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Prevention of hepatocellular carcinoma

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Is there a need to prevent hepatocellular carcinoma?

Hepatocellular carcinoma (HCC) is considered to be one of the major malignant diseases in the world today. Among the reasons for this are two that are especially relevant in considering the need to prevent this tumor. The first is the high incidence of HCC, and the second its grave prognosis.

HCC is now the fifth most common global cancer (fifth in males and eighth in females) if colon and rectal cancers and mouth and pharyngeal cancers are grouped together [1,2]. Moreover, it is either the most common tumor or among the three most common tumors in many of the most populous regions of the world [1–3]. Assuming that HCC constitutes 85% or more of primary liver cancers, 480 000 new cases of HCC were estimated to occur worldwide in the year 2000, and the tumor accounted for 4.8% of all new human cancers (6.4% among men and 3.0% among women) [1]. The incidence of HCC differs appreciably in different geographical regions, with 80% of cases occurring in low-income countries in sub-Saharan Africa or low-income countries (or occasionally countries that were low-income until relatively recently) in eastern or south-eastern Asia and the Pacific islands [1–3].

In addition to its high incidence, HCC carries a particularly poor prognosis, even more so in populations in which the tumor is very common. HCC ranks fourth in annual global cancer mortality rates [1,4]. In low-income countries with a high incidence of HCC, the tumor is responsible for as much as two-thirds of cancer deaths [1], and its annual mortality rate is virtually identical to its annual incidence [3,4]. For example, in a year in which 371 400 new cases of HCC were estimated to have occurred, 363 000 patients died as a result of the tumor, giving an annual fatality ratio of 0.97 [3]. Because of the often rapid growth rate of HCC, especially in Black African and Chinese populations, and the absence of symptoms during the early stages of the disease, the tumor is frequently advanced when the patient is first seen. Moreover, when symptomatic, HCC is seldom amenable to, and has a high recurrence rate after, resection or ablation by other means and is almost always refractory to nonoperative forms of treatment.

Thus, it is obvious that so prevalent is HCC, particularly in populous low-income countries, and so poor are the results of treatment when the tumor is symptomatic, that prevention of HCC is an urgent priority.

Definition of cancer prevention

Cancer prevention can be attempted at three levels [5].

- **Primary prevention** is preventing an etiological agent from initiating the carcinogenic process. This is the premier strategy and is achieved by eliminating, avoiding, or neutralizing the carcinogen, or by stopping the *in vivo* conversion of a precarcinogen into a carcinogen.
- **Secondary prevention** is interfering with the metabolism of a carcinogen, or preventing it from reaching its target or interacting with tissue nucleophiles, especially DNA.
- **Tertiary prevention** is preventing precancerous lesions from progressing to cancer.

The term 'secondary prevention' has in the past been loosely used to refer to the detection and surgical resection or ablation by other means of small presymptomatic tumors in population screening or case detection and surveillance programs. This intervention is a commendable attempt to improve the dismal prognosis of patients with symptomatic HCC by early diagnosis and treatment of the tumor, but it is not, by definition, prevention.

Primary prevention of a tumor depends upon knowing the risk factor or factors for the tumor, and secondary and tertiary prevention require, in addition, knowledge of the mechanisms involved in its

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pathogenesis. HCC is multifactorial in etiology and has a complex multi-step pathogenesis. A number of major and minor causal associations have been identified, although less is known about the genesis of the tumor.

Risk factors for hepatocellular carcinoma

The geographically most widely distributed of the major causal associations of HCC are chronic hepatitis B (HBV) and C virus (HCV) infections and cirrhosis, whatever its cause. Other risk factors are important in certain geographical regions only: dietary exposure to aflatoxins in parts of sub-Saharan Africa, China and Taiwan; dietary iron overload in Black Africans in parts of sub-Saharan Africa; and ditch, pond or river water contaminated by blue-green algae that produce microcystins in parts of China. Minor risk factors are oral contraceptive steroids, cigarette smoking, a number of inherited metabolic diseases (the most common of which is hereditary hemochromatosis), insulin resistance, and membranous obstruction of the inferior vena cava. The main thrust of any prevention program should be directed against the more important etiological forms of HCC.

Hepatitis B virus

Chronic HBV infection is the predominant global cause of HCC and accounts for as much as 80% of the tumor in regions with a very high incidence of HCC. More than 2 billion of the world's population are estimated to show serological evidence of present or past HBV infection, and some 385 million (approximately 6% of the global population) are chronic carriers of the virus. Of the latter, 25% or more will develop HCC [1–3] (in hyperendemic regions of the virus, such as Taiwan, more than 40% will die from HCC, cirrhosis, or both diseases [6]). HBV is today considered to be (with tobacco) the most important environmental carcinogen to which humans are exposed. In those geographical regions where the virus is hyperendemic, with carrier rates as high as 15% or more, and the incidence of HCC very high (in eastern and south-eastern Asia, some of the Pacific islands, and sub-Saharan Africa), the infection is predominantly acquired in infancy or early childhood. The main route of infection in Chinese populations is perinatal, with fewer infections occurring later by horizontal spread of the virus [7]. In contrast, relatively few infections in Black African populations occur as a result of perinatal transmission, and most of the infections are acquired a little later by horizontal spread [8]. The exact routes of horizontal transmission of HBV between young children are uncertain. HBV infections acquired this early in life have an 80–90% chance of becoming chronic, and it is these early-onset carriers that are at extremely high risk of HCC development, with lifetime relative risks as high as 100 or more [6]. The risk of malignant transformation is greater when cirrhosis is present.

Although it is known that HBV may be both directly and indirectly carcinogenic (the latter by causing chronic necroinflammatory hepatic disease), much remains to be learnt about the precise mechanisms involved.

