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Novel and innovative approaches for treatment of β-thalassemia

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ABSTRACT

Basic science studies have provided new insights into the pathophysiology of β-thalassemias. Studies of genotypic and phenotypic heterogeneity among patients and better understanding of control of erythropoiesis have provided new targets for designing novel agents that can be tailored to individual patient needs. JAK-2 kinase inhibitors and agents targeting the GDF-11/SMAD pathway are in clinical trials. Recent understanding of the control of switch of HbF to HbA during infancy has provided new targets for development of drugs and gene-editing strategies. Advancement in vector design, purification and transduction of human stem cells have led to multiple gene therapy clinical trials that are exploring the clinical benefits, safety and durability of these approaches. HSCT remains the only curative approach at present and continued improvements in unrelated and haplo-identical transplants is helping to expand the donor pool. Even though the future looks promising, carefully designed clinical trials in adults and children, with suitable end-points are still needed to confirm the efficacy, toxicity of these new agents, and improvements in quality of life of patients with β-thalassemia.

1. Introduction

β-thalassemias are monogenic disorders characterized by reduced or absent synthesis of the β-globin chain, one of the main components of hemoglobin A (HbA, α2β2). Several hundred mutations are now described in the β-globin gene (HBB gene cluster on chromosome 11) or its regulatory elements, leading to decreased or no synthesis of the β-globin [1]. This results in a relative increase in the unattached α-globin chains (α/β-chain imbalance) that form insoluble hemi-chromes in the erythrocyte progenitors. The hemichromes damage the erythrocyte membrane, leading to severe intramedullary erythrocyte apoptosis (ineffective erythropoiesis, IE) and severely shortened red blood cell (RBC) life-span due to extra-medullary hemolysis, leading to severe anemia [2,3].

Although the switch from γ-to β-globin synthesis begins before birth, replacement of the HbF (α2γ2) by HbA occurs in the postnatal period [4]. Consequently, infants with severe β-globin chain abnormality become symptomatic around 6 months of age. Based on their transfusion needs, β-thalassemia patients are classified as transfusion dependent (TDT) or non-transfusion dependent thalassemia (NTDT), although these definitions are also fluid, as some NTDT patients may need regular transfusions as they become older [5].

The phenotype of β-thalassemias is variable depending upon the reduction or absence of β-globin chain synthesis and other genetic variables like co-inheritance of α- and γ-mutations, as well as co-inheritance of other hemoglobinopathies (Hb E, Lepore and sickle hemoglobin) [6]. Some mutations may also alter the fetal to adult Hb switching and may lead to higher production of HbF into adulthood (hereditary persistence of fetal Hb, HPFH) and less severe anemia [7,8]. Therefore, though the thalassemia severity can be usually be predicted based on the mutation analysis of the HBB cluster, other genetic factors may modify the actual phenotype and transfusion needs.

1.1. New insights from erythropoiesis

Erythropoietin (EPO), primarily produced in the kidney, regulates the erythropoietic activity in response to cellular hypoxia, by regulating the activity of the erythroid progenitor cells (BFUe, CFUe). Upon binding of EPO to its receptor (EPOR), the tyrosine kinase –Janus kinase 2 (JAK2) is phosphorylated, which in turn activates multiple signal transduction pathways (e.g. an

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Abbreviations

NCT	National Clinical Trials Identifier (clinicaltrials.gov)
HPFH	hereditary persistence of fetal hemoglobin
IE	ineffective erythropoiesis
TF	transcription factor

important pathway is by activation of Signal Transducer and Activator of Transcription 5, Stat5), that are crucial to erythropoiesis [9]. Increased EPO levels due to severe anemia lead to stress erythropoiesis (bone marrow hyperactivity and extramedullary hematopoiesis) as a compensatory mechanism in thalassemia patients [10], but this still proves 'ineffective' in preventing anemia due to increased rates of erythrocyte apoptosis and hemolysis.

