m^2	PO	Every other day Start taper day 10		
VEPEMB				28	244
Vinblastine	6 mg/m^2	IV	1		
Cyclophosphamide	500 mg/m^2	IV	1		
Procarbazine	100 mg/m^2	PO	1–5		
Prednisone	30 mg/m^2	PO	1–5		
Etoposide	60 mg/m^2	PO	15–19		
Mitoxantrone	6 mg/m^2	IV	15		
Bleomycin	10 mg/m^2	IV	15		
VBM				21–28	245
Vinblastine	6 mg/m^2	IV	1, 8		
Bleomycin	10 mg/m^2	IV	1, 8		
Methotrexate	30 mg/m^2	IV	1, 8		

IV, intravenous; PO, by mouth.

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rather than eight cycles of escalated BEACOPP. It is also clear that approximately two-thirds of patients with advanced HL may not need intensive therapy such as escalated BEACOPP, because they will be cured with ABVD. Clinical risk factors, treatment burden and cost, fertility issues, the risk of long-term relapses, as well as potential short- and long-term complications should be considered as physicians and patients decide which regimen to use as initial treatment for advanced-stage HL.

Positron-Emission Tomography–Directed Approaches

A strategy to potentially optimize therapy for HL, by possibly increasing efficacy and decreasing toxicity, is to utilize PET scans during treatment. Because changes in glucose metabolism precede changes in tumor size, responses can be assessed earlier during treatment with PET scans than with CT. Early interim PET scan imaging after chemotherapy for HL has been shown to be a sensitive prognostic indicator of outcome in patients with advanced disease.[123] In prospective studies, interim PET scans after two cycles of ABVD chemotherapy was a significant predictor of progression-free or event-free survival in patients with advanced-stage disease.[101,102] Similar findings were reported for patients treated with Stanford V or escalated BEACOPP.[124,125] Current clinical trials are now testing whether patient outcomes can be improved by modifying treatment based on the interim PET scan results. In patients who have an inadequate response based on the interim PET scan, treatment is either intensified or salvage therapy is contemplated.

Initial studies testing whether deescalation to less intense or abbreviated therapy maintains efficacy in patients who have a complete response by the interim PET scan suggest that this approach is feasible. Avigdor et al.[126] treated advanced-stage HL patients with two cycles of escalated BEACOPP and deescalated to ABVD chemotherapy for four cycles if the PET scan after the initial two cycles was negative. Patients who did not achieve a negative scan were removed from the study and considered for salvage therapy followed by high-dose chemotherapy and autologous stem-cell transplantation. Seventy-two percent of patients had a negative scan, and deescalation to ABVD resulted in a 4-year progression-free survival of 87%.[126] In a similar fashion, the GHSG HD18 trial is testing whether the number of cycles of escalated BEACOPP can be reduced from six to four in patients with a negative interim PET scan.

An alternative approach is to intensify therapy in patients who do not have a negative interim PET scan. Initial studies have explored whether patients can start treatment with two cycles of ABVD and escalate to BEACOPP if the interim scan is positive.[127,128] Initial reports suggest that this strategy, with BEACOPP intensification only in interim PET-positive patients, showed better results than ABVD-treated historic controls, and spared BEACOPP toxicity in the majority of patients.[127] A similar strategy is being prospectively explored in the UK National Cancer Research Institute Response Adapted Therapy using FDG-PET imaging in the advanced HL (RATHL) trial. In this study, all patients receive two courses of ABVD chemotherapy, and PET-negative patients are randomized between ABVD and AVD, to test whether the omission of bleomycin reduces lung toxicity while achieving an equivalent outcome. Patients who remain PET positive undergo treatment escalation with BEACOPP, thereby attempting to improve remission rates. These studies are still in progress.

Consolidation Radiotherapy

An alternative strategy to modifying the initial treatment for advanced-stage HL is to attempt to consolidate the response following initial chemotherapy. Radiotherapy is commonly used as consolidation following primary chemotherapy, with the goal of improving responses or preventing progression in patients with residual masses.