Hepatitis C virus

Chronic HCV infection is another important global cause of HCC: 170 000 million people are currently estimated to be chronically infected with this virus (about 3% of the global population). The infection is predominantly acquired in adulthood, mainly as a result of the illicit use of intravenous drugs and transfusion of contaminated blood or blood products. Eighty per cent or more of those acutely infected become chronic carriers, and approximately 20% of the latter progress to cirrhosis. The annual risk of HCC formation in those chronically infected with HCV ranges from 8–9% in Japan to 1.9% in the United States, and the risk is at least four times greater in those with cirrhosis than in those without [9]. Complications of chronic HCV infection are currently the most frequent indication for liver transplantation in the western world.

During recent decades, the incidence of HCV-related HCC has increased markedly in Japan and to a lesser extent the USA, the UK, and some western European countries. HCV is now the major risk factor for HCC in Japan (which has a high incidence of HCC), Spain and Italy (with intermediate incidences), and many industrialized countries with a low incidence of the tumor [9]. There is a 70–80% 5-year recurrence rate after resection of HCV-induced HCC [10]. Recurrences are more likely in those with persistent viremia. When chronic HCV and HBV infections occur together, the hepatocarcinogenic potentials of the two viruses are synergistic.

HCC formation in patients with chronic HCV infection has been thought to be the result of virally induced necroinflammation [9]. However, more recent evidence suggests that the virus may also be directly carcinogenic.

Cirrhosis

All etiological forms of cirrhosis may be complicated by the development of HCC, although not with equal frequency [11]. The main culprits are chronic HBV and HCV infections and alcohol abuse. Chronic HCV infection and alcohol abuse often occur together as risk factors in industrialized countries, but the exact nature of their interaction remains uncertain. Apart from the cause of the cirrhosis, increasing age and duration of cirrhosis and male sex are the major risk factors for malignant transformation in cirrhotic patients [11]. The increased hepatocyte turnover rate in cirrhosis acts as a potent tumor promoter. In addition, reactive oxygen and nitrogen species generated by the chronic

necroinflammatory process are mutagenic and carcinogenic [11].

Aflatoxins

Aflatoxins are structurally related difuranocoumarin derivatives produced mainly by certain species of *Aspergillus flavus* and *A. parasiticus*. These molds are ubiquitous, but because humidity and moisture content of plants are important factors in determining growth and toxin production by the molds, contamination of crops occurs particularly in tropical and subtropical countries with warm, humid climates. Certain staple foodstuffs, such as maize, groundnuts, fermented soy beans, and soy source, are prone to contamination [12–14]. Contamination takes place both during growth of the crops and as a result of their improper storage. Of the aflatoxins (B_1, B_2, G_1, G_2), aflatoxin B_1 (AFB_1) is the one most often found in contaminated human foodstuffs and is the most potent hepatocarcinogen in both humans and a variety of experimental animals [12–14]. The importance of AFB_1 as a human hepatocarcinogen is enhanced when highs levels of dietary exposure to this toxin occur in regions highly endemic for HBV infection. The carcinogenic effects of the two agents are then synergistic and multiplicative relative risks for HCC are recorded [15].

The liver is the primary site for biotransformation of ingested aflatoxins. The parent molecule is innocuous but it is converted by cytochrome P450 to electrophilic intermediates that are mutagenic [12–14]. During phase I metabolism the parent molecule undergoes a two electron oxidation by CYP3A4 and CYP1A2 to form AFB_1-8,9-*exo*-epoxide and AFB_1-8,9-*endo*-epoxide. AFB_1-8,9-*exo*-epoxide is the more highly reactive of the two. Other metabolites are formed from AFB_1, including AFQ_1, AFM_1, and AFP_1. These are poorer substrates for epoxidation and, consequently, are less mutagenic than AFB_1. CYP3A4 is the predominant cytochrome P450 in the human liver and it forms *exo*-epoxide and AFQ_1, whereas CYP1A2 forms some *exo*-epoxide but also a high proportion of *endo*-epoxide and AFM_1.

The reactive *exo*- and *endo*-epoxides are detoxified by a number of pathways. The principal route is by glutathione-S-transferase-mediated conjugation with reduced glutathione to form AFB_1-*exo*- and -*endo*-epoxide-glutathione-S-transferase conjugates [12]. The *exo*-epoxide-glutathione-S-transferase conjugate is converted to AFB_1-mercapturic acid, which is excreted in the urine. Both *exo*- and *endo*-epoxides can also undergo rapid nonenzymatic hydrolysis to AFB_1-8,9-dihydrodiol [12]. If the quantity of AFB_1 ingested in the diet exceeds the capacity of the phase II pathways to detoxify the AFB_1-8,9-*exo*-epoxide formed or if, for any reason, the activity of these pathways is decreased (for example, by polymorphisms of the glutathione-S-transferase gene), the highly reactive metabolite accumulates and binds with high affinity to guanine bases in cellular DNA to form the 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy-AFB_1 DNA (AFB_1-N^7-guanine) adduct [12]. This adduct can give rise to guanine to thymine transversions in cellular DNA. AFB_1 and AFG_1 dialdehydes do not bind to DNA but form Schiff bases with primary amine groups (e.g. lysine), to form protein adducts such as AFB_1-albumin [12].

Iron overload

Iron overload of the liver occurs mainly in two diseases, hereditary hemochromatosis and dietary iron overload in the Black African (formerly called Bantu visceral siderosis). HCC often complicates hereditary hemochromatosis, and the longer the patient survives the greater is the risk (the relative risk may be as high as 200) [16]. Cirrhosis is a very common complication of the excess hepatic iron and was thought to be an essential precursor of the malignant transformation. However, in recent years about 30 patients with hereditary hemochromatosis but without cirrhosis have been reported to develop HCC, raising the possibility that excess hepatic iron may be directly carcinogenic as well as inducing HCC indirectly by causing chronic necroinflammatory hepatic disease. In some patients with hereditary hemochromatosis HCC has developed after therapeutic iron depletion. This should not discount the potential role of excess iron in hepatocarcinogenesis. The carcinogenic effects of chemicals may become evident only 20 or more years after exposure, and it may be argued that the pathogenetic mechanisms responsible for malignant transformation were irreversibly under way before iron depletion was accomplished.

