Diamond-Blackfan anemia: pathogenesis, management and development of future therapies

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A B S T R A C T

Diamond-Blackfan anemia (DBA) is an inherited bone marrow failure syndrome characterized by a macrocytic anemia, reticulocytopenia and reduction in erythroid precursors in an otherwise normocellular bone marrow. The disease usually presents before one year of age. Fifty percent of the patients have congenital anomalies. The mainstay of current therapy is corticosteroids and if the patients do not respond to steroids, chronic blood transfusion therapy is needed. The disease can be cured by allogeneic bone marrow transplantation. DBA is a genetic disorder and is inherited in an autosomal dominant manner with variable penetrance in 50% of cases while the remainders represent new mutations. To date, mutations have been identified in 60-70% of DBA patients. Practically all of these patients have a mutation in or a deletion of, a ribosomal protein gene. Ten different ribosomal protein genes have now been identified as DBA genes and recently a handful of patients have been found with mutations in *GATA1*. Abnormal ribosome biogenesis and ribosomal stress leads to activation of the tumor suppressor p53. The p53 response appears to be particularly prominent in erythroid progenitors and may explain many features of the DBA phenotype and symptoms.

Learning goals

At the conclusion of this activity, participants should have:
- learnt the key clinical manifestations, diagnostic criteria and current treatment options for Diamond-Blackfan anemia;
- got an insight into the molecular and cellular pathogenesis of Diamond-Blackfan anemia and how mechanism-based therapies may be developed to reduce side effects or cure the disease.

Introduction

Bone marrow failure syndromes consist of diverse disorders characterized by the dysfunction of bone marrow to produce cells of one or more blood lineages. In one-third of the pediatric marrow failure cases the disease is inherited involving a genetic component causing the bone marrow dysfunction[1]. Inherited bone marrow failure syndromes (IBMFS) usually present in childhood and are associated with physical abnormalities and cancer predisposition. Recent progress in genetics and molecular biology investigations has revolutionized the understanding of IBMFS pathophysiology. Many of the genes mutated in these disorders encode components of fundamental cellular processes such as DNA damage repair (Fanconi anemia) or telomere maintenance (Dyskeratosis congenita). Diamond-Blackfan anemia (DBA) is a congenital bone marrow failure syndrome that is emerging as a paradigm for diseases associated with defects in ribosome biogenesis and function. Similarly to other IBMFS, physical abnormalities and cancer predisposition are both characteristic for DBA. However, why defects in ribosome biogenesis result in anemia, a relatively tissue-specific phenotype, is intriguing and not perfectly understood.

Clinical symptoms and diagnosis

Diamond-Blackfan anemia is a congenital bone marrow failure syndrome that manifests early in life. It classically presents at 2-3 months of age, and the majority of patients (approx. 90%) are diagnosed during their first year of life. However, in some rare cases DBA may present in adulthood.[2,3] The main hematology findings at presentation include macrocytic anemia, reticulocytopenia and selective absence of erythroid precursors in an otherwise normocellular bone marrow.[4] Together with the early onset of symptoms (<1 year), these criteria have remained the accepted standard for DBA diagnosis. As a supporting hematologic feature, the vast majority of patients have elevated erythrocyte adenosine deaminase (eADA) activity.[5,6] Elevated fetal hemoglobin is also often observed. Although DBA is sometimes referred to as pure red cell aplasia, this term may be misleading since other hematopoietic lineages may be affected. Some patients present with a modest neutropenia, thrombocytosis or thrombocytopenia.[2] Furthermore, neutropenia and thrombocytopenia become increasingly common during the course of the disease.[7]

Similarly to other IBMFS, physical defects and cancer predisposition are characteristic of

DBA. Congenital abnormalities are present in approximately 40-50% of the patients.[2,3,8,9] The majority of these involve head and eyes, upper limbs, heart and the genitourinary system. Furthermore, one-third of cases show retarded growth. Patients with DBA have an increased risk of developing cancer.[10] The mechanism of increased carcinogenesis is unknown. The observed-to-expected ratio of all cancers combined is 5.4-fold higher than in the general population with the highest risk for myelodysplastic syndrome (MDS, 287-fold), acute myeloid leukemia (AML, 28-fold), colon carcinoma (36-fold) and osteogenic sarcoma (33-fold). The cancer risks appear lower than in Fanconi anemia and dyskeratosis congenital.[11] Specific cancer screening approaches may be difficult to design in practice due to diversity of the cancers that develop in DBA.

For diagnosis, laboratory blood analysis, bone marrow analysis (aspiration and biopsy) and genotyping are required (Table 1). The differential diagnosis of DBA includes other IBMFS and several acquired disorders, for example, transient erythroblastopenia of childhood and infections by parvovirus B19.[12] Findings from National Patient Registries in North America and Europe have provided extensive clinical data and, together with the recent advances in gene discovery, have provided key clinical insights.[2,3,8,9,13] Detailed and extensive descriptions of the recommended approach to clinical diagnosis and management of DBA have recently been described in the report from the DBA Clinical Consensus Conference and a scholarly written "How I Treat Diamond Blackfan anemia" overview.[12,13]

Current treatment

Corticosteroids form the main therapeutic regimen in DBA and approximately 80% of the patients initially respond to this treatment. However, because of the progressive loss of response or unacceptable side effects, only half of these patients (40% of total) can be sustained on corticosteroids.[3,13] If the patient responds to corticosteroids, an attempt is made to reduce the dose gradually to reduce side effects that include slow growth rate, cataracts and demineralization of bone leading to pathological fractures. It is recommended to treat congenital anomalies by surgery before steroid treatment starts to facilitate wound healing.[13] The remaining patients require chronic transfusion therapy every 3-5 weeks to maintain sufficient hemoglobin levels (>8 g/dL) that allows for adequate growth and development, while not suppressing the endogenous red blood cell production. Chronic transfusion therapy must be combined with iron chelation to avoid the accumulation of iron in the liver, heart and other organs. Approximately 20% of the patients enter spontaneous remission in which physiologically acceptable hemoglobin level is maintained without therapeutic interventions.