The precise subgroup of patients with advanced-stage HL who benefit from consolidative radiotherapy has changed over time with the use of different chemotherapy regimens and the routine use of PET scans in clinical practice. For patients treated with standard anthracycline-based chemotherapy, those with only a partial response to treatment as determined by conventional restaging may convert to complete remissions after consolidation radiotherapy. Patients with a complete response to initial treatment, however, do not appear to benefit from consolidation radiotherapy.[129]

As more intensive regimens have been used, resulting in more complete responses, the need for consolidation radiotherapy has decreased. This may be particularly true when intensive approaches are coupled with a PET-based evaluation of residual masses to confirm a complete response. In three successive GHSG trials for advanced HL, the use of radiotherapy was reduced in each study as treatment was intensified and a PET scan analysis was included. In the HD9 trial, two-thirds of patients treated with COPP/ABVD or BEACOPP received radiotherapy. In contrast, in the HD15 trial where PET scans guided the decision, only 11% of patients were treated with radiotherapy after escalated BEACOPP without compromising patient outcome.[117] These studies suggest that the use of radiotherapy can possibly be restricted to patients with PET-positive residual masses after escalated BEACOPP treatment; however, the exact role of radiotherapy in ABVD-treated patients in the era of PET scans is not well defined.

Incorporating Novel Agents into Frontline Therapy

Previous strategies to improve the outcome of patients with advanced-stage HL have largely focused on the intensification of therapy. This has resulted in trials becoming focused on younger patients who are in good health and has also resulted in increased toxicity of therapy. However, not all newly diagnosed patients with HL are young with a good performance score. Also, patients and physicians are concerned about toxicity associated with treatment and want to minimize complications. New treatment approaches that benefit a greater proportion of patients and that are associated with less toxicity are, therefore, needed. The most promising strategy to achieve this may be to add novel agents to less intense chemotherapy regimens. Novel agents currently being used in combination with chemotherapy in the frontline setting include brentuximab vedotin, rituximab, and lenalidomide.

The use of brentuximab vedotin is currently attracting substantial interest, and this agent is being combined with modified forms of the ABVD and BEACOPP combinations. Brentuximab vedotin was initially combined with ABVD, and then substituted for bleomycin in a phase 1 study.[130] In this study, complete responses after the conclusion of front-line therapy were achieved in 95% of the 22 patients receiving ABVD plus brentuximab and in 96% of the 25 receiving AVD plus brentuximab. Significant pulmonary toxicity, however, was seen when brentuximab vedotin was given with the bleomycin-containing regimen, resulting in the concurrent use of bleomycin and brentuximab vedotin being contraindicated. Based on the very high response rate, and the fact that brentuximab vedotin when given with AVD was well tolerated, a randomized phase 3 trial comparing ABVD and AVD plus brentuximab vedotin (ECHELON-1 trial) has been initiated.

The GHSG is also exploring the use of brentuximab in combination with BEACOPP variants, namely a more conservative variant BrECAPP (brentuximab vedotin, etoposide, cyclophosphamide, doxorubicin, procarbazine, and prednisone) and a more aggressive variant BrECADD (brentuximab vedotin, etoposide, cyclophosphamide, doxorubicin, dacarbazine, and dexamethasone). The interim results of a randomized phase 2 trial suggest that use of these anti-CD30 targeted BEACOPP variants is feasible without compromising the efficacy associated with escalated BEACOPP.[131]

Two clinical trials have added rituximab to ABVD chemotherapy to deplete intratumoral B cells and that express CD20 and which may support the growth and survival of the malignant cells. Both studies demonstrated high complete response rates, and the event-free survival in both studies suggested promising activity of the combination. Furthermore, the combination was also effective in patients with high IPS scores. However, the efficacy of this combination will need to be confirmed in a randomized trial.[132,133]

A further strategy being evaluated by the GHSG is the addition of lenalidomide to moderate-dose chemotherapy for newly diagnosed advanced-stage patients. In a recent phase 1/2 study, the efficacy and safety of four to eight cycles of AVD chemotherapy plus lenalidomide at doses of 5 to 35 mg per day, followed by radiotherapy, was tested in elderly patients.[134] The regimen was well tolerated and the preliminary response results were encouraging, suggesting that adding new drugs to modified chemotherapy regimens holds significant promise for the future.