Similar levels of hepatic iron may be present in dietary iron overload in the Black African [17]. This condition results from the consumption of large quantities of alcoholic beverages that are brewed in iron drums or pots and have a high iron content. During the process of fermentation, the pH of the ferment drops to a very low level, causing iron to leach out of the container into the contents. Cirrhosis accompanies iron overload in fewer of these patients than occurs in hereditary hemochromatosis [16], but this condition too is complicated by HCC formation, with a relative risk of 10.6 (95% confidence limits 1.5–76.8) and a population attributable risk of 29 in one study [18]. Support for a direct carcinogenic effect of excess hepatic iron is provided by a recent report of the formation of iron-free foci and HCC in the absence of cirrhosis or portal fibrosis in an animal model (Asare, Paterson, Kew and Mossando, unpublished observations).

Microcystins

In most rural regions in China with a high incidence of HCC, the population drinks primarily pond or ditch water. Drinking water from these artificial sources or

(to a lesser extent) river water rather than deep-well water, is a risk factor for HCC in some of these regions; relative risks of 1.9 (95% confidence interval 1.01–4.74) and 2.9 (95% confidence interval 2.59–3.27) have been recorded in Haimen and Fusui, respectively [19]. Microcystins derived from blue-green algae have been identified in pond and ditch water in high incidence regions of HCC and differences noted in the microcystin content of the drinking water of HCC patients and controls [19]. In experimental studies in rats microcystins act as a tumor promoter in aflatoxin-induced liver cancers [20].

Prevention of hepatocellular carcinoma

Attempts to prevent HCC are of relatively recent origin, but there is every prospect that it will eventually be possible to prevent most cases of this common and devastating tumor. For the immediate future the emphasis should be on practical and economical interventions in countries with high incidences of HCC, especially low-income countries.

Primary prevention

Primary prevention is, at least theoretically, the most effective form of cancer prevention. It lends itself particularly well to intervention in viral, chemical, and physical causes of cancer. Given that oncogenic hepatitis viruses contribute to the development of 75–80% of global HCC, prevention of these chronic infections would have a huge impact on the global occurrence of the tumor. Primary prevention could best be accomplished by immunization against the viruses. This approach became possible when an effective and safe vaccine against HBV became available in the 1970s.

Immunization against hepatitis B virus infection in the prevention of hepatocellular carcinoma

HBV vaccine has been incorporated into the Expanded Program of Immunization (EPI) in 181 countries, including most, if not all, countries in eastern and south-eastern Asia and the Pacific islands. As a result, 80–90% of babies in these countries are now being immunized against HBV. This accomplishment has already resulted in a 90% to 15% decrease in the percentage of chronically infected babies born to highly infectious carrier mothers in these countries, and a 10-fold or more decrease in the rate of chronic HBV carriage in the age groups immunized [21]. Because of the long interval between initial infection with HBV and the development of HCC, it will take 30–50 years for a decrease in incidence of HBV-induced HCC to be realized in adults in these countries. Nevertheless, in Taiwan, where immunization of babies against HBV began in 1984 and universal coverage was achieved by 1986, the prevalence of HCC among children in the age group that had received the vaccine has decreased by 70% in comparison with children in the nonvaccinated age groups [21]. In the early reports, the decrease was greater in boys than girls [22], but in later studies based on a larger number of children this difference was not evident (70% decrease in boys, 62% in girls) [23]. These findings augur well for the eventual elimination of HBV infection in that region. Unfortunately, in sub-Saharan Africa, for a number of reasons but mainly because of financial constraints, competing health care priorities (HIV/AIDS, malaria, tuberculosis, measles, and diarrheal illnesses) and poor delivery services not able to access large parts of the populations, $<10\%$ of babies have until recently been immunized against the virus. However, with the provision of financial backing from the Global Alliance for Vaccines and Immunization (GAVI), the Vaccine Fund, and other governmental and nongovernmental sources, the dismal picture in sub-Saharan Africa is now changing for the better [24]. The extremely encouraging results achieved in the Far East give promise that the universal incorporation of HBV vaccine into the EPI in all countries in this region and in sub-Saharan Africa will prevent approximately 1 000 000 deaths per year from HCC and cirrhosis in future birth cohorts [24], and that with universal immunization throughout the world HCC caused by this virus could ultimately be completely prevented.

Because of the early onset of chronic HBV infection in highly endemic regions, immunization should be performed shortly after birth. In populations in which perinatal transmission from highly infectious HBV e antigen-positive mothers to their babies predominates, the highest level of protection against the virus (86%) is achieved when the first dose of the vaccine is given as soon after birth as possible together with hyperimmune hepatitis B globulin as passive prophylaxis, followed by the second and third doses of the vaccine at 1 and 6 months, respectively [21]. In practice, because of its high cost, hyperimmune globulin is seldom given, and this decreases the rate of protection by 5–10%. Active immunization with three or four doses of vaccine without hyperimmune globulin is immunogenic in 90% of neonates born to noncarrier mothers or HBeAg-negative carrier mothers [21]. In regions in which the majority of the infections that become chronic are acquired a little later in life by the horizontal route, the first dose of the vaccine is being given slightly later and without passive immunization. Three injections are normally given. The need for a booster dose at the time of entering school has not yet been resolved.

The introduction of HBV vaccine into the EPI in most countries and the beneficial effect this has already had in reducing viral carriage rates and the occurrence of HCC in vaccinated children is undoubtedly the most promising and far-reaching development in the prevention of this tumor and indeed, indirectly, other virally induced tumors. HBV vaccine can rightfully claim to be the first 'anti-cancer vaccine'. The universal

inclusion of HBV vaccine in the EPI throughout the world has already required and will continue to require considerable investment of resources and time. Regrettably, health workers and epidemiologists concerned with the prevention of cancer are still largely unaware of this momentous development in global cancer control [24].

Immunization against hepatitis C virus in the prevention of hepatocellular carcinoma

Despite considerable research over a number of years into the development of a vaccine against HCV, there appears to be little likelihood of such a vaccine becoming available in the near future. Difficulties impeding the development of this vaccine include the extreme variability of the genomic structure of the virus, especially in the hypervariable region, the large number of quasispecies in the blood of infected individuals, and the lack of evidence for an effective neutralizing antibody against the virus.