Growth differentiation factor-11 (GDF-11) levels are increased in patients with thalassemia. GDF-11 is a cytokine, which belongs to the transforming growth factor (TGF)-beta superfamily and blocks terminal erythroid maturation through an autocrine amplification loop involving oxidative stress and α-globin precipitation. The effects of GDF-11 are mediated via SMAD signaling pathway, which leads to a decrease in erythroid differentiation [11]. This contributes further to the severity of IE.

1.2. Thalassemia and iron metabolism

There is a close connection between erythropoiesis and iron (Fe) metabolism. The hormone that controls the iron absorption is hepcidin, which is synthesized in the liver and secreted into blood. Hepcidin synthesis is controlled by transferrin saturation, iron storage, inflammation and demand for the erythropoiesis [12]. A variety of erythroid factors e.g. growth differentiation factor (GDF-15) and Erythroferrone (Erfe) affect the production of hepcidin [13,14]. Increased IE, anemia and inflammation leads to increased production of GDF-15 that leads to suppression of hepcidin levels. This in turn leads to increased absorption of Fe from the gut. Increased gut absorption of Fe contributes to the increased iron overload, especially in NTDT patients and further contributes to the Fe overload from transfusions in TDT patients.

Methods and treatments that are new to the clinic or are in the development phase are discussed in the following sections and are highlighted in Table 1. Three potential innovative approaches for treatment of thalassemia are discussed- 1) novel agents, 2) gene-therapy approaches, and 3) innovations in stem cell transplant.

2. New agents

2.1. Agents for induction of HbF in β-thalassemia

Recent studies of HbF regulation have energized the field for the development of clinical HbF inducers. Increased production of γ-globin can ameliorate the severity of β-thalassemias by decreasing the α/β-chain imbalance, which is roughly proportional to the clinical severity [15].

2.1.1. Hydroxyurea

Hydroxyurea (HU) therapy exerts a 2- to 9-fold increase in γ-mRNA expression in thalassemia patients [16]. However, increase in the HbF levels did not always correlate with increases in total Hb levels in clinical studies. HU therapy is not associated with considerable effects on the erythrocyte deformability in β-thalassemia, which may explain the reduced benefits of the drug in this condition, compared to sickle cell anemia [17]. HU (10–20 mg/kg per day) has been reported to lead to some reduction (40–70% decrease) in transfusion needs in TDT patients, especially in HbE/β-thalassemia genotypes [18,19]. Caution should be exercised in interpreting results of the single arm trials in thalassemia patients that use transfusion reduction as their ends-point, as patients could be kept at a lower nadir Hb level during follow-up and may be at risk of increased morbidity later in life.

In smaller studies, HU therapy was also associated with improvements in endocrine function, leg ulcers and extra-medullary hematopoiesis [20,21]. Due to perceived safety concerns (dose dependent myelotoxicity, skin reactions) and lack of long term benefit in thalassemia patients, HU use is still restricted to small subgroup of patients (thalassemia intermedia, Hb-E thalassemia or patients with splenectomy) and long term studies proving benefits are still pending.

2.1.2. Thalidomide/lenalidomide

In-vitro studies have shown that these compounds slow erythroid maturation, increase proliferation of immature erythroid cells and regulate transcription of globin genes, resulting in HbF induction [22,23]. Some early studies of thalidomide have shown benefit for increasing HbF production in thalassemia patients [24], although the risk-benefit still needs to be established in view of potential toxicity of these drugs. These drugs should be administered in the context of clinical studies, where benefits and side effects can be studied and data on clinical and quality of life collected over years.

2.1.3. Sirolimus

This is another potential drug for inducing HbF production in thalassemia patients, as it also increases γ-mRNA expression and a corresponding increase in HbF [25,26]. Risk-benefit of Sirolimus also needs to be confirmed in large patient studies.

Table 1
Novel Agents and their mechanism of action.