Allogeneic bone marrow transplantation is the only curative treatment for the hematopoietic manifestation of DBA, and it is normally considered among the young patients (<10 years) who are transfusion-dependent and have access to a matched sibling donor.[3,12] However, although matched sibling donor bone marrow transplantations have been reported with satisfactory results, transplantation using a matched alternative donor is associated with a poor outcome.

Numerous alternative therapies (growth factors, prolactin, immunosuppressants) have been applied in the treatment of DBA but these are not routinely used since they have either been ineffective or only found to be effective in rare cases.[12,13] Of special interest is the recent case report demonstrating a complete remission in response the amino acid L-leucine.[14] Supporting this report, therapeutic experiments with L-leucine improved the erythroid defect in zebrafish and mouse models for DBA.[15,16] With the current therapies, the overall survival at over 40 years is 75.1%.[3] A high proportion of deaths are treatment-related and corticosteroid-responsive patients have a significant survival advantage compared to transfusion-dependent patients.

Inheritance and genetics of DBA

The incidence of DBA is estimated to be 5-7 cases per million live births without ethnic predilection or biased sex ratio.[2,8,9] Almost 50% of DBA cases are familial and inherited as an autosomal dominant trait with variable penetrance.[6] Family members who share a common genetic alteration may show dramatic variation in the severity of anemia and treatment response.

Mutations in or deletions of genes encoding ribosomal protein (RP) S19, RPS24, RPS17, RPL35a, RPL5, RPL11, RPS7, RPS10, RPS26 and RPL26 collectively explain the genetic basis for approximately 60-70% of DBA cases[17-25] (Figure 1). Furthermore, alterations in additional RP genes have been identified in isolated patients, although the pathogenic significance of these rare variants is not clear.[21-23] All reported mutations are heterozygous, which

Table 1. Diagnostic criteria, genetic analysis and current therapeutic approaches for Diamond-Blackfan anemia. This is a simplified overview based on the report from the DBA Clinical Consensus Conference[12] and a recent clinical review.[13]

Main diagnostic criteria
Age less than one year
Macrocytic anemia with no significant cytopenias
Reticulocytopenia
Normal marrow cellularity with a relatively low number of erythroid precursors
Minor diagnostic criteria
Elevated erythrocyte adenosine deaminase activity
Elevated fetal hemoglobin (HbF)
Congenital anomalies described in classical DBA
Inheritance and genetic analysis
Gene mutation in one of the ribosomal protein genes described in classical DBA
Positive family history (found in 50% of cases)
Differential diagnosis
Other IBMFS: Fanconi anemia, Schwachman Diamond Syndrome, Dyskeratosis congenita
Acquired disorders: transient erythroblastopenia of childhood, Pearson syndrome
Viral infections, *e.g.* B19 parvovirus
Current therapies
The natural therapy: remission (20%)
Corticosteroids
Blood transfusion
Allogeneic transplantation (relatively rare, see text)

is consistent with the dominant inheritance pattern.

Twenty-five percent of the patients have mutations in the gene coding for RPS19 making it the most common DBA gene. More than 120 unique alterations have been identified (Available from: www.dbagenes.unito.it Accessed January 2013[26]). The mutations may completely disrupt the expression of *RPS19*, or interfere with the folding of RPS19 or its assembly into the 40S ribosomal subunit, and thus result in a functional haploinsufficiency. Nearly all mutations in the other DBA genes are predicted to cause premature termination, splicing disruption, frame shifting or complete deletion of one allele, supporting functional haploinsufficiency as the basis for the disease pathology.[27-29]

Recently, patients with *GATA1* mutations were identified in two unrelated families.[30] However, the identification and phenotypic characterization of additional DBA patients with *GATA1* mutations will eventually determine whether these patients present 'classical' DBA.

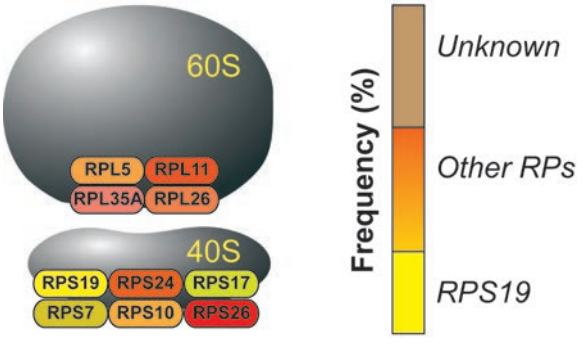

Figure 1. The ribosomal proteins mutated in DBA, their frequency and distribution within the ribosomal subunits. The figure shows the two ribosomal subunits, the large 60S and the small 40S subunit. Approximately half of the ribosomal mass consists of the ribosomal RNA and the other half of the ribosomal proteins, which are referred to as the RPL when they are found in the large 60S subunit and RPS in the small 40S subunit. In approximately 30-40% of patients the mutation is unknown. *RPS19* is by far the most common disease gene and it is found mutated in 25% of patients. *GATA1* mutations are not shown here since GATA1 is a transcription factor and these mutations are rare.

Erythropoiesis

The erythrocyte is the most common cell type in blood. Mature erythrocytes have a limited life span, approximately 120 days in humans and 40 days in mice, and they must be continuously produced in order to renew the red cell mass. The erythroid lineage consists of erythroid progenitor and precursor cell compartments (Figure 2). Erythroid progenitor cells are relatively infrequent and can be divided into the early and late progenitor cells based on their colony-forming potential *in vitro*. The early progenitor cells (burst-forming unit-erythroid, BFU-E) are the first solely erythroid-restricted cells and give rise to large multi-clustered colonies.[32] BFU-Es also possess a limited self-renewal capacity. The late progenitor cells (colony-forming unit-erythroid, CFU-E) give rise to smaller colonies than BFU-Es. The proliferation and survival of BFU-Es is mainly dependent on stem cell factor (SCF) and interleukin-3 (IL-3) signaling, while erythropoietin (Epo) alone is sufficient to support CFU-Es. CFU-Es differentiate into morphologically distinguishable erythroid precursor cells. The first recognizable precursor, proerythroblast, undergoes 3-5 cell divisions giving rise to basophilic, polychromatic and orthochromatic erythroblasts. These differentiation divisions are characterized by a