Other forms of primary prevention against HBV and HCV

Because the full beneficial effects of immunization against HBV will not be felt for many years and there is no early prospect of a vaccine against HCV, other methods of preventing the spread of these viruses must continue to be rigidly enforced in an attempt to prevent HCC induced by these viruses. These precautions are more important in preventing HCV-related HCC because persistent infection with this virus occurs predominantly in adulthood as a result of intravenous drug abuse and transfusion of contaminated blood or blood products [25], routes that are more amenable to prevention than those responsible for the great majority of the chronic infections leading to HBV-induced HCC [7,8]. In spite of recent advances in treating HCV infection, the overall impact of therapy is relatively small because the majority of chronically infected individuals are unaware that they are infected [25]. Consequently, prevention of infection with this virus by means other than vaccination and anti-viral treatment will continue to be an important strategy for the foreseeable future. Efforts to prevent infection should focus on identifying persons at increased risk of HCV infection and providing them with counselling and testing for the presence of the virus, as well as reducing both the incidence of new infections and the risk of progression to chronic liver disease. The following practices should be introduced on as wide a scale as possible [25].

1. *Safe injection practices.* These are based on education of medical, paramedical and dental practitioners to avoid the use of unnecessary injections and to improve the safety of injection and infusion techniques. The latter includes rigid adherence to the use of needles and syringes on a single occasion only or, if this is not possible, the unfailing use of fool-proof methods of sterilization of needles or syringes that have to be re-used. Also important is the avoidance of, or correct use of, multi-dose vials, and lessening the risk of nosocomial infections resulting from needle-stick injuries by the proper disposal of used needles and, and whenever possible, the use of 'disposal-proof' needles.

Preventing HCV and HBV infections in illicit drug users remains a difficult and sometimes contentious issue. Changes in injection practices that will minimize sharing of contaminated equipment by providing 'needle and syringe exchange programs' (which should include exchange not only of needles and syringes but also all the other drug paraphenalia) on as wide a scale as is possible is a pivotal part of any program to prevent infection spread of HCV or HBV among drug addicts.

2. *Screening of donated blood for the presence of hepatitis viruses.* Transfusion-associated HCV and HBV infections have been virtually eliminated in industrialized countries by screening all donated blood with sensitive assays for detecting these viruses. Regrettably, screening of donated blood for blood-borne viruses is not performed in many low-income countries. Rectifying this hazardous practice is an essential step in preventing HCV-induced HCC and to a lesser extent HBV-induced HCC in these countries.

3. *The rational use of viral inactivation steps in the manufacture of blood products.*

4. *Passive immunization.* Passive immunization with hepatitis B hyperimmune globulin is useful in preventing transmission of HBV, but it is expensive and its effect is of limited duration. Its use alone will not prevent all cases of perinatal HBV infection, and the fact that it is more expensive and less effective than HBV vaccine precludes its use in this situation. The value of immune globulin in preventing HCV infections has still to be ascertained.

5. *Anti-viral agents.* Treatment with currently used anti-viral agents has limited efficacy in the sustained eradication of hepatitis B and C viruses, and so does relatively little in preventing the spread of these viruses. Nevertheless, treatment with interferon-α (IFN-α) of individuals chronically infected with these viruses reduces the risk of, or delays the development of, HCV-induced HCC and possibly HBV-induced HCC (see Tertiary prevention, below).

Aflatoxin B_1 exposure

Contamination of staple foodstuffs by AFB_1 does not occur in industrialized countries because those foodstuffs that might be affected are screened for their

aflatoxin content by governmental agencies and do not enter the market if unacceptably high levels are found. The problem occurs in low-income countries where the crops are either consumed by the subsistence farmer or are sold locally or regionally without ever coming under the scrutiny of a governmental agency. Because contamination by *A. flavus* or *A. parasiticus* takes place both during growth of the crops and as a result of their improper storage, attempts at primary prevention need to be focused on minimizing both sources of fungal contamination and AFB_1 production [12–14]. One possible intervention is to alter agricultural practices in regions of high dietary AFB_1 intake by replacing crops that are highly susceptible to fungal contamination with others, such as rice, at lower risk. This approach has been successfully used in one limited study in China when a change to a rice-based diet resulted in an appreciable decrease in AFB_1 intake, but for most communities in low-income countries a change in diet is not feasible [12–14]. Relatively simple pre-harvest prevention could involve spraying with fungicides and, because damaged plants are more susceptible to fungal contamination, increasing the resistance of the plants to fungal infection by ensuring adequate irrigation and spraying with insecticides [12–14]. In the longer term contamination of growing crops might be prevented by the introduction of non-aflatoxigenic strains of *Aspergillus* to compete with the aflatoxin-producing strains, or by genetically engineering foodstuffs that are resistant to infection by *Aspergillus* species. However, these methods may not be affordable in countries with the greatest need to prevent dietary exposure to AFB_1.

The likelihood of contamination during storage is increased by excessive moisture and any form of damage to the crops. Methods of combating this include sun-drying of the crops before storage and drying on cloth rather than directly on the earth; well-ventilated, rain-proof storage facilities; storage in jute rather than plastic sacks and in wooden containers rather than on the earth; removal of visibly moldy plants by hand sorting; and the use of insecticides to control insect damage and fungicides to prevent spread of fungal spores [12,13]. A study of the effectiveness of these preventive methods is underway in Conakry, Guinea [26]. If these interventions are to be successful in practice, education of subsistence farmers in their use and the provision of financial assistance for their institution will be required. Even so, they are unlikely to completely eliminate contamination of stored staple foodstuffs by AFB_1.

Dietary iron overload in the African

Dietary iron overload has virtually disappeared from urban Black African populations as a result of a change in their drinking habits from home-brewed sorghum-based beverages with a high iron content to commercially available iron-free varieties of alcohol. However, the pattern of alcohol consumption in rural areas remains largely unchanged. Attempts at intervention would require education about the health hazards of alcohol brewed in iron drums or pots, backed up by the provision of suitably sized aluminium or other iron-free containers. Such a program has yet to be attempted on a large scale.