Agent	MOA
Hydroxyurea	Induction of HbF
Thalidomide, Lenalidomide	Induction of HbF
Sirolimus	Induction of HbF
Ruxolitinib, Pacritinib	JAK2 inhibitor (inhibits EPO signaling pathway)
Luspatercept, Sotatercept	Activin receptor-II trap ligands (inhibit effect of GDF-11)
Mini-hepcidins	Reduce iron overload
Apo-transferrin	Reduce toxicity of iron
Gene therapy	Multiple mechanisms – Gene- addition or Gene-editing to increase production of HbA or F.

2.2. Agents modulating erythropoiesis

2.2.1. JAK2 inhibitors (Ruxolitinib, Pacritinib)

These agents have significant anti-proliferative and anti-inflammatory activity to control extra-medullary hematopoiesis and splenomegaly in β-thalassemia patients [27]. By inhibiting the main signaling pathway for EPO on erythrocyte progenitors, these agents can have a beneficial effect on splenomegaly and amount of blood sequestered by the spleen, leading to less transfusion requirements. Hence, these agents, once proven safe, can be utilized in lieu of splenectomy and to decrease extra medullary hematopoiesis.

2.2.2. Activin receptor—II trap ligands (Luspatercept/Sotatercept)

These designer ligands inhibit over-activated SMAD signaling proteins in the erythroid precursors, thereby inhibiting the effect of GDF-11 cytokine on late stage erythrocyte differentiation and maturation. Notably, these compounds have been shown to mitigate disease complications of IE, reduce iron overload, and improve bone pathology in animal models [28,29]. Clinical trials are underway and have shown early promise in reducing transfusion requirements in β-thalassemia patients when these agents are administered for a short period of 12 weeks [30]. Studies on the effect of these medications on IE and transfusion burden are being performed (NCT 01571635 and NCT 02604433).

2.3. Agents modulating iron metabolism

2.3.1. Mini-hepcidins

Since, low hepcidin levels are associated with increased gut absorption of Fe, hepcidin agonists might improve the iron burden in thalassemia patients. Mini-hepcidins are short peptide mimetics that are sufficient to induce hepcidin actions; thereby decreasing serum iron levels and ameliorating iron overload [31]. Use of these compounds significantly reduces iron overload and erythroid cell damage in $Hbb^{th3/l}$ mice [32].

2.3.2. Apo-transferrin administration

Decreased transferrin saturation can be beneficial in thalassemia, therefore, administration of Apo-transferrin can decrease labile plasma iron concentrations, normalize RBC survival, and increase Hb production [33]. This protein is also in early stages of clinical trials.

3. Gene-therapy

The era of genome sequencing and understanding of the HBB gene cluster and its strict regulation and control, has provided new options for the treatment for thalassemia patients. The complete understanding of the switch from γ-globin to β-globin and the control of this switch by various transcription factors (TF) has provided new targets for gene-modifications. BCL11a is now recognized to be an important TF that controls the switch from production of HbF to HbA [34,35]. After birth, as the level of BCL11a increase, the level of HbF production decreases and HbA increases.

Also, the advances in vector development, transduction of human stem cells and various gene-editing tools, provide a new hope for availability of curative options in the near future, making this one the most promising treatment options.

Currently, the gene-therapy approaches can be divided into two broad groups-

A)Gene-addition, and B) Gene-editing approaches.

