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Review Article

Current and future alternative therapies for beta-thalassemia major

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ABSTRACT

Beta-thalassemia is a group of frequent genetic disorders resulting in the synthesis of little or no β-globin chains. Novel approaches are being developed to correct the resulting α/β-globin chain imbalance, in an effort to move beyond the palliative management of this disease and the complications of its treatment (e.g. life-long red blood cell transfusion, iron chelation, splenectomy), which impose high costs on healthcare systems. Three approaches are envisaged: fetal globin gene reactivation by pharmacological compounds injected into patients throughout their lives, allogeneic hematopoietic stem cell transplantation (HSCT), and gene therapy. HSCT is currently the only treatment shown to provide an effective, definitive cure for β-thalassemia. However, this procedure remains risky and histocompatible donors are identified for only a small fraction of patients. New pharmacological compounds are being tested, but none has yet made it into common clinical practice for the treatment of beta-thalassemia major. Gene therapy is in the experimental phase. It is emerging as a powerful approach without the immunological complications of HSCT, but with other possible drawbacks. Rapid progress is being made in this field, and long-term efficacy and safety studies are underway.

Beta-thalassemia was first discovered in the Mediterranean Basin and is highly prevalent in countries also affected by malaria, but human migration has resulted in the establishment of this disease in many areas of the world [1]. All patients display defects of hemoglobin (Hb) beta-chain production, but the resulting phenotypes are highly variable, ranging from severe anemia to an absence of clinical symptoms. Classification and severity grading are based principally on spontaneous Hb levels and clinical tolerance, regardless of the underlying genotype. Patients with beta-

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thalassemia intermedia have blood Hb concentrations of 7–10 g/dL and do not require regular transfusion. They may display a broad spectrum of clinical signs, depending on the degree of alpha to non-alpha globin chain imbalance and several genetic and environmental factors. They may suffer from numerous complications, including pulmonary hypertension, thrombotic events, infection, endocrine dysfunction and leg ulcers [2]. Patients with beta-thalassemia major require regular transfusions of red blood cells to survive [3]. However, repeated transfusions cause iron overload, with life-threatening complications, such as endocrine dysfunction, cardiomyopathy, liver disease and, ultimately, premature death. In the absence of transfusion, patients with beta-thalassemia major die within the first five years of life, and even with transfusions, only 50–65% of patients live beyond the age of 35 years in high-income countries [4–6]. Research is highly active [Fig. 1], and progress has been made towards the development of new drugs, including biological products, some of which have recently reached the clinical trial stage.

Pharmacological therapies

Trace amounts of fetal Hb (HbF) persist into adulthood, accounting for less than 1% of total Hb in most adults [7]. However, HbF levels may exceed this threshold in some individuals. Beta-thalassemia patients with inherited persistent high levels of HbF production have a milder clinical course than other patients with this disease, and many do not require transfusions [8]. Therapeutic approaches reactivating HbF, and increasing its concentration, are, therefore, attractive. Variant HbF levels are highly inheritable [9]. Genome-wide association studies (GWAS) have compared individuals with low and persistently high levels of HbF, with the aim of identifying quantitative trait loci (QTL) to serve as a source of plausible candidate causal genes or regulatory regions [10] accounting for the persistence of γ-globin gene expression. Strong associations between HbF level and single-nucleotide polymorphisms (SNPs) have been identified for at least four genomic loci, including the HBB (hemoglobin, beta) and olfactory receptor gene clusters on chromosome 11p15.4, the chromosome 6q23.3 HBS1L-MYB (HBS1-like translational GTPase - v-myb avian myeloblastosis viral oncogene homolog) intergenic region (HMIP), the BCL11A (B-cell CLL/lymphoma 11A) locus on chromosome 2p16.1, and the KlF1 (Kruppel-like factor 1) gene on chromosome 19p13.13 [11–18]. Variants at the HMIP-BCL11A-HBB loci account for 20–50% of the variability of HbF levels and the relative contributions of these variants differ between ethnic groups [19–23].

The −158C > T (rs7482144) SNP located at the *Xmn*I site of the HBG2 (hemoglobin gamma G gene) promoter in the HBB locus was shown to be correlated with HbF levels in pioneering studies conducted on normal individuals and patients with sickle cell disease or β-thalassemia [24–27]. In the absence of a demonstrated functional role for this site, it has been suggested that HbF phenotype is modified by *cis*-linked elements located elsewhere in the β-globin cluster and in linkage disequilibrium with this SNP [28,29]. Indeed, a quasi-palindromic structure located at the 5′ DNAse hypersensitive site 4 (HS4) of the locus control region (LCR), a polymorphism of which is in linkage disequilibrium with the *Xmn*I site [28], may affect direct or indirect interactions with the transcriptional repressor BCL11A [30]. This transcriptional repressor directly regulates HbF levels during the globin switch after birth [31]. Erythroid-specific BCL11A knockdown blocks the silencing of fetal globin genes [32], and BCL11A SNPs associated with HbF level variations (located in intron 2) colocalize with target sites for erythroid transcription factors [33]. The KLF1 transcription factor represses γ-globin expression by activating BCL11A, with haploinsufficiency causing high HbF levels [17,34,35]. The MYB gene is a key regulator of the balance between proliferation and differentiation during erythropoiesis [36]. It regulates HbF levels through an as yet undetermined mechanism [37,38]. The intergenic HBS1L-MYB region contains MYB enhancer sites and DNA targets for erythroid transcription factors [39]. Genetic variants associated with the persistence of HbF, located in a 24 kb region of HMIP [15], affect MYB expression [16] by reducing erythroid transcription factor binding and long-range promoter activation [40].

The regulatory transcription factors involved in γ-globin gene regulation or F-cell differentiation and survival are potentially of considerable interest as targets for increasing HbF levels. However, it remains difficult to modulate the function of factors other than enzymes or signal-dependent nuclear factors by disrupting DNA/protein or protein/protein interactions [41] and such modulation is particularly problematic for factors with important non-erythroid functions [42]. Furthermore, any interference with erythroid transcription factors may result in the inappropriate disruption of erythropoiesis. Efforts are being made to design endonucleases capable of precisely disrupting the genomic sequences involved in the erythroid-specific expression of γ-globin repressors, as a means of activating HbF, but this remains a difficult challenge [43].

The S-phase cell-cycle inhibitor hydroxyurea (HU) has proved clinically effective in patients with sickle cell anemia (SCA) [44,45]. It is also of clinical benefit to some patients with β-thalassemia intermedia and it reduces the need for transfusions in a subset of individuals with β-thalassemia major [46–53]. However, side effects have been reported, including cytopenia, hyperpigmentation, weight gain, opportunistic infections, azoospermia in approximately 80% of men (even years after the end of treatment), and marked hypomagnesemia [54]. There is little or no risk of leukemia [55], but HU is believed to be teratogenic [54]. Given the potential adverse effects and reported efficacy in only a subset of patients, it is important to identify likely responders and nonresponders before initiating treatment, to ensure that prescriptions are efficiently targeted. The increase in HbF levels following HU injection in patients with β-thalassemia major seems to be correlated with the *Xmn*I polymorphism [46,49,50,53], although this result has not been confirmed by all studies [47]. The correlation is less evident for non-transfusion-dependent thalassemia [48,51,52]. This is problematic, as treatments aiming to increase Hb levels by a few grams per deciliter are clearly more promising for the treatment of patients with thalassemia intermedia than for the treatment of transfusion-dependent patients. Baseline HbF level is clearly correlated with BCL11A SNP markers [11,12,14] and levels of the γ-globin repressor