Blue-green algae and microcystins

Since 1973 the Chinese government has been urging the rural population to drink water from deep wells [27]. This has resulted, for example in Qidong county, in 80% of the population now drinking deep well-water compared with 20% in the 1970s [27]. In addition, in some regions the drinking water is treated by granular-activated carbon filtration. It is too soon to assess the effect of these interventions on the occurrence of HCC.

Secondary prevention

Secondary chemoprevention entails the use of natural or synthetic chemicals to block, retard, or reverse the carcinogenic process. Effective strategies need to be safe, inexpensive, and mechanistically simple. There are currently two etiological forms of HCC in which preliminary evidence indicates that chemoprevention might be possible. These are AFB_1-induced and alcohol-induced HCC.

Chlorophyllin

The possibility of modulating the balance between metabolic activation and detoxification of AFB_1 lends itself to chemoprevention of this cause of HCC. Sodium copper chlorophyllin is derived from natural chlorophylls and has been used as a food colorant and in a number of over-the-counter medicines for controlling odor in geriatric and osteomy patients and as an accelerant in wound healing [14,28]. It is an effective anti-carcinogen in a number of experimental models [29], including AFB_1-induced HCC. Chlorophyllin forms tight molecular complexes with a number of chemical carcinogens, including aflatoxins, thereby reducing their bioavailability and hence their carcinogenic potential [30]. It also acts as an antioxidant to inhibit lipid peroxidation [31]. A single randomized, double-blind, placebo-controlled trial has thus far been conducted in Qidong county, China [28]. This showed that 100 mg of chlorophyllin, administered three times a day for 4 months, caused a 55% reduction in the median level of urinary excretion of the AFB_1 DNA adduct, AFB_1-N^7-guanine, when compared with placebo. No toxic side effects were observed and compliance to the drug was good. Further trials are needed to ascertain whether the long-term administration of this drug will be feasible and safe. Supplementation of the diet with foods that are rich in chlorophylls, such as spinach and other leafy green vegetables, might be a more practical alternative.

Oltipraz

A second approach is to modify the detoxification pathway of AFB_1 in such a way as to render its reactive metabolite innocuous. The anti-schistosomal drug, oltipraz (a substituted 1,2-dithiole-3-thione) is structurally similar to the dithiolethiones found in cruciferous vegetables that may play a role in cancer prevention [14,32]. Oltipraz is a potent inducer of the expression of the phase II detoxifying enzyme, glutathione-S-transferase, and also regulates the transcription of genes encoding other conjugating or antioxidative enzymes, and might therefore be effective in the secondary prevention of AFB_1-induced HCC [32]. Keap1 (Kelch-like ECH-associated factor 1) sequesters Nrf2, a member of the nuclear factor erythroid-derived 2 family, in the cytoplasm by binding to its amino-terminal regulatory domain [33]. Treatment with oltipraz disrupts the interaction between KEAP1 and NRF2, allowing NRF2 to translocate to the nucleus, where it forms heterodimers with small MAF-family proteins to activate the expression of glutathione-S-transferase and other genes [13]. More recent studies of the pharmacodynamic effects of oltipraz have shown that the drug also has an inhibitory effect on certain phase 1 enzymes, including CYP3A4 and CYP1A2 [34]. It therefore reduces the activation of AFB_1 to AFB_1-8,9-*exo*-epoxide. Oltipraz thus affects the metabolism of AFB_1 at two levels.

A randomized, placebo-controlled, double-blind trial conducted in Qidong county, China, showed a 2.6-fold increase in the urinary excretion of the AFB_1-8,9-*exo*-epoxide metabolite, AFB_1-mercapturic acid, and lesser increases in excretion of other AFB_1 biomarkers [35]. However, because of cost and safety considerations, it is doubtful whether this drug could be used on a wide scale in the secondary prevention of HCC.

Polyprenoic acid

Crucial events in alcohol-induced hepatic damage are an altered homeostasis and depletion of retinoids (retinyl esters, retinal, and retinoic acid) [36] and the proliferative activation of hepatocytes, changes that may provide a promoting environment for hepatocarcinogenesis. Malfunction of the retinoid nuclear receptors may do likewise [36]. Possible mechanisms for these actions may involve 'cross-talk' with the alcohol-activated JNK-dependent signalling pathway and inhibition of c-jun and c-fos activity and the induction of apoptosis. The restoration of retinoids and their homeostasis by either dietary supplementation or the use of an inhibitor of retinoid metabolism might thus have a secondary preventive effect.

Polyprenoic acid, an acyclic retinoid, was originally shown to have chemopreventive properties in experimental models of liver cancer [36]. In addition, the agent suppresses cell growth and induces differentiation and apoptosis in human cancer cell lines [36]. Oral administration of polyprenoic acid over 12 months has been reported in a double-blind, placebo-controlled trial in Japanese patients to delay and perhaps prevent second HCCs after resection of the initial tumor or its eradication using percutaneous ethanol injection, and to significantly improve survival rate [7,38]. Serum lactin-reactive α-fetoprotein (AFP-L3), which indicates the presence of transformed hepatocytes in the remnant liver, disappeared after 12 months of administration in the polyprenoic acid group but not in the placebo group. This observation suggests that the AFP-L3-producing latent malignant clones were eliminated from the remnant liver by the polyprenoic acid and prevented recurrence of HCC [38,39]. The findings in this trial will need to be confirmed in further studies with a prolonged follow-up period before its use for this indication can be advocated.

A whole host of chemicals have been shown in animal models or tissue culture experiments to prevent cancer formation or to have properties that suggest that they might have chemopreventive potential. However, until the use of these substances can be shown to be effective, safe and feasible in humans, they cannot be considered to have a role in the secondary prevention of human HCC. The same is true of a number of molecular manuevers performed in transgenic animal models, and of dietary changes in animals known to spontaneously develop liver cancers.