3.1. Gene-addition

This involves insertion of a lenti-viral/retroviral vector that contains the whole regulatory and the β-or γ-producing genes into autologous human stem cells 'in-vitro', and then infusing these modified stem cells back to the patient after myeloablation [36,37]. Though conceptually easy, the field has technologically advanced only recently, where the vectors (packaged with all the HBB genes and it's regulatory elements) can now be produced at large scale, achieve extreme purification and potency to transfect large number of 'non-proliferating' human stem cells to provide clinical meaningful responses [38,39]. For a long lasting correction (and with the hope of one time curative treatment), the insertions are done in stem and progenitor cells ($CD34^+$ selected population). In that case the globin producing genes have to be placed under the control of an erythroid specific promoter, so that the transcription of the inserted genes can only occur in erythroid precursors, and not in white blood cells or platelets, which are also derived from the modified stem cells. There are multiple lenti-viral vectors in clinical trials now for thalassemia (NCT 02453477, NCT 02906202, NCT 02151526). Once a large enough population of stem cells have been transduced and infused back to patient, it is expected that the erythrocyte progenitors derived from these stem cells will produce enough β (or γ) globin to combine with α-chains and reduce the α/β imbalance. This will lead to correction of IE and improved RBC lifespan, with larger number of erythrocytes with higher Hb persisting in the peripheral blood, leading to the correction of anemia and reduction in transfusion needs [40,41].

Since the vector insertions into the stem cells occur 'semi-randomly' and remains largely an uncontrolled process, there is a small risk that some insertions into human stem cells can occur in the vicinity of proto-oncogenes and can stimulate clonal proliferation leading to leukemia/myelodysplastic syndrome (MDS) [42]. Currently, FDA requires all patients treated with gene therapy to be followed for a period of 15 years to clearly establish the incidence of this risk. Fortunately, to date of all the patients treated with lenti-viral vectors, none has developed any leukemia or MDS [43].

All patients treated till date have tolerated the conditioning regimen with myeloablative doses of busulfan without any unexpected toxicity and infusion reactions related to the administration of modified stem cells. All patients have engrafted, though efficacy analysis of the first few patients treated with BB305 lentiviral vector, show variable responses and total Hb production. This variability is expected, as patients with β-thalassemia have large genetic heterogeneity due to varied mutations in the HBB cluster and various genetic modifiers and therefore, the level of Hb required to become transfusion independent is variable. The initial results of two concurrent trials (HGB 204 and 205 using BB305 vector), show an average production of 4–5 g/dl of HbA^{T87Q} from the gene-insertions (HbA^{T87Q} is the gene insertion derived Hb that can be detected separately from transfusion derived HbA by HPLC due to presence of one AA substitution: Threonine at 87 position instead of Glutamine). An increase of Hb by ~5 g/dl is enough to lead to transfusion independence in HbE/β-thalassemia and β0/β+ patients, but only leads to partial improvement in β0/β0 patients, where there is a need for higher levels of Hb production to become transfusion independent [44]. Improvements in technology are in progress to improve the transduction efficiency of the stem cells.

3.2. Gene-editing

Availability of the new tools in the last few years is leading a rapid development of gene-editing approaches to ameliorate the

anemia in thalassemia patients. Last few years have seen advances in availability of different nucleases- Zinc-Finger Nucleases (ZFN), Transcription Activator-Like Effector Nucleases (TALENS), and Clustered Regularly Interspaced Short Palindromic Repeats and Crispr-associated-nuclease 9 (Crispr-Cas9), which are all nucleases that can cut the human DNA at precise locations [45–48]. Of these techniques, Crispr-Cas9 is the most appealing as it leads to precise double stranded breaks in the DNA helix, using a pre-designed 42-nucleotide guide sequence, which has bases complimentary to its target site of the desired break in the DNA [49,50].

BCL11a (the TF that controls the switch from HbF to HbA) provides an excellent target for gene-editing approaches. By suppressing BCL11a, it is postulated that HbF production can be triggered again in thalassemia patients. Making specific deletions in the erythroid enhancer of the BCL11a is a promising approach that is being explored currently [51]. Another approach to increasing HbF production by gene editing is to recreate the mutations seen in patients with HPFH. This can be achieved by –

i) creating small deletions e.g. in the γ-δ intergenic region leads to significant enhancement of the γ-gene expression [52];
ii) creating small deletions in the area of HBB cluster where TF bind, so the effect of TF can be inhibited [53,54]; and
iii) creating point mutations in the γ-globin promoter region that can also lead to overexpression of the mutated gene [55].

