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Chapter

Updates in Thalassemia

Tamer Hassan and Marwa Zakaria

Abstract

Beta-thalassemia is a genetic disease caused by mutations in the β-globin gene, resulting in partial or complete deficiency of β-chain. Deficiency of β-chain was accompanied by excess unmatched α-globin chains with subsequent dyserythropoiesis, oxidative stress, and chronic anemia. The main therapeutic option is blood transfusion that improves the anemic status but unfortunately exacerbates iron overload status. Till now, the only curative measure is allo-hematopoietic stem cell transplantation. New diagnostic and therapeutic modalities are now available. These include the preimplantation genetic diagnosis and new tools in the assessment of iron overload. Also, new therapeutic options aimed at different targets are being developed; for example, therapies that stimulate the synthesis of γ-globin and reduce the synthesis of α-globin, as well as the iron excess, dyserythropoiesis, and oxidative stress. However, the most likely ideal approach is efficient prevention, through genetic counseling, carrier detection, and prenatal diagnosis.

Keywords: β-thalassemia, genetic diagnosis, gene therapy, gene editing, new iron chelators, HSCT

1. Introduction

1.1 Updates in diagnosis

1.1.1 Preimplantation genetic diagnosis

Prenatal diagnosis (PND) services are the best practices to control prevalence of disease. Wide spread practicing of PND proved effective in reducing the number of new cases but on the other hand high abortion rate is hided, which ethically unaccepted and for many couples is not a suitable choice. Preimplantation genetic diagnosis is considered in a similar fashion to prenatal diagnosis (PGD) and is a strong alternative to conventional PND. PGD means genetic profiling of embryos prior to implantation (embryo profiling). Its main advantage is that it avoids selective pregnancy termination as the method makes it highly likely that the baby will be free of the disease under consideration. At present PGD is the only abortion free fetal diagnostic process [1].

The most important indications of PGD [1] are:

- monogenic disorders, e.g., Thalassemias;
- HLA matching (savior baby);
- cancer predisposition; and
- sex discernment.

Technical aspects (Figure 1) [2]:

1. obtaining embryos (assisted reproductive technology)
   - controlled ovarian stimulation (COH) to obtain large number of oocytes and
   - ICSI/IVF;
2. obtaining biopsy (blastomere or blastocyst biopsy);
3. genetic analysis techniques (PCR, FISH); and
4. embryo transfer and cryopreservation of surplus embryos.

Mutation testing:

PGD method to diagnose beta thalassemia was initially reported in 1998 and used either denaturing gradient gel electrophoresis or restriction enzyme digestion methods to perform the mutation analysis. The possibility of misdiagnosis due to allele drop out (ADO) and DNA contamination is considered to be the major problems associated with preimplantation genetic diagnosis [3]. Recently, the European Society of Human Reproduction and Technology (ESHRE) recommends doing both direct and indirect mutation testing using short tandem repeat (STR) linkage analysis to achieve the highest accuracy rate [4].

1.1.2 Assessment of iron overload in thalassemia

a. Well established methods for assessment of iron overload in thalassemia [5]:
   - serum ferritin;
   - liver biopsy for liver iron content (LIC);
   - echocardiography, MUGA for cardiac iron; and
   - MRI T2* for LIC and cardiac iron.

b. New methods—monitoring of liver iron overload by SQUID:
   - Superconducting QUantum Interference Device (SQUID) has been recently introduced as an integral part of thalassemia care in few centers worldwide. The SQUID allows the thalassemia team to monitor iron concentration in the livers of patients and gives them a reliable tool to help adjust medication in order to avoid serious complications [6].

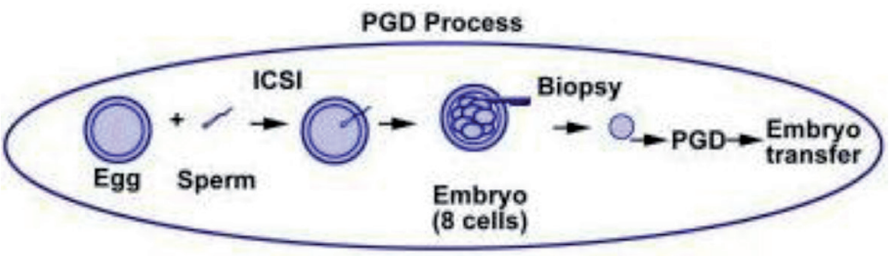

Figure 1.
Technical steps of PGD [2].

Mechanism of action of SQUID:
SQUID has high-power magnetic field. Iron interferes with the field and changes in the field are detected [6].

Advantages and disadvantages of SQUID:
Advantages and disadvantages of SQUID are listed in **Table 1** [6].

Advantages	Disadvantages
Linear correlation with LIC assessed by liver biopsy	Indirect measure of LIC
May be repeated frequently	Limited availability
	High cost
	Highly specialized: needs dedicated technician
	May underestimate LIC levels

Table 1.
Advantages and disadvantages of SQUID.