Complications of Treatment

The initial treatment of patients with HL with chemotherapy, often in combination with radiotherapy, results in a significant proportion of patients who are cured of their disease. The toxicity of treatment, however, is a significant limitation to its use. Although early toxicities of therapy are commonly manageable and of short duration, late toxicities are often irreversible and may result in life-threatening complications. The late effects of treatment determine the long-term morbidity, mortality, and quality of life of patients with HL. In the first 10 years after treatment, most deaths are due to disease progression or relapse, but beyond this time point, deaths due to late effects predominate.[135]

Acute hematologic toxicity, with possible infectious complications and treatment-related mortality, is associated with the intensity of the treatment combination, the age of the patient, and their comorbid conditions.[136,137] These toxicities are commonly managed by dose modifications and growth factor support. For patients receiving bleomycin, pulmonary toxicity is a concern. Bleomycin lung toxicity is a potentially life-threatening complication and may be more prevalent in patients receiving ABVD chemotherapy.[138]

A significant complication after treatment is the development of second malignancies. These can involve solid organs (most commonly lung, skin, breast, or gastrointestinal) or be hematologic (leukemia, myelodysplasia, or secondary lymphomas).[139] The risk of second malignancies is highest after treatment for childhood HL.[140,141] In those patients treated for HL before adulthood, the risk of developing a second malignant disease has been estimated to be almost 20 times greater than the general population, with a 30-year cumulative risk of 18% for male patients and 26% for female patients.[141] The most common second malignancy in female patients is breast cancer. Important risk factors for therapy-associated breast cancer are age of younger than 20 years at the time of treatment and treatment with extended field radiotherapy that includes the mediastinum.[142,143] The risk of breast cancer is estimated to be approximately 30% in patients who received 40 Gy to the mediastinum before 25 years of age.[144]

Chemotherapy drugs, especially alkylating agents, contribute to the risk of hematologic malignancies, particularly acute myeloid leukemia (AML) and myelodysplasia. The cumulative risk of developing AML is approximately 1.5% for patients treated for advanced stage Hodgkin lymphoma with chemotherapy regimens such as ABVD.[145] There may be an increase in the incidence of myelodysplasia and AML when more intensive regimens such as escalated BEACOPP are used. The overall rate of other second malignancies, however, appears similar when more intensive and less intensive chemotherapy regimens are compared.[115]

Other late effects include infertility, cardiac effects, endocrine dysfunction, peripheral neuropathy, and local effects from radiotherapy. Alkylating agents may induce male and female sterility, but this is far less frequent in patients treated with ABVD-like regimens than alkylating-containing regimens such as BEACOPP.[146–149] An increase in myocardial infarction, congestive cardiac failure, asymptomatic coronary disease, valvular dysfunction, and stroke have been recorded after treatment for HL, and the risk of cardiac mortality may persist for many years after completing therapy.[150]

SPECIAL CIRCUMSTANCES

Elderly Patients

Elderly patients with HL are a heterogeneous population, particularly when life expectancy, comorbidities, and functional status are considered. Patients older than 65 years constitute approximately 20% of the HL population, but less than 10% of patients included in clinical trials are >60 years. The results of clinical trials are, therefore, not broadly applicable to the elderly who often have difficultly tolerating aggressive treatment approaches. Elderly patients may even have difficulty tolerating ABVD chemotherapy, and response rates to ABVD in elderly patients are typically lower than those seen in younger patients. Older patients often have a poorer event-free survival after ABVD treatment when compared to younger patients.[151]

One reason for the relatively poor outcome in elderly patients is their susceptibility to the toxic effects of intensive therapy, and many have coexisting conditions that affect their ability to tolerate standard treatments. Although fit elderly patients can be treated with curative intent using the same therapeutic regimens as used in younger patients, toxicities and complications are more frequent.[151,152] For more frail elderly patients or those with significant comorbidities, alternative regimens such as VEPEMB (vinblastine, cyclophosphamide, procarbazine, prednisolone, etoposide, mitoxantrone, and bleomycin) or VBM (vinblastine, bleomycin, and methotrexate) could be considered.[153–155] New targeted agents such as brentuximab vedotin, alone or in combination with less toxic agents, are being studied in the treatment of elderly patients with HL.