Animal studies are being performed currently to prove which of these methods and targets lead to significant production of HbF with minimal toxicity to stem cells and 'off-target' (cutting DNA at unintended sites) activity [56].

Performing these small edits in the stem cells of thalassemia patients 'ex-vivo' by Crispr-Cas9 tool, may provide an inexpensive way (compared to stem cell transplant and gene addition) in future to ameliorate the disease.

All these methods, once refined and scaled to human applications, are expected to go into clinical trials in the near future. If considered efficacious, safe and durable, they will vastly improve the outcomes of thalassemia patients, obviating the need for finding donors for a cure, as this is the primary barrier to stem cell transplantation. The main differences between gene therapy and stem cell transplantation are highlighted in Table 2.

Another strategy that is still in early phase of investigation is to use induced pluripotent stem cells (iPSC) instead of hematopoietic stem and progenitor cells from patients. Patient specific iPSCs can be generated from somatic cells (eg. skin fibroblasts) and can be genetically modified and then infused back to the patient to make erythroid cells [57,58]. The main advantage of this approach will be to obviate the need of collecting hematopoietic stem cells from the patients and iPSCs can provide a ready resource of corrected cells that will make the gene therapy process simpler. Also the risk of insertional mutagenesis may be lower in the iPSCs, making this a better approach for safety reasons [59].

4. Innovation in hematopoietic stem cell transplantation (HSCT)

The topic of HSCT is covered in detail in the excellent review by Dr. Alok Sirivastava and hence, is only discussed here to complete the list of new treatment options. HSCT from a well-matched donor remains the standard curative option for patients with β-thalassemia [60]. Unrelated donor transplants, cord blood transplants and haplo-identical transplants provide new avenues now to increase the donor pool [61–63]. Each of these options need to be studied further in well-designed studies with the endpoints of not only transfusion independence, but also focusing on quality of life, effect on iron overload and associated morbidity due to HSCT related complications like graft versus host disease (GVHD) in the post-transplant period. It is my strong recommendation that the end-point of all HSCT trials in thalassemia should not just focus on transfusion-free survival, but should also include 'GVHD-free survival' and quality of life parameters.

5. Combination treatments

It is envisaged that many of these future treatments may not work individually for all β-thalassemia patients due to heterogeneity of genotype/phenotypes, but combination therapies may improve outcomes and quality of life. Combining various treatments based on a design based on their mechanism of action and non-overlapping toxicity will be important in the future. For example, patients who are converted to NTDT after gene therapy (transfusion independent, but maintain Hb levels between 8 and 10 g/dl), may benefit from Hb-F inducing drugs currently in trials [64].

6. Summary

- Major advances in the understanding of pathophysiology of β-thalassemia and control of erythropoiesis has provided new targets for design of novel agents.
- Multiple novel, rationally designed, and targeted therapeutic agents are expected to be available in the near future that lead to decrease in ineffective erythropoiesis, increase HbF production or decrease gut absorption of iron.
- Gene-therapy is progressing rapidly with multiple clinical trials being conducted in many countries with the promise of commercial products to be available in the near future.
- Improvements in unrelated and haplo-identical HSCT will expand the donor pool for patients willing to undergo allogeneic HSCT.
- Carefully designed studies are still needed to confirm the efficacy, toxicities and long-term durability of all these approaches.
- Quality of life parameters using validated tools should be assessed as part of all experimental studies, so that risk-benefits of each approach can be clearly delineated.

Table 2
Differences between allogeneic HSCT and autologous gene-therapy.

Allogeneic HSCT	Autologous Gene Therapy
Toxicity is from conditioning and immunosuppression	Toxicity is related to intensity of conditioning
Immunosuppression required	No immunosuppression needed
Risk of immune-mediated rejection	None
Graft- versus-Host Disease risk	None
Dependent on donor availability	None
Long-term risks: organ toxicities	Potential risk of oncogenesis or 'off-target' activity to be ascertained in clinical studies

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