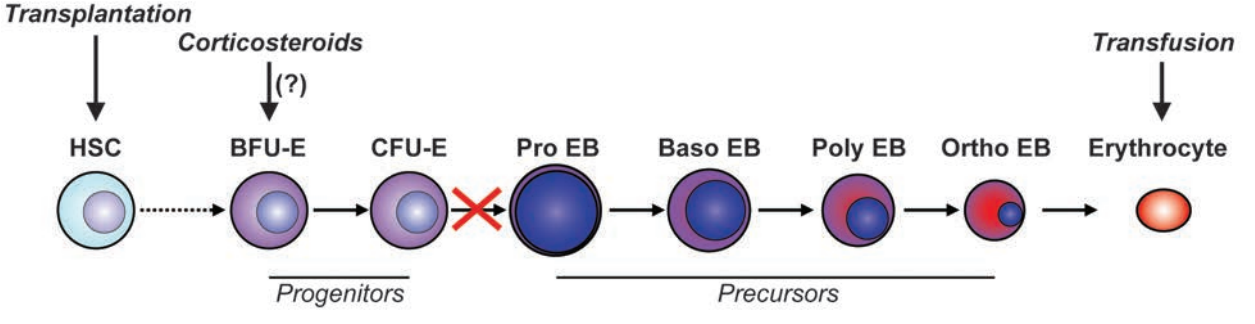

Figure 2. Erythroid development and differentiation. Cells of the erythroid lineage can be divided into erythroid progenitor and precursor cells. Erythroid progenitor cells are distinguished based on their differential growth factor requirements and colony-forming capacity *in vitro*. BFU-E progenitor cells are dependent on SCF and IL-3 signaling, while CFU-E progenitor cells are solely dependent on Epo. In contrast to the erythroid progenitor cells, erythroid precursor cells are recognized based on their morphology, which reflects the accumulation of erythroid-specific proteins, decrease in size and nuclear condensation. Scientific evidence suggests that although there is a proliferation deficiency throughout the hierarchy including at the level of hematopoietic stem cells and early progenitors, the main deficiency is at the level of the CFU-E - proerythroblast transition. The arrows indicate at which level of the hierarchy the different treatment modalities act. Blood and marrow transplantation rebuilds the hematopoietic system from the level of the stem cells and is curative if successful whereas chronic red cell transfusions just treat the anemia temporarily. It is known that corticosteroids increase the self-renewal of BFU-Es and thereby increase the total erythroid output but they may also have additional DBA-specific mechanisms of action. More detailed knowledge about the mechanism of action may allow a reduction in the corticosteroid dose by using other drugs that synergize with corticosteroids in combination.[31]

rapid G1 cell cycle phase, which results in a progressive decrease in the cell size.[33] Simultaneously, maturating precursor cells undergo alterations in morphology that reflect the accumulation of erythroid-specific proteins and nuclear condensation. Orthochromatic erythroblasts withdraw from the cell cycle and form reticulocytes by extruding their nuclei. Reticulocytes loose their mitochondria and ribosomes within a couple of days and mature into erythrocytes.[32] The main intrinsic regulator of erythropoiesis apart from the Epo receptor and c-Kit (SCF receptor) is the transcription factor GATA-1.[34,35]

The hematopoietic defect and cellular mechanisms in DBA

The success of bone marrow transplantation and studies using cultured cells from patients demonstrate the intrinsic cell nature of the hematopoietic defect in DBA. Erythroid progenitor cells are usually present, often in normal numbers, in the marrow of young patients suggesting that the main erythroid failure of DBA results from impaired terminal differentiation of erythroid progenitor cells rather than from their absence.[36,37] Consistent with these studies, Ohene-Abuakwa *et al.* used a liquid erythroid culture system in order to locate the erythroid defect at the onset of Epo-dependent terminal erythroid differentiation.[38] Furthermore, recent studies using mouse models for RPS19-deficient DBA located the most severe erythroid defect at the CFU-E-proerythroblast transition, corroborating the previous findings.[39] Some patients develop hypocellular bone marrow over time and this is often associated with neutropenia and thrombocytopenia.[7] Although the frequency of immature hematopoietic stem and progenitor cells in patients appears normal, their proliferative capacity is significantly lower compared to controls.[7,41] These findings suggest that the hematopoietic defect in DBA involves hematopoietic progenitors or even hematopoietic stem cells (HSCs) resulting in bone marrow failure. Supporting these conclusions are recent findings from an inducible Rps19-deficient mouse model.[39] In this study, transplantation of HSCs derived from mice that had been transiently exposed to Rps19 deficiency led to significantly reduced engraftment in the peripheral blood, demonstrating the irreversible exhaustion of HSCs.[39]

Disease severity and spontaneous remission

Despite recent advances in understanding the molecular basis of DBA, the natural course of the disease remains largely unpredictable. Approximately 20% of the patients enter spontaneous remission, often during the first decade of life, in which physiologically acceptable hemoglobin level is maintained without therapeutic intervention. Interestingly, there appears to be no clear correlation between the chance of remission and the type and duration of the therapy. The failure of the genotype to predict the hematopoietic phenotype is highlighted by the variable penetrance of genetic lesions in DBA pedigrees. However, there is a genotype-phenotype relationship when it comes to orofacial clefts since these are found in patients with *RPL5* and *RPL11* mutations and not in patients with mutated *RPS19*.[13,29]

It is of interest that the vast majority of patients in remission continue to exhibit elevated eADA and macrocytosis.[2,6] These findings suggest a continuous presence of the erythroid defect, which is compensated through extrinsic factors that stimulate the hematopoietic stem and progenitor cells, leading to increased influx of cells into the Epo-responsive stage. Indeed, Ohene-Abuakwa *et al*. demonstrated a consistent erythroid defect of patient cells *in vitro* regardless of the clinical severity.[38] Intriguingly, a similar defect was observed when culturing cells from asymptomatic first-degree relatives who shared the genetic lesion. Relapses tend to occur under conditions of hematopoietic stress, such as pregnancy, indicating the importance of the dynamics of the hematopoietic system in determining whether the patient is symptomatic or not.[41] Presentation of anemia in DBA normally coincides with the neonatal decline in HSC turnover.[42] Dynamics of the hematopoietic system could also directly influence the severity of the cellular defect of DBA. This is supported by the fact that the chance of relapse in remitted patients appears low, except during stress conditions.