1.2 Updates in treatment

1.2.1 Gene therapy

The first obstacle against gene therapy was the extremely complex regulation of the globin genes. The second and equally important obstacle has been the lack of an optimal vector for gene transfer into quiescent hematopoietic stem cells (HSC) [7].

The first successful gene therapy for β-thalassemia major was in 2007. The process is as follow: autologous HSCs are harvested from the patient and then genetically modified with a lentiviral vector expressing a normal globin gene. After the patient has undergone appropriate conditioning therapy to destroy existing defective HSCs, the modified HSCTs are reintroduced to the patient [8].

Two large clinical trials have been recently conducted. The first one was entitled "ß-Thalassemia Major with Autologous CD34+ Hematopoietic Progenitor Cells Transduced with TNS9.3.55 a Lentiviral Vector Encoding the Normal Human ß-Globin Gene." This trial was sponsored by Memorial Sloan Kettering Cancer Center. The second one was entitled "A Study Evaluating the Efficacy and Safety of the LentiGlobin® BB305 Drug Product in Subjects with Transfusion-Dependent β-Thalassemia, who do Not Have a β0/β0 Genotype." It was sponsored by bluebird bio. Expected success awaits these clinical trials (**Figure 2**) [9].

1.2.2 Gene editing

A newer approach employs genome editing techniques, such as transcription activator-like effectors nucleases (TALEN), zinc finger nucleases (ZFN), or the clustered regularly interspaced short palindromic repeats (CRISPR) with Cas9 nuclease system. They replace the target single-mutation sites with the correct sequence, restoring the functional gene configuration. Producing a sufficiently large number of corrected genes is the major challenge with this new approach (**Figure 3**) [7].

1.2.3 Targeting ineffective erythropoiesis

A large number of preclinical and early clinical studies investigating erythropoiesis modulators are currently studied. These modulators include

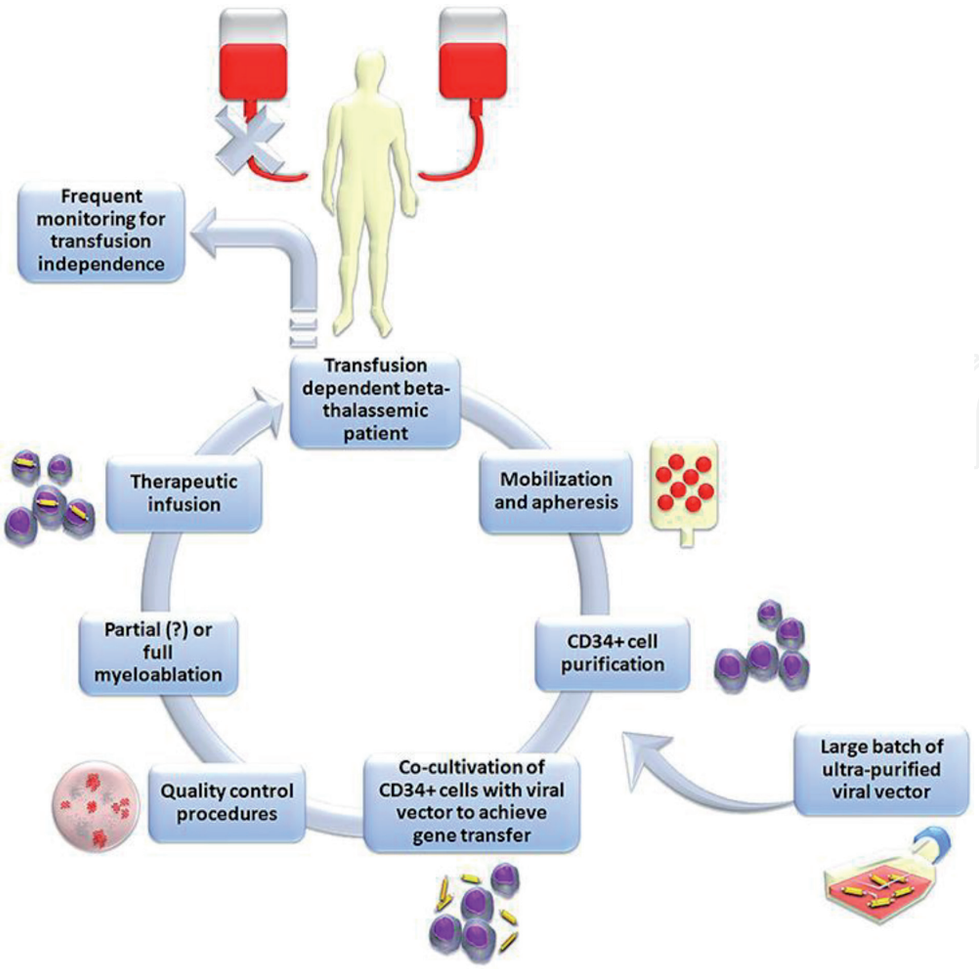

Figure 2.
Gene therapy of beta thalassemia [9].