Pregnancy

HL is one of the most common cancers in pregnant patients, with concurrent pregnancy reported in approximately 3% of all patients.[156,157] Overall, the prognosis and clinical course of HL diagnosed in pregnant women are similar to other patients.[158]

If possible, treatment of asymptomatic, early-stage, pregnant patients should be delayed until after the second trimester or until they complete their pregnancy. If treatment is required, it may be possible to control the disease with single-agent vinblastine to allow the pregnancy to go to term.[156,158,159] Patients who progress while receiving vinblastine can be treated with ABVD chemotherapy during the second or third trimester. Although radiotherapy should be generally avoided during pregnancy, advances in radiotherapy techniques have significantly reduced the risk of fetal complications and radiotherapy could be used if needed.[160] Treatment should not be delayed if the patient has symptomatic, advanced-stage, or progressive HL. If treatment is required and the patient does not want a therapeutic abortion, the successful completion of pregnancy without fetal malformation is possible with the use of ABVD or similar regimens.[161]

Salvage Chemotherapy and Stem Cell Transplantation

Salvage chemotherapy followed by autologous stem cell transplantation (ASCT) has become the treatment of choice in patients with relapsed HL or if the disease is refractory to initial chemotherapy.[162,163] Two randomized phase 3 clinical trials showed improved progression-free survival in patients receiving high-dose chemotherapy (HDCT), compared to those treated with standard-dose salvage

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chemotherapy, although there was no statistically significant difference in overall survival.[164,165]

Although these randomized controlled trials form the basis for the management of patients with relapsed or refractory HL (RR-HL), the challenge to clinicians remains how best to apply these data to patients as primary treatment strategies evolve. Improvements in the management of patients undergoing ASCT (the use of peripheral blood stem cells [PBSC] and modern supportive care) and allogeneic stem cell transplantation (alloSCT; the use of nonmyeloablative or reduced-intensity conditioning techniques and increased experience with matched unrelated and alternative donor stem cell sources) have led to improved safety, increasing age, and comorbidity cutoffs for transplant patients. These technical advances have granted further accessibility to stem cell transplant therapies. With the advent of active novel agents, the role of stem cell transplantation in the management of HL may need to be addressed again in randomized controlled trials.

Prognostic Factors in Relapsed/Refractory Hodgkin's Lymphoma

Multiple studies have identified prognostic factors in RR-HL who undergo salvage chemotherapy and ASCT. The largest studies of prognostic factors in patients not specifically selected for ASCT have been performed by the GHSG. Separate studies have examined prognostic factors in primary refractory HL (defined as progressing while on primary treatment or within 3 months of completion) and the second paper examined patients who relapsed beyond 3 months after completion of primary therapy. In the primary treatment setting, 206 patients were identified with the significant adverse prognostic factors identified from multivariate analysis being poor performance status (ECOG >0), age >50 years, and failure to obtain a temporary remission to initial therapy.[166] In the relapse setting, 422 patients were studied and the significant adverse prognostic factors for overall survival identified in multivariate analysis were anemia (hemoglobin <120 in males, <105 in females), advanced clinical stage (III or IV), and time to treatment failure of <12 months.[167]

In summary, other series and institutional reviews generally confirm that that time to relapse after initial therapy along with advanced stage and poor performance status at relapse are consistent predictors of poor outcome. Time to relapse is of clinical significance because the GHSG primary refractory series had a 5-year OS of 26% compared to 46% for early relapsers after chemotherapy (3 to 12 months) and 71% for late relapsers (after 12 months) in their series studying relapsers.[16,18] Prospective validation of the predictors of outcome identified by Josting et al. [16,18] have yet to be performed.

TREATMENT

Salvage Chemotherapy Prior to Autologous Stem Cell Transplantation and Peripheral Blood Stem Cell Mobilization