5q minus syndrome

MDS comprise a heterogeneous group of clonal disorders characterized by dysplastic bone marrow and peripheral cytopenia. The 5q- syndrome is a distinct subtype of MDS, defined by an isolated interstitial deletion of chromosome 5q, and is characterized by macrocytic anemia, normal or elevated platelet counts, dysplastic megakaryocytes and elevated risk of AML.[43] Most patients respond to the treatment with lenalidomide, resulting in reduced transfusion requirement that is often combined with a complete cytogenetic response.[44] The 5q- common deleted region encompasses forty protein-coding genes.[45] By a systematic targeting of each gene using the short hairpin RNA (shRNA) technology, Ebert *et al*. identified *RPS14* as the critical gene for the erythroid phenotype.[46] Therefore, a similar mechanism underlies the erythroid phenotype in both 5q minus syndrome and DBA.

The molecular pathology in DBA

With the exception of a few DBA patients with *GATA1* mutations, all the identified mutations in DBA are found in ribosomal proteins. Therefore, defects in ribosome biogenesis are considered the key pathogenic mechanism in DBA. However, it is still not yet fully understood why the main phenotype, ineffective erythropoiesis, is relatively tissue-specific since ribosomal proteins have a generic function in all cell types. Below, we will discuss ribosomal stress, a possible role for p53, and the regulation of protein translation as possible molecular mechanisms causing the DBA phenotype.

Ribosome biogenesis and ribosomal stress

Ribosome biogenesis takes place in a specialized nuclear compartment, the nucleolus, which is formed around the actively transcribed rRNA genes. Transcription of rRNA genes by RNA polymerase I gives rise to a 47S precursor rRNA (pre-rRNA), which simultaneously asso-

ciates with trans-acting factors to form the 90S pre-ribosome. After a series of remodeling and pre-rRNA processing, 90S pre-ribosome splits into pre-40S and pre-60S subunits that are exported into the cytoplasm where the final maturation steps occur.[47,48] The modified pre-rRNA undergoes hierarchical endonucleolytic and exonucleolytic cleavages, eventually giving rise to 18S, 28S and 5.8S mature rRNAs.[47] Ribosomal proteins assemble with pre-rRNA in a hierarchical manner and facilitate its processing, nuclear export and cytoplasmic maturation, and deficiency of ribosomal proteins impairs the rRNA processing at distinct stages.[49-51] Perturbations to the dynamics and flow of this process have been associated with alterations in the regulation of cell size and cell cycle progression, leading to developmental defects and increased cancer susceptibility.[52] Pharmacological or genetic disruption of rRNA transcription and processing has shown to result in the activation of the tumor suppressor p53.[53-56] Similarly, numerous studies have demonstrated the activation of p53 in response to ribosomal protein deficiencies.[57,58] During normal growth conditions, the activity of p53 is kept low by the oncoprotein mouse double minute 2 (Mdm2). In the absence of stress, Mdm2 binds to p53 and functions as an ubiquitin ligase, targeting p53 for proteosomal degradation. Various cellular stresses disrupt the interaction between Mdm2 and p53, resulting in the stabilization and activation of p53. In case of ribosomal stress, impaired rRNA synthesis or processing leads to nuclear accumulation of free ribosomal proteins, which are able to bind to Mdm2 and inhibit its ubiquitin ligase function, resulting in the accumulation of p53 (Figure 3). Although multiple ribosomal proteins have been shown to interact with Mdm2, the recent evidence suggests that only RPL5 and RPL11, in a mutually dependent manner, are required for Mdm2 inhibition.[58]

Disease models suggest a role for p53

Several animal models with reduced expression of ribosomal proteins have been generated to define the role of ribosomal proteins in hematopoiesis and generate model systems for DBA (reviewed in McGowan and Mason[59]). rps19-deficient zebrafish models were generated using morpholino technology.[60,61] These models showed developmental and hematologic abnormalities. Furthermore, the loss of p53 rescued the phenotypic abnormalities observed upon rps19 haploinsufficiency.[60] In 2008,