Other chemoprevention possibilities

A decrease in the risk of HCC correlates with an increased consumption of leafy green vegetables [40]. These vegetables contain a range of biologically active phytochemicals. Plants belonging to the family Cruciferae and the genus *Brassica* (including broccoli, cauliflower, and Brussels sprouts) contain large quantities of isothiocyanates, mostly in the form of their glycosinolate precursors. Some of these isothiocyanates have been shown to inhibit tumor formation in rats [41]. Trials of the use of glycosinolates and isothiocyanates from broccoli sprouts in preventing HCC in cohorts of subjects at high risk for the tumor are in progress [13].

Hereditary hemochromatosis

Whether or not excess hepatic iron is proved to be directly hepatocarcinogenic, 'de-ironing' of patients with hereditary hemochromatosis by repeated venesection would be expected to have a secondary preventive effect against HCC formation both by reversing the accumulation of iron and preventing the development of cirrhosis. Analyses have shown that removing the iron dramatically improves prognosis. Life expectancy reverts to normal and the number of cases of HCC decreases if cirrhosis is not present, and survival improves considerably in those with cirrhosis [42]. 'De-ironing' aims to lower the serum ferritin concentration to a normal level and to keep it there. This

intervention is preferably commenced as soon as the diagnosis of hereditary hemochromatosis is made and should be continued for life.

Tertiary prevention

Chronic necroinflammatory hepatic disease, commonly in the form of cirrhosis, less often chronic hepatitis, and rarely 'reversed lobulation' resulting from chronic suprahepatic portal hypertension, is a premalignant condition [11]. The three most common causes of chronic necroinflammatory hepatic disease complicated by the development of HCC are HBV and HCV infections and alcohol abuse. In the first two, attempts have been made to prevent progression of chronic hepatitis or cirrhosis to HCC by treating the preneoplastic virally induced necroinflammation. IFN has been most widely used for both HCV- and HBV-induced diseases, and glycyrrhizin has been tried in chronic HCV infection. These agents may be regarded as being immunopreventive by functioning as biological response modifiers.

Use of IFN-α in preventing HCV-related HCC

HCV-related HCC is becoming an increasingly important problem in many industrialized countries. Its development is closely related to the duration and progression of chronic HCV hepatitis and cirrhosis. A large number of studies of the use of IFN treatment in preventing the development of HCC in patients with chronic HCV-related hepatitis or cirrhosis have now been reported, particularly from Japan and Europe [43]. Most, but not all, have shown that such treatment reduces the risk of progression to HCC or delays the development of the tumor [43]. However, many of the published analyses, particularly the earlier ones, have had defects in design, although some of these have been difficult to avoid because of ethical and logistic considerations. Particularly important among the defects have been poor matching of treated and untreated patients and insufficient length of patient follow-up. It takes at least 5 years to show a statistically significant difference between the hepatocarcinogenesis rate in IFN-treated and -untreated patients. Another difficulty in proving a beneficial effect of IFN treatment is the high rate of carcinoma development even in the treated group.

IFN-induced prevention or delay in HCC formation has been shown to be likely in patients with chronic HCV hepatitis, but conflicting results have been obtained when cirrhosis has been present. However, a recent meta-analysis of 11 studies involving 2178 patients with HCV-related cirrhosis confirmed that IFN significantly reduced the risk of tumor formation [44]. The reduction in rates of malignant transformation complicating both chronic hepatitis and cirrhosis has been far greater in patients in whom a sustained response to IFN was obtained [45–47]. In some studies the beneficial effect of IFN was confined to patients in whom a sustained viral clearance was achieved, whereas in others it also accompanied a sustained biochemical response (normalization of serum transaminase levels) [45–48]. Nevertheless, the tumor may develop even with complete elimination of the virus and normalization of the serum transaminase levels, and IFN does not prevent HCC development in nonresponders.

HCC is more likely to develop in the presence of severe hepatic fibrosis [48]. Fibrosis has been shown either to progress more slowly or to regress in patients in whom IFN induces a sustained virological response and normalization of serum transaminase levels, indicating that the antifibrotic effects of IFN may contribute to its chemoprevention capability [49,50].

Support for a tertiary preventive effect of IFN is provided by studies that show that this treatment prevents recurrences of HCC after initial resection or ablative treatment [51,52], although further more carefully controlled trials are needed to put the issue beyond doubt.

The mechanism or mechanisms by which IFN reduces the risk for HCC are uncertain. Clearance of the virus and reduction in hepatic inflammation are obvious factors, but another mechanism may be upregulating the function of tumor suppressor genes [53]. IFN is known to have a tumor inhibiting effect on HCC cell lines [54].

The combination of pegylated IFN and ribavirin has improved the sustained virological clearance rates in patients with chronic HCV infection. However, the use of this combination in the prevention of HCC development has not yet been reported.

Use of IFN-α in preventing chronic HBV-induced HCC

In contrast to the many studies of the effect of IFN in preventing the development of HCC in patients with HCV-induced chronic hepatitis or cirrhosis, few studies have addressed this issue in patients with HBV-related disease and the findings have been conflicting. Most studies have failed to provide convincing evidence that treating patients with HBV-related cirrhosis or chronic hepatitis with IFN will lessen the risk of malignant transformation. A recent meta-analysis of the available literature showed that consistent results were observed only with studies emanating from European countries and these did not indicate a protective effect of IFN [55]. Chronic HBV carriers with low serum HBV DNA levels seldom progress to tumor formation [56]. Those with very high levels may prove to the ones in whom IFN does protect against the development of HCC.

Glycyrrhizin

Glycyrrhizin, the active principle of licorice, has a chemical structure similar to cortisone. It is composed of one molecule of glycyrretinic acid and two molecules

of glucuronic acid. Glycyrrhizin enhances IFN-γ production, has immune-modulating activity, stimulates natural killer cells, and suppresses hepatic inflammation [57,58]. The compound also has antioxidative properties. It has been used for over 30 years in Japan, partly in the form of the Chinese herbal medicine, Sho-saiko-to, of which glycyrrhizin is one of the main ingredients, in the treatment of chronic hepatitis (by far the most common cause of which is HCV). Daily injections of glycyrrhizin reduce the levels of the serum aminotransferases in a dose-dependent manner in these patients, although antiviral activity *per se* is not evident [59]. In a prospective trial performed in Japan over 15–20 years, a 2.5-fold lower risk of developing HCC was reported in patients who received parenteral glycyrrhizin compared with those who did not [59]. HCV-RNA titers did not decrease, and the beneficial effect of the drug was attributed to controlling or retarding necroinflammatory and fibrotic processes in the liver. These conclusions were supported by two further studies [60,61]. In addition, glycyrrhizin reduced the risk of HCC recurrence after surgical resection [62].