Despite a multitude of published phase 2 studies reporting results of salvage regimens for RR-HL,[168–178] Randomized control trial (RCTs) of second-line regimens have not been performed and, thus, there is no obvious *standard of care* regimen. The published RCTs of ASCT for RR-HL employed mini-BEAM or dexa-BEAM and the control arm of the most recent GHSG trial used dexamethasone, cytarabine, and cisplatin (DHAP), so these regimens can be considered as *standard* regimens in this setting.[164,165,179] Because the goal of salvage chemotherapy is to enable patients to proceed to ASCT, the ideal regimen should have a high response rate with minimal toxicity, and not impair the collection of peripheral blood stem cells for ASCT. Although the RCTs of ASCT support the use of multidrug regimens including carmustine (BCNU), etoposide, cytarabine, and melphalan (mini-BEAM), these regimens have significant hematologic toxicity, requiring frequent hospitalization for febrile neutropenia, and a high incidence of transfusion support (Table 102.11). Stem cell mobilization appears to be compromised following treatment with mini-BEAM.[180]

Given the multicenter experience with DHAP reported by the GHSG, a platinum-based regimen such as DHAP is a reasonable choice given comparable response rates and less toxicity.[179] When given prior to randomization in the HD-R2 study, DHAP lead to complete response (CR)/complete response unconfirmed (CRu) in 24%, PR in 46%, and SD in 20%. As the trial allowed patients to proceed to randomization as long as they did not have PD, 90% of patients proceeded toward transplant. Several published and widely used salvage chemotherapy regimens are summarized in Figure 102.1. These trials report similar response rates to DHAP, and there is no evidence to demonstrate that one is superior over others. Although the dexa-BEAM regimen had an overall response rate (ORR) of 81% in the GHSG/EBMT phase 3 ASCT trial, treatment related mortality (TRM) from salvage chemotherapy in that study was 5%. Other trials have reported a lower TRM between zero to 2%, a more acceptable level given the typically young age and lack of comorbidity typical of patients in this setting. Although the optimal number of cycles of salvage chemotherapy is unknown, two to three cycles of treatment are usually given by convention with a need to balance optimizing response and the risk of further toxicity.

The available institutional series reporting response rates to salvage chemotherapy often include a mixture of patients with primary refractory and relapsed disease with most series likely unable to demonstrate differences due to a lack of statistical power. Patients with primary refractory HL have an inferior response rate to second-line chemotherapy (51% versus 83%; $p < 0.0001$),[181] which highlights the unique and inferior biology in this group of patients. The proportion of primary refractory patients in reported series along with other imbalances of prognostic factors and typically small sample sizes in these series likely explain any potential variation in reported response rates.[163,166,176,182–184]

Despite aggressive combination chemotherapy, between 10% to 40% of patients do not achieve a response to salvage chemotherapy and there are no RCT data supporting ASCT in nonresponders. Courses of alternative salvage chemotherapy have been given in an attempt to demonstrate chemosensitive disease prior to transplant. Studies have largely assessed responses using CT scan–based criteria. These series have largely reported selected patient populations and are characterized by small numbers, although the goal of achieving a response and proceeding to ASCT occurs in approximately half of the patients.[185–188]

An important issue related to salvage chemotherapy is the potential for second-line therapy to impair the ability to mobilize peripheral blood stem cells to support potentially curative high-dose chemotherapy. The efficacy of salvage chemotherapy for HL must be balanced by toxicity and the impact on subsequent PBSC mobilization. Success rates for PBSC mobilization have not been consistently reported in the RCTs or trials assessing the efficacy of salvage therapy. Some studies report that regimens containing melphalan, such as dexa-BEAM or mini-BEAM, may result in reduced stem cell mobilization.[189–191] Available results for commonly employed regimens demonstrate that at least 80% of patients undergoing PBSC mobilization reach a minimum threshold of 2.0×10^6 CD34 cells per kilogram.[180,192]

The Role of Functional Imaging in Response Assessment Prior to Autologous Stem Cell Transplantation

The use of FDG-PET in response assessment postsalvage chemotherapy and prior to ASCT is increasing despite a lack of large prospective data. Outside of response assessment, FDG-PET

TABLE 102.11

Salvage Regimens Commonly Used for the Treatment of Relapsed and Refractory Hodgkin's Lymphoma