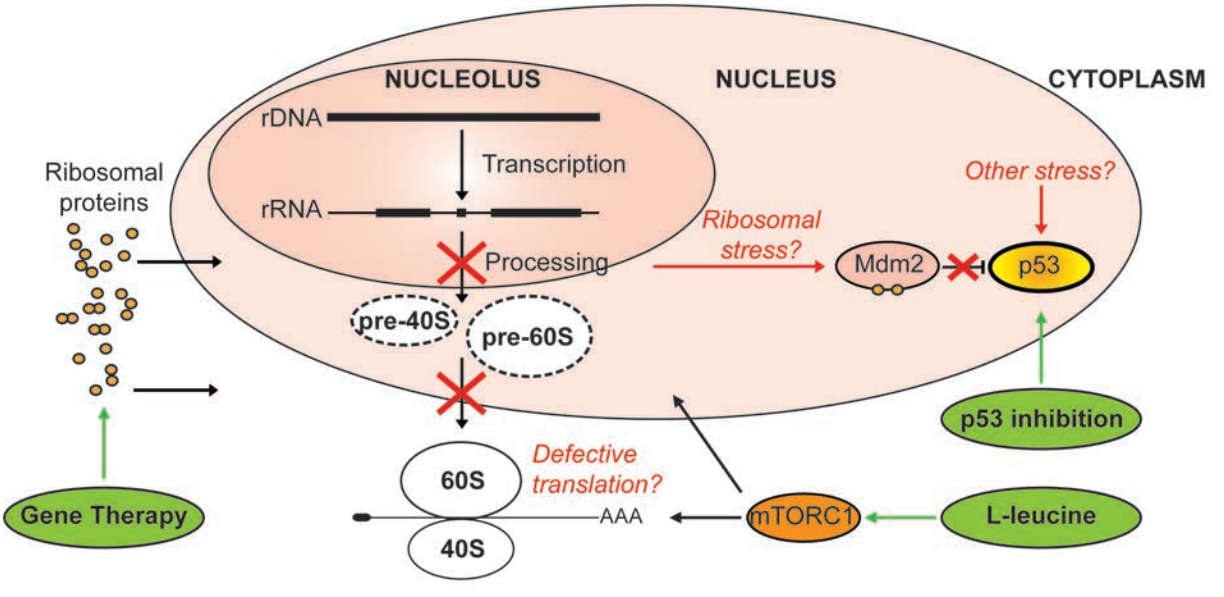

Figure 3. Ribosome biogenesis, ribosomal stress and development of possible mechanism-based therapies. Transcription of rDNA by RNA polymerase I gives rise to a 47S rRNA precursor, which associates with trans-acting factors that mediate a series of chemical modifications and nucleolytic cleavages. This results in the formation of pre-40S and pre-60S ribosomal subunits that are exported into the cytoplasm where the final maturation takes place. Ribosomal proteins associate with pre-rRNA in a hierarchical manner and facilitate its processing, nuclear export and maturation. Deficiency of ribosomal proteins impairs rRNA processing (indicated by the red X). Ribosomal stress is believed to be a key pathogenic mechanism in DBA. During steady state conditions, the levels of p53 are kept low through proteosomal degradation by Mdm2. Impaired rRNA synthesis or processing leads to nuclear accumulation of free ribosomal proteins, which are able to bind to Mdm2 and inhibit its ubiquitin ligase function, resulting in the accumulation of p53.58 The figure also shows three possible mechanistic approaches to treat DBA. Gene replacement therapy will cure the hematologic disease. Reduction in p53 activity will improve erythropoiesis in DBA although p53 reduction is not without risks. L-Leucine can activate the mTORC1 pathway. mTORC1 regulates ribosome biogenesis by promoting rRNA and ribosomal protein synthesis and enhancing translation initiation and elongation. Of these three possible approaches, L-Leucine therapy is least likely to cause severe side effects. Clinical trials using L-Leucine are under way.

McGowan *et al.* reported a novel mouse model for RPS19-deficient DBA that presents a missense mutation resulting in a single amino acid substitution in the Rps19 protein.[62] This mutation was embryonic lethal in a homozygous setting. However, the heterozygous mice exhibited dark skin, retarded growth and a mild macrocytic anemia with a reduction in erythrocyte number. Importantly, all of these features were rescued in a p53-deficient background. RPS19 can be down-regulated in hematopoietic cells using knockdown technology to generate a DBA-like phenotype *in vitro*.[63-65] This approach was taken to generate mouse models with inducible and graded downregulation of Rps19.[38] Depending on the level of Rps19 downregulation, mice with mild to lethal macrocytic anemia could be generated. Strikingly, crossing these mice into p53-deficient background almost fully corrected the lethal hematopoietic phenotype.[38]

As the studies using DBA animal models demonstrate an activation of p53 in response to ribosomal protein deficiencies, it is tempting to speculate that the erythroid failure in DBA patients is caused through p53-dependent mechanisms. Recently, downregulation of RPS19 or RPS14 in primary human bone marrow cells was shown to result in the erythroid-pronounced activation of p53.[66] Furthermore, the treatment of bone marrow cells with nutlin-3, a compound that activates p53 by preventing its interaction with Mdm2, led to an erythroid-biased activation of p53. Finally, inhibition of p53 with a small molecule pifithrin alpha rescued the erythroid defect in RPS19-deficient and RPS14-deficient human bone marrow cell cultures. Immunohistochemistry for p53 in the bone marrow biopsies from DBA patients demonstrated elevated levels of p53, although variation was observed in terms of the intensity and cell type-specificity of p53 staining.[66] However, a generic defect in ribosomal biogenesis may influence the translational apparatus in cells and influence other regulatory pathways than just p53.

Translational defects

Ribosomal protein haploinsufficiency has been shown to result in reduced rate of protein synthesis.[67] However, whether the global reduction in translation contributes to the severe anemia of DBA is not known. Studies in mice deficient for Flvcr, a heme exporter protein, have led to a hypothesis that defective globin synthesis contributes to the erythroid defect of DBA.[68] These findings suggest that the accumulation of free heme in proerythroblasts is toxic, raising a hypothesis that the dysregulation of heme synthesis and globin translation, resulting in a transient excess of free heme, could in part explain the erythroid defect of DBA.

Development of future therapies

Lenalidomide

Lenalidomide has proven to be highly effective in the treatment of patients with 5q- syndrome, causing both hematologic and cytogenetic responses.[44] Although the underlying mechanism remains elusive, lenalidomide has been reported to promote the erythroid differentiation of human CD34-positive bone marrow cells and the production of fetal hemoglobin.[69] This is due to its ability to stimulate CFU-E progenitor cells, possibly through the modulation the Epo receptor turnover.[70,71] As corticosteroids and lenalidomide promote erythropoiesis at distinct stages, use of these agents in combination could provide a more profound therapeutic effect in DBA.[70]

L-Leucine

Recently, based on the theory of inefficient translation as the underlying cause for the severe anemia in DBA, Pospisilova *et al*. reported one patient who became transfusion-independent in response to treatment with the amino acid L-leucine.[14] Similarly, L-leucine administration alleviated the developmental defects and in some cases also the anemia of rps19-deficient and rps14-deficient zebrafish models.[15] Furthermore, dietary L-leucine was shown to improve the anemia of Rps19-deficient mice.[16] L-leucine is an essential branched chain amino acid that plays an important role in the regulation of protein synthesis, and this response involves the mammalian/mechanistic target of rapamycin complex 1 (mTORC1) pathway.[72] Thus the enhanced translation of ribosomal proteins could underlie the therapeutic effect of L-leucine. Irrespective of the mechanism, several large clinical trials are now ongoing or about to start. The future outcome of these trials could be exciting since the side effects of L-Leucine, if used in the correct dose, are expected to be relatively modest compared to the potential toxic effects of corticosteroids.

Targeting the p53 pathway

Based on the current experimental findings, it is tempting to speculate that the erythroid defect in DBA is largely caused through a p53-dependent mechanism. The identification of p53 could provide a novel therapeutic avenue for the treatment of DBA and related disorders. Inhibition of p53 with a small molecule pifithrin alpha rescues the erythroid defect of RPS19-deficient and RPS14-deficient human bone marrow cell cultures.[66] Indeed, a transient dampening of the p53 pathway could provide a therapeutic benefit in patients. However, direct interference with p53 raises concerns because of its role as a tumor suppressor. Strategies targeting disease-specific factors either upstream or downstream of p53 could provide a more promising alternative.