References

[1] Ferlay J, Bray J, Pisani P, Parkin DM. GLOBOCAN 2000: Cancer incidence, mortality and prevalence worldwide. Version 1.0. IARC CancerBase No. 5. International Agency for Research on Cancer. Lyon: IARC Press, 2001.

[2] WHO Mortality Database. World Health Statistics Annuals, 1998.

[3] Bosch FX, Ribes J. The epidemiology of primary liver cancer: global epidemiology. In: Tabor E, ed. Viruses and liver cancer. Amsterdam: Elsevier, 2002:1–16.

[4] Bosch FX, Ribes J, Borras J. Epidemiology of primary liver cancer. Semin Liver Dis 1999;19:271–85.

[5] Farber E. Approaches to cancer prevention: introduction and perspective. Proc Am Assoc Cancer Res 1988;29:527–8.

[6] Beasley RP, Hwang L-Y, Lin CC, Chien CS. Hepatocellular carcinoma and HBV: a prospective study of 22,707 men in Taiwan. Lancet 1981;22:1129–33.

[7] Stevens CE, Szmuness W. Vertical transmission of hepatitis B and neonatal hepatitis B. In: Bianchi L, Gerok W, Sickinger K, Stalder GA, eds. Virus and the liver. Lancaster, UK: MTP Press, 1980:71–4.

[8] Botha JA, Ritchie MJJ, Dusheiko GM, Mouton HWK, Kew MC. Hepatitis B virus carrier state in Black children in Ovamboland: role of perinatal and horizontal infection. Lancet 1984;2:1209–12.

[9] Kew MC. Hepatitis C virus and hepatocellular carcinoma in developing and developed countries. Vir Hepatit Rev 1998;4: 259–69.

[10] Kumada T, Nakano S, Takeda I, Sugiyama K, Osada T, Kiriyama S, et al. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. Hepatology 1997;25:87–92.

[11] Kew MC. The role of cirrhosis in hepatocarcinogenesis. In: Bannasch P, Keppler D, Weber G, eds. Liver cell carcinoma. Dordrecht: Kluwer Academic Publishers, 1989:37–46.

[12] Wild CP, Turner PC. The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis 2002;17:471–81.

[13] Kensler TW, Qian GS, Chen JG, Groopman JD. Translational strategies for cancer prevention in liver. Nature Rev 2003;3: 321–9.

[14] Sudakin DL. Dietary aflatoxin exposure and chemoprevention of cancer: a clinical review. J Toxicol Clin Toxicol 2003;41: 195–204.

[15] Kew MC. Synergistic interaction between aflatoxin B_1 and hepatitis B virus in hepatocarcinogenesis. Liver Int 2003;23: 1–5.

[16] Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and non-cirrhotic patients with hereditary hemochromatosis. N Engl J Med 1985;313:1256–62.

[17] Isaacson C, Seftel HC, Keeley KJ, Bothwell TH. Siderosis in the Bantu: the relationship between iron overload and cirrhosis. J Lab Clin Med 1981;58:845–53.

[18] Mandishona E, MacPhail AP, Gordeuk VR, Kedda MA, Paterson AC, Roualt TA, et al. Dietary iron overload as a risk factor for hepatocellular carcinoma in Black Africans. Hepatology 1998;27:1563–6.

[19] Yu SZ, Chen G. Blue green algae toxins and liver cancer. China J Cancer Res 1994;6:9–17.

[20] Zhou TL, Yu SZ. Laboratory study on the relationship between drinking water and hepatoma: quantitative evaluation using GGT method. China J Prev Med 1990;234:203–5.

[21] Chang M-H. Decreasing incidence of hepatocellular carcinoma among children following universal hepatitis B immunization. Liver Int 2003;23:309–14.

[22] Chang W-H, Shau WY, Chen CJ, Wu TC, Kong MS, Liang DC, et al. The effect of universal hepatitis B vaccination on hepatocellular carcinoma rates in boys and girls. JAMA 2000; 284:3040–2.

[23] Lee CL, Hsieh KS, Ko YC. Trends in the incidence of hepatocellular carcinoma in boys and girls in Taiwan after large-scale hepatitis B vaccination. Cancer Epidemiol Biomarkers Prev 2003;12:57–9.

[24] Kane MA. Global control of primary hepatocellular carcinoma with hepatitis B vaccine: the contributions of research in Taiwan. Cancer Epidemiol Biomarkers Prev 2003;12:2–3.

[25] Kew MC, Francois G, Lavanchy D, et al. Prevention of hepatitis C virus infection. J Med Virol 2004 (in press).

[26] Turner PC, Sylla A, Diallo MS, Castegnaro JJ, Hall AJ, Wild PC. The role of aflatoxins and hepatitis viruses in the etiopathogenesis of hepatocellular carcinoma: a basis for primary prevention in Guinea-Conakry, West Africa. J Gastroenterol Hepatol 2002;17:S441–S448.

[27] Yu SZ. Primary prevention of hepatocellular carcinoma. J Gastroenterol Hepatol 1995;10:674–82.

[28] Egner PA, Wang J-B, Zhu YR, Zhang BC, Wu Y, Zhang QN, et al. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk of liver cancer. Proc Natl Acad Sci U S A 2001;98:14601–6.

[29] Dashwood R, Negishi T, Hayatsu H, Breinholt V, Hendricks J, Bailey G. The importance of using pure chemical in (anti) mutagenicity studies: chlorophyllin as a case in point. Mutat Res 1998;399:245–53.