Regimen/Drug	Dose	Route	Schedule (Day)	Cycle Length (Days)	Reference
GVD				21	246
Gemcitabine	1,000 mg/m^2	IV	1, 8		
Vinorelbine	20 mg/m^2	IV	1, 8		
Liposomal doxorubicin	15 mg/m^2	IV	1, 8		
IGEV				21	247
Vinorelbine	20 mg/m^2	IV	1		
Gemcitabine	800 mg/m^2	IV	1, 4		
Ifosfamide	2,000 mg/m^2	IV	1–4		
Prednisone	100 mg	PO	1–4		
MESNA	1,200 mg/m^2	IV	1–4, 30 min prior then at 4 and 8 h		
DHAP				14–21	248
Cisplatin	100 mg/m^2	IV	1		
Cytarabine	2,000 mg/m^2	IV	Day 2, Q12 h × 2 doses		
Prednisone	40 mg	IV	1–4		
ICE				14	249
Ifosfamide	5,000 mg/m^2	IV	2		
Carboplatin	AUC5	IV	2		
Etoposide	100 mg/m^2	IV	1–3		
MESNA	5,000 mg/m^2	IV	2		
Augmented ICE				14	195
Ifosfamide	5,000 mg/m^2	IV	1, 2		
Carboplatin	AUC5	IV	3		
Etoposide	200 mg/m^2	IV	Day 1, Q12 hours × 3 doses		
MESNA	5,000 mg/m^2	IV	1, 2		
Brentuximab vedotin	1.8 mg/kg	IV	1	21	229
Dexa-BEAM				28	168, 250
Dexamethasone	8 mg	PO	Day 1–10, Q8 hour		
Carmustine	60 mg/m^2	IV	2		
Etoposide	75–150 mg/m^2	IV	4–7		
Cytarabine	100 mg/m^2	IV	Day 4–7, Q12 hour × 8 doses		
Melphalan	20 mg/m^2	IV	3		
Mini-BEAM				28	164, 169
Carmustine	60 mg/m^2	IV	1		
Etoposide	75 mg/m^2	IV	2–5		
Cytarabine	100 mg/m^2	IV	Day 2–5, Q12 hour × 8 doses		
Melphalan	30 mg/m^2	IV	6		
ASHAP				21	171
Doxorubicin	10 mg/m^2	IV, continuous infusion	1–4		
Cisplatin	25 mg/m^2	IV, continuous infusion	1–4		
Cytarabine	1,500 mg/m^2	IV	5		
Methylprednisolone	500 mg	IV	1–5		
VIP				28	172
Etoposide	75 mg/m^2	IV	1–5		
Ifosfamide	1,200 mg/m^2	IV	1–5		
Cisplatin	20 mg/m^2	IV	1–5		
GDP				21	174
Gemcitabine	1,000 mg/m^2	IV	1, 8		
Dexamethasone	40 mg	PO	1–4		
Cisplatin	75 mg/m^2	IV	1		

(continued)

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TABLE 102.11

Salvage Regimens Commonly Used for the Treatment of Relapsed and Refractory Hodgkin's Lymphoma *(continued)*

Regimen/Drug	Dose	Route	Schedule (Day)	Cycle Length (Days)	Reference
GEM-P				28	175
Gemcitabine	1,000 mg/m^2	IV	1, 8		
Methylprednisolone	1,000 mg	PO or IV	1–5		
Cisplatin	75 mg/m^2	IV	15		
MINE				28	176
Mitoguazone	500 mg/m^2	IV	1, 5		
Ifosfamide	1,500 mg/m^2	IV	1–5		
Vinorelbine	15 mg/m^2	IV	1, 5		
Etoposide	150 mg/m^2	IV	1–3		
IVE				21	251
Epirubicin	50 mg/m^2	IV	1		
Etoposide	200 mg/m^2	IV	1–3		
Ifosfamide	3,000 mg/m^2	IV	1–3		
MESNA	3,000 mg/m^2	IV	1–3		

IV, intravenous; PO, by mouth.

scanning can also be viewed as a biomarker with a positive test after salvage therapy, suggesting a higher rate of relapse post-ASCT (whether this is due to tumor-related or other factors in the FDG-PET avid lesion remains to be elucidated). Retrospective institutional series suggest that abnormal functional imaging (FI; either gallium or FDG-PET scan) after salvage therapy and prior to ASCT are predictive of poor outcome (3-year OS of 58% versus 87% if negative FI). In particular, patients who had achieved a PR with CT imaging could be discriminated by FI; in those with negative FI, outcome was similar to patients in CR (3-year OS of 90% in CR, 80% in PR with negative functional imaging) but significantly inferior if positive (65%).[193] A large series studying FI after ifosfamide, carboplatin, etoposide (ICE) chemotherapy reported similar results with a 5-year event-free survival (EFS) of 31% for FI-positive disease compared to 75% if negative.[194]