Gene therapy

Gene therapy is the only approach apart from allogeneic transplantation that can cure the hematopoietic defect in DBA. In a recent proof-of-principle experiment, the lethal bone marrow failure in Rps19-deficient mice could be cured by gene therapy.[73] However, as the current therapies, especially those with corticosteroids, have a relatively good outcome, moving gene therapy to the clinic will require a careful assessment of the risk-benefit ratio for this approach. We envisage that the first clinical trials could be applied to patients with a chronic transfusion-dependent DBA. Lentiviral vectors, in which the potent spleen focus-forming vector (SFFV) promoter drives the expression of codon-optimized human *RPS19* cDNA, were used to correct the DBA phenotype in mice.[73] However, for future clinical application, more moderate cellular promoters must be validated, as they are potentially safer with regards to the probability of insertional mutagenesis. Clinical trials for Fanconi anemia employing similar lentiviral vectors, in which the *PGK* promoter drives the expression of *FANCA* cDNA, are

being conducted.[74] However, the elongation factor 1α (EF1α) short promoter may prove to be an even more viable alternative.[75] Furthermore, a lentiviral vector utilizing the EF1α promoter combined with the locus control region of β-globin has been shown to allow a constitutive but erythroid-pronounced transgene expression.[76] The safety and efficacy of ongoing clinical trials using lentiviral vectors to treat disorders other than DBA will largely determine the future of DBA gene therapy. Although the follow-up time for these trials is still relatively short, no severe genotoxic side effects have been reported.[77] The development of a human gene therapy protocol for RPS19-deficient DBA is estimated to take approximately five years

References

1. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev. 2010;24:101-22.
2. Willig TN, Niemeyer CM, Leblanc T, Tiemann C, Robert A, Budde J, et al. Identification of new prognosis factors from the clinical and epidemiologic analysis of a registry of 229 Diamond-Blackfan anemia patients. DBA group of Société d'Hématologie et d'Immunologie Pédiatrique (SHIP), Gesellshaft für Pädiatrische Onkologie und Hämatologie (GPOH), and the European Society for Pediatric Hematology and Immunology (ESPHI). Pediatr Res. 1999;46:553-61.
3. Lipton JM, Atsidaftos E, Zyskind I, Vlachos A. Improving clinical care and elucidating the pathophysiology of Diamond Blackfan anemia: an update from the Diamond Blackfan Anemia Registry. Pediat Blood Cancer. 2006; 46:558-64.
4. Diamond LK, Wang WC, Alter BP. Congenital hypoplastic anemia. Adv Pediatr. 1976;22: 349-78.
5. Glader BE, Backer K, Diamond LK. Elevated erythrocyte adenosine deaminase activity in congenital hypoplastic anemia. N Engl J Med. 1983;309:1486-90.
6. Orfali KA, Ohene-Abuakwa Y, Ball SE. Diamond Blackfan anaemia in the UK: clinical and genetic heterogeneity. Br J Haematol. 2004;125:243-52.
7. Giri N, Kang EM, Tisdale JF, Follman D, Rivera M, Schwartz GN, et al. Clinical and laboratory evidence for a trilineage haematopoietic defect in patients with refractory Diamond-Blackfan anaemia. Br J Haematol. 2000;108:167-75.
8. Ball SE, McGuckin CP, Jenkins G, Gordon-Smith EC. Diamond-Blackfan anaemia in the U.K.: analysis of 80 cases from a 20-year birth cohort. Br J Haematol. 1996;94:645-53.
9. Ramenghi U, Garelli E, Valtolina S, Campagnoli MF, Timeus F, Crescenzio N, et al. Diamond-Blackfan anaemia in the Italian population. Br J Haematol. 1999;104:841-8.
10. Alter BP, Giri N, Savage SA, Peters JA, Loud JT, Leathwood L, et al. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. Br J Haematol. 2010;150:179-88.
11. Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. Blood. 2012;119:3815-9.
12. Vlachos A, Ball SE, Dahl N, Alter BP, Sheth S, Ramenghi U, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. Br J Haematol. 2008;142:859-76.
13. Vlachos A, Muir E. How I treat Diamond-Blackfan anemia. Blood. 2010;116:3715-23.
14. Pospisilova D, Cmejlova J, Hak J, Adam T, Cmejla R. Successful treatment of a Diamond-Blackfan anemia patient with amino acid leucine. Haematologica. 2007;92:66-7.
15. Payne E, Virgilio M, Narla A, Sun H, Levine M, Paw BH, et al. L-Leucine improves anemia and developmental defects associated with Diamond-Blackfan anemia and del(5q)MDS by activating the mTOR pathway. Blood. 2012;120:2214-24.