[30] Breinholt V, Schimerlik M, Dashwood R, Bailey GS. Mechanisms of chlorophyllin anticarcinogenesis against aflatoxin B_1: complex formation with the carcinogen. Chem Res Toxicol 1995;8:506–14.

[31] Kamat JP, Boloor KK, Devasgayan TP. Chlorophyllin as an effective anti-oxidant against the membrane damage in vitro and ex vivo. Biochim Biophys Acta 2000;1487:113–27.

[32] Morel F, Fardel O, Meyer DJ, Langouet S, Gilmore KS, Meunier B, et al. Preferential increase of glutathione-S-transferase class α transcripts in cultured human hepatocytes by phenobarbital, 3-methylcholanthrene and dithiolethiones. Cancer Res 1993;53:231–4.

[33] Itoh K, Wakabayashi N, Kotoh H, Ishii T, Igarashi K, Engel JD, et al. Keap1 represses nuclear activation of antioxidant

responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev 1999;13:76–86.

[34] Langouet S, Coles B, Morel F, Becquemont L, Beaune P, Guengerich FP, et al. Inhibition of CYP1A2 and CYP3A4 by oltipraz results in the reduction of aflatoxin B_1 metabolism in human hepatocytes in primary culture. Cancer Res 1995;55: 5574–9.

[35] Jacobson LP, Zhang BC, Zhu YR, Wang JB, Wu Y, Zhang QN, et al. Oltipraz chemoprevention trial in Qidong, People's Republic of China: study design and clinical outcomes. Cancer Epidemiol Biomarkers Prev 1997;6:257–65.

[36] Okuno M, Kojima S, Moriwaki H. Chemoprevention of hepatocellular carcinoma: concept, progress and perspectives. J Gastroenterol Hepatol 2001;16:1329–35.

[37] Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, et al. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. N Engl J Med 1996;334:1561–7.

[38] Muto Y, Moriwaki H, Saito A. Prevention of second primary tumors by an acyclic retinoid in patients with hepatocellular carcinoma. N Engl J Med 1999;340:1046–7.

[39] World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997.

[40] Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acd Sci USA 1997;94: 10367–72.

[41] Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, et al. Hepatitis C genotypes and the risk of hepatocellular carcinoma in cirrhosis: a prospective study. Hepatology 1997;25:754–8.

[42] Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer D, et al. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110: 1107–19.

[43] Tabor E. Interferon for preventing and treating hepatocellular carcinoma associated with hepatitis B and C viruses. Dig Liv Dis 2003;35:297–305.

[44] Yuen MF, Hui CK, Cheng CC, Wu CH, Lai YP, Lai CL. Long-term follow-up of interferon-α treatment in Chinese patients with chronic hepatitis B infection: the effect of hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. Hepatology 2001;34:139–45.

[45] Marcellin P, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, et al. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-α therapy. Ann Intern Med 1997;127:875–81.

[46] Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Hepatology 1998;27:1394–402.

[47] Ikeda K, Saitoh S, Kobayashi M, Suzuki Y, Suzuki F, Tsubota A, et al. Long-term interferon for 1 year or longer reduces the hepatocellular carcinogenesis rate in patients with liver cirrhosis caused by hepatitis C virus: a pilot study. J Gastroenterol Hepatol 2001;16:406–15.

[48] Ikeda A, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999; 29:1124–30.

[49] Omata M, Yoshida H. Resolution of liver cirrhosis and prevention of hepatocellular carcinoma by interferon therapy against chronic hepatitis C. Scand J Gastroenterol Suppl 2003;237:47–51.

[50] Takimoto M, Ohkoshi S, Ichida T, Takeda Y, Nomoto M, Asakura H, et al. Interferon inhibits progression of liver fibrosis and reduces the risk of hepatocarcinogenesis in patients with chronic hepatitis C. Dig Dis Sci 2002;47:170–6.

[51] Ikeda K, Arase Y, Saitoh S, Kobayashi M, Suzuki Y, Suzuki F, et al. Interferon-β prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor: a prospective randomised study of hepatitis C virus-related liver cancer. Hepatology 2000;32:228–32.

[52] Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Yamazaki O, et al. Effects of long-term postoperative interferon-α therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. Ann Intern Med 2001;134:963–7.

[53] Melen K, Keskinen P, Lehtonen A, Julkinen I. Interferon-induced gene expression and signalling in human hepatoma cell lines. J Hepatol 2000;33:764–72.

[54] Yamashita Y, Koike K, Takaoki M, Matsuda S. Suppression of HBsAg production in PLC/PRF/5 human hepatoma cell line by interferons. Microbiol Immunol 1988;32:1119–26.

[55] Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol 2001;34:593–602.

[56] Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Fukuda M, et al. Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by hepatitis B virus: a pilot study. Cancer 1998;82:827–35.

[57] Abe N, Ebina T, Ishida N. Interferon induction by glycyrrhizin and glycyrrhetinic acid in mice. Microbiol Immunol 1982; 26:535–9.

[58] Kimura M, Watanabe H, Abe T. Selective activation of extrathymic T cells in the liver by glycyrrhizin. Biotherapy 1992; 5:167–76.

[59] Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, et al. The long-term efficacy of glycyrrhizin in chronic hepatitis C patients. Cancer 1997;79:1494–500.

[60] van Rossum TGJ, Vulto AG, de Man RA, Brouwer JT, Schalm SW. Glycyrrhizin as a potential treatment for chronic hepatitis C: a double-blind, randomised, placebo-controlled phase 1/2 trial. Aliment Pharmacol Ther 1998;12:199–205.

[61] Kumada H. Long-term treatment of chronic hepatitis C with glycyrrhizin for preventing liver cirrhosis and hepatocellular carcinoma. Oncology 2002;62(Suppl 1):94–100.

[62] Tarao K, Takemiya S, Tamai S, Sugimasa Y, Ohkawa S, Akaike M, et al. Relationship between the recurrence of hepatocellular carcinoma and serum aminotransferase levels in hepatectomised patients with hepatitis C virus-associated cirrhosis and hepatocellular carcinoma. Cancer 1997;79: 688–94.