The group at MSKCC has reported results of a prospective study that tested the strategy of attempting to achieve a negative FDG-PET scan prior to ASCT.[195] In patients that had a positive FDG-PET scan following ICE salvage chemotherapy, a non–cross-resistant chemotherapy regimen to ICE (GVD [gemcitabine, vinorelbine, and liposomal doxorubicin]) was given as a second-line salvage chemotherapy regimen. A positive FDG-PET scan was seen in 38% of cases post-ICE; 26 of 33 patients that received GVD achieved a response (CR, PR, or MR) and went onto transplant. Of these 33 patients, a negative FDG-PET scan was achieved in 52% and their outcome appeared similar to the patients who were FDG-PET negative after ICE. These data demonstrate that the goal of FDG-PET negativity prior to autograft is likely of value and that the use of a non–cross-resistant regimen can be successful in approximately half of patients. Unfortunately, the outcome of FDG-PET–avid patients that were transplanted remains poor with an EFS of 25% at a median follow-up beyond 4 years. Validation of this observation in other series and with other commonly used regimens would help to confirm this treatment approach.

Autologous Stem Cell Transplantation High-Dose Therapy Regimens and Strategies

The role of ASCT in HL has been defined by two published phase 3 RCTs.[164,165] The GHSG/EBMT assigned 161 patients with relapsed HL to receive two cycles of dexa-BEAM chemotherapy, and randomized responding patients to either two additional cycles of dexa-BEAM or high-dose therapy and ASCT. Freedom from treatment failure at 3 years was significantly improved in the ASCT group (55 versus 34%; p = 0.02), although there was no difference in overall survival.[165] These trials of ASCT did not include chemorefractory patients; only cohort and registry data address the benefit of ASCT in these patients.[162,163,166]

The role of ASCT in lymphoma overtly refractory to chemotherapy has not been well defined in the modern literature. The Seattle group reported the outcome of 64 chemoresistant (defined as less than a partial remission) HL patients who were transplanted on protocols conducted between 1986 and 2005. At a median follow-up of 4.2 years post-ASCT, 5-year PFS and OS were 17% and 31%, respectively, suggesting inferior outcomes when compared to ASCT in chemosensitive patients.

The two randomized trials of ASCT for RR-HL used BCNU, etoposide, Ara-C, melphalan (BEAM) HDCT. Other single institution studies report outcomes with diverse regimens.[50,196–200] The lack of randomized comparisons of HDCT regimens makes it difficult to conclude that there is an optimum regimen in terms of toxicity and efficacy. Late effects including second primary malignancies, cognitive deficits, and chronic fatigue are important considerations, but the impact of HDCT on these outcomes and if they vary between regimens remain unclear.

Further intensification of high-dose regimens has not been a successful strategy in RR-HL,[50] but single-arm studies augmented-dose mobilization regimens,[47] or additional therapy after stem cell collection[52] have been reported to improve outcomes. The Cologne high-dose sequential (HDS) protocol begins with an induction phase of two cycles of DHAP chemotherapy followed by response assessment. Responders proceed to HDS, which consists of 4g/m^2 of cyclophosphamide followed by G-CSF and subsequent PBSC collection, 8 g/m^2 of methotrexate with vincristine 1.4 mg/m^2, etoposide 2 g/m^2 with G-CSF and an optional second PBSC collection, and finally, BEAM HDCT and ASCT. Based on a multicenter phase 2 pilot trial showing HDS to be feasible with acceptable toxicity, the GHSG subsequently led an RCT.[201] The HD-R2 trial, a randomized comparison of HDS therapy followed by ASCT to standard DHAP and ASCT failed to show any benefit for the experimental HDS arm over the standard arm with no significant differences in freedom from treatment failure, progression-free, or overall survival were observed.[202]

An alternative intensification strategy that has been tested is the use of tandem autologous transplants.[203] This approach was prospectively tested in a large GELA cohort study. The multicenter