16. Jaako P, Debnath S, Olsson K, Bryder D, Flygare J, Karlsson S. Dietary L-leucine improves the anemia in a mouse model for Diamond-Blackfan anemia. Blood. 2012;120:2225-8.
17. Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, et al. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. Nat Genet. 1999;21:169-75.
18. Gazda HT, Grabowska A, Merida-Long LB, Latawiec E, Schneider HE, Lipton JM, et al. Ribosomal protein S24 gene is mutated in Diamond-Blackfan anemia. Am J Hum Genet. 2006;79:1110-8.
19. Cmejla R, Cmejlova J, Handrkova H, Petrak J, Pospisilova D. Ribosomal protein S17 gene (RPS17) is mutated in Diamond-Blackfan anemia. Hum Mutat. 2007;28:1178-82.
20. Farrar JE, Nater M, Caywood E, McDevitt MA, Kowalski J, Takemoto CM, et al. Abnormalities of the large ribosomal subunit protein, Rpl35A, in diamond-blackfan anemia. Blood. 2008;112:1582-92.
21. Gazda HT, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Schneider H, et al. Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. Am J Hum Genet. 2008;83:769-80.
22. Doherty L, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Clinton C, et al. Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. Am J Hum Genet. 2010;86:222-8.
23. Farrar JE, Vlachos A, Atsidaftos E, Carlson-Donohoe H, Markello TC, Arceci RJ, et al. Ribosomal protein gene deletions in Diamond-Blackfan anemia. Blood. 2011;118:6943-51.
24. Gazda HT, Preti M, Sheen MR, O'Donohue MF, Vlachos A, Davies SM, et al. Frameshift mutation in p53 regulator RPL26 is associated with multiple physical abnormalities and a specific pre-ribosomal RNA processing defect in diamond-blackfan anemia. Hum Mutat. 2012;33:1037-44.
25. Kuramitsu M, Sato-Otsubo A, Morio T, Takagi M, Toki T, Terui K, et al. Extensive gene deletions in Japanese patients with Diamond-Blackfan anemia. Blood. 2012;119:2376-84.
26. Boria I, Quarello P, Avondo F, Garelli E, Aspesi A, Carando A, et al. A new database for ribosomal protein genes which are mutated in Diamond-Blackfan Anemia. Hum Mutat. 2008; 29:263-70.
27. Farrar JE, Dahl N. Untangling the phenotypic heterogeneity of Diamond Blackfan anemia. Semin Hematol. 2011;48:124-5.
28. Devlin EE, Dacosta L, Mohandas N, Elliott G, Bodine DM. A transgenic mouse model demonstrates a dominant negative effect of a point mutation in the RPS19 gene associated with Diamond-Blackfan anemia. Blood. 2010;116:2826-35.
29. Boria I, Garelli E, Gazda HT, Aspesi A, Quarello P, Pavesi E, et al. The ribosomal basis of Diamond-Blackfan anemia: mutation and database update. Hum Mutat. 2010;31:1269-79.
30. Sankaran VG, Ghazvinian R, Do R, Thiru P, Vergilio JA, Beggs AH, et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. J Clin Invest. 2012;122:2439-43.
31. Flygare J, Rayon Estrada V, Shin C, Gupta S, Lodish HF. HIF1alpha synergizes with glucocorticoids to promote BFU-E progenitor self-renewal. Blood. 2011;117:3435-44.
32. Testa U. Apoptotic mechanisms in the control of erythropoiesis. Leukemia. 2004;18:1176-99.
33. Von Lindern M. Cell-cycle control in erythropoiesis. Blood. 2006;108:781-2.
34. Cantor AB, Orkin SH. Transcriptional regulation of erythropoiesis: an affair involving multiple partners. Oncogene. 2002;21:3368-76.
35. Hattangadi SM, Wong P, Zhang L, Flygare J, Lodish HF. From stem cell to red cell: regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. Blood. 2011;118:6258-68.
36. Lipton JM, Kudisch M, Gross R, Nathan DG. Defective erythroid progenitor differentiation system in congenital hypoplastic (Diamond-Blackfan) anemia. Blood. 1986;67: 962-8.
37. Casadevall N, Croisille L, Auffray I, Tchernia G, Coulombel L. Age-related alterations in erythroid and granulopoietic progenitors in Diamond-Blackfan anaemia. Br J Haematol. 1994;87:369-75.
38. Ohene-Abuakwa Y, Orfali KA, Marius C, Ball SE. Two-phase culture in Diamond Blackfan anemia: localization of erythroid defect. Blood. 2005;105:838-46.
39. Jaako P, Flygare J, Olsson K, Quere R, Ehinger M, Henson A, et al. Mice with ribosomal protein S19 deficiency develop bone marrow failure and symptoms like patients with Diamond-Blackfan anemia. Blood. 2011;118:6087-96.
40. Hamaguchi, I, Flygare J, Nishiura H, Brun AC, Ooka A, Kiefer T, et al. Proliferation deficiency of multipotent hematopoietic progenitors in ribosomal protein S19 (RPS19)-deficient diamond-Blackfan anemia improves following RPS19 gene transfer. Mol Ther. 2003;7:613-22.
41. Faivre L, Meerpohl J, Da Costa L, Marie I, Nouvel C, Gnekow A, et al. High-risk pregnancies in Diamond-Blackfan anemia:

a survey of 64 pregnancies from the French and German registries. Haematologica. 2006;91:530-3.
42. Rufer N, Brümmendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, et al. Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. J Exp Med. 1999;190:157-67.
43. Giagounidis AA, Germing U, Haase S, Hildebrandt B, Schlegelberger B, Schoch C, et al. Clinical, morphological, cytogenetic, and prognostic features of patients with myelodysplastic syndromes and del(5q) including band q31. Leukemia. 2004;18:113-9.
44. List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E, et al. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med. 2006;355:1456-65.
45. Boultwood J, Fidler C, Strickson AJ, Watkins F, Gama S, Kearney L, et al. Narrowing and genomic annotation of the commonly deleted region of the 5q- syndrome. Blood. 2002;99:4638-41.
46. Ebert BL, Lee MM, Pretz JL, Subramanian A, Mak R, Golub TR, et al. An RNA interference model of RPS19 deficiency in Diamond-Blackfan anemia recapitulates defective hematopoiesis and rescue by dexamethasone: identification of dexamethasone-responsive genes by microarray. Blood. 2005;105:4620-6.
47. Hadjiolova KV, Nicoloso M, Mazan S, Hadjiolov AA, Bachellerie JP. Alternative pre-rRNA processing pathways in human cells and their alteration by cycloheximide inhibition of protein synthesis. Eur J Biochem. 1993;212:211-5.
48. Rouquette J, Choesmel V, Gleizes PE. Nuclear export and cytoplasmic processing of precursors to the 40S ribosomal subunits in mammalian cells. EMBO J. 2005;24:2862-72.
49. O'Donohue MF, Choesmel V, Faubladier M, Fichant G, Gleizes PE. Functional dichotomy of ribosomal proteins during the synthesis of mammalian 40S ribosomal subunits. J Cell Biol. 2010;190:853-6.
50. Flygare J, Aspesi A, Bailey JC, Miyake K, Caffrey J, Karlsson S, et al. Human RPS19, the gene mutated in Diamond-Blackfan anemia, encodes a ribosomal protein required for the maturation of 40S ribosomal subunits. Blood. 2007;109:980-6.
51. Choesmel V, Bacqueville D, Rouquette J, Noaillac-Depeyre J, Fribourg S, Crètien A, et al. Impaired ribosome biogenesis in Diamond-Blackfan anemia. Blood. 2007;109:1275-83.
52. Ruggero D, Pandolfi PP. Does the ribosome translate cancer? Nat Rev Cancer. 2003;3:179-82.
53. Andera L, Wasylyk B. Transcription abnormalities potentiate apoptosis of normal human fibroblasts. Mol Med. 1997;3:852-63.
54. Pritchard DM, Watson AJ, Potten CS, Jackman AL, Hickman JA. Inhibition by uridine but not thymidine of p53-dependent intestinal apoptosis initiated by 5-fluorouracil: evidence for the involvement of RNA perturbation. Proc Natl Acad Sci USA. 1997;94:1795-9.
55. Yuan X, Zhou Y, Casanova E, Chai M, Kiss E, Gröne HJ, et al. Genetic inactivation of the transcription factor TIF-IA leads to nucleolar disruption, cell cycle arrest, and p53-mediated apoptosis. Mol Cell. 2005;19:77-87.
56. Pestov DG, Strezoska Z, Lau LF, Evidence of p53-dependent cross-talk between ribosome biogenesis and the cell cycle: effects of nucleolar protein Bop1 on G(1)/S transition. Mol Cell Biol. 2001;21:4246-55.
57. Sulic S, Panic L, Barkic M, Mercep M, Uzelac M, Volarevic S. Inactivation of S6 ribosomal protein gene in T lymphocytes activates a p53-dependent checkpoint response. Genes Dev. 2005;19:3070-82.
58. Fumagalli S, Ivanenkov VV, Teng T, Thomas G. Suprainduction of p53 by disruption of 40S and 60S ribosome biogenesis leads to the activation of a novel G2/M checkpoint. Genes Dev. 2010;26:1028-40.
59. McGowan KA, Mason PJ. Animal models in Diamond Blackfan anemia. Semin Hematol. 2011M48:106-16.
60. Danilova N, Sakamoto K, Lin S. Ribosomal protein S19 deficiency in zebrafish leads to developmental abnormalities and defective erythropoiesis through activation of p53 protein family. Blood. 2008;112:5228-37.
61. Uechi T, Nakajima Y, Chakraborty A, Torihara H, Higa S, Kenmochi N. Deficiency of ribosomal protein S19 during early embryogenesis leads to reduction of erythrocytes in a zebrafish model of Diamond-Blackfan anemia. Hum Mol Genet. 2008;17:3204-11.
62. McGowan KA, Li JZ, Park CY, Beaudry V, Tabor HK, Sabnis AJ, et al. Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. Nat Genet. 2008;40:963-70.
63. Flygare J, Kiefer T, Miyake K, Utsugisawa T, Hamaguchi I, Da Costa L, et al. Deficiency of ribosomal protein S19 in CD34+ cells generated by siRNA blocks erythroid development and mimics defects seen in Diamond-Blackfan anemia. Blood. 2005;105:4627-34.
64. Ebert BL, Lee MM, Pretz JL, Subramanian A, Mak R, Golub TR, et al. An RNA interference model of RPS19 deficiency in Diamond-Blackfan anemia recapitulates defective hematopoiesis and rescue by dexamethasone: identification of dexamethasone-responsive genes by microarray. Blood. 2005;105:4620-6.
65. Miyake K, Flygare J, Kiefer T, Utsugisawa T, Richter J, Ma Z, et al. Development of cellular models for ribosomal protein S19 (RPS19)-deficient diamond-blackfan anemia using inducible expression of siRNA against RPS19. Mol Ther. 2005;11:627-37.
66. Dutt S, Narla A, Lin K, Mullally A, Abayasekara N, Megerdichian C, et al. Haploinsufficiency for ribosomal protein genes causes selective activation of p53 in human erythroid progenitor cells. Blood. 2011;117:2567-76.
67. Cmejlova J, Dolezalova L, Pospisilova D, Petrtylova K, Petrak J, Cmejla R. Translational efficiency in patients with Diamond-Blackfan anemia. Haematologica. 2006;91:1456-64.
68. Keel SB, Doty RT, Yang Z, Quigley JG, Chen J, Knoblaugh S, et al. A heme export protein is required for red blood cell differentiation and iron homeostasis. Science. 2008;319:825-8.
69. Moutouh-de Parseval LA, Verhelle D, Glezer E, Jensen-Pergakes K, Ferguson GD, Corral LG, et al. Pomalidomide and lenalidomide regulate erythropoiesis and fetal hemoglobin production in human CD34+ cells. J Clin Invest. 2008;118:248-8.
70. Narla A, Dutt S, McAuley JR, Al-Shahrour F, Hurst S, McConkey M, et al. Dexamethasone and lenalidomide have distinct functional effects on erythropoiesis. Blood. 2011;118:2296-304.
71. Basiorka AA, McGraw K, Clark J, Caceres G, Johnson J, Hall L, et al. Lenalidomide upregulates erythropoietin receptor expression in hematopoietic progenitors by modulating receptor turnover. Blood. 2011;118:2382.
72. Stipanuk MH. Leucine and protein synthesis: mTOR and beyond. Nutr Rev. 2007;65:122-9.
73. Jaako P, Debnath S, Olsson K, Schambach A, Baum C, Flygare J, Karlsson S. Gene therapy corrects the anemia and lethal bone marrow failure in mouse model for RPS19-deficient Diamond-Blackfan anemia. Blood. 2012;120:513.
74. Tolar J, Becker PS, Clapp DW, Hanenberg H, de Heredia CD, Kiem HP, et al. Gene therapy for fanconi anemia: one step closer to the clinic. Hum Gene Ther. 2012;23:141-4.
75. Zychlinski D, Schambach A, Modlich U, Maetzig T, Meyer J, Grassman E, et al. Physiological promoters reduce the genotoxic risk of integrating gene vectors. Mol Ther. 2008;16:718-25.
76. Montiel-Equihua CA, Zhang L, Knight S, Saadeh H, Scholz S, Carmo M, et al. The β-globin locus control region in combination with the EF1α short promoter allows enhanced lentiviral vector-mediated erythroid gene expression with conserved multilineage activity. Mol Ther. 2012;20:1400-9.
77. Biffi A, Bartolomae CC, Cesana D, Cartier N, Aubourg P, Ranzani M, et al. Lentiviral vector common integration sites in preclinical models and a clinical trial reflect a benign integration bias and not oncogenic selection. Blood. 2011;117:5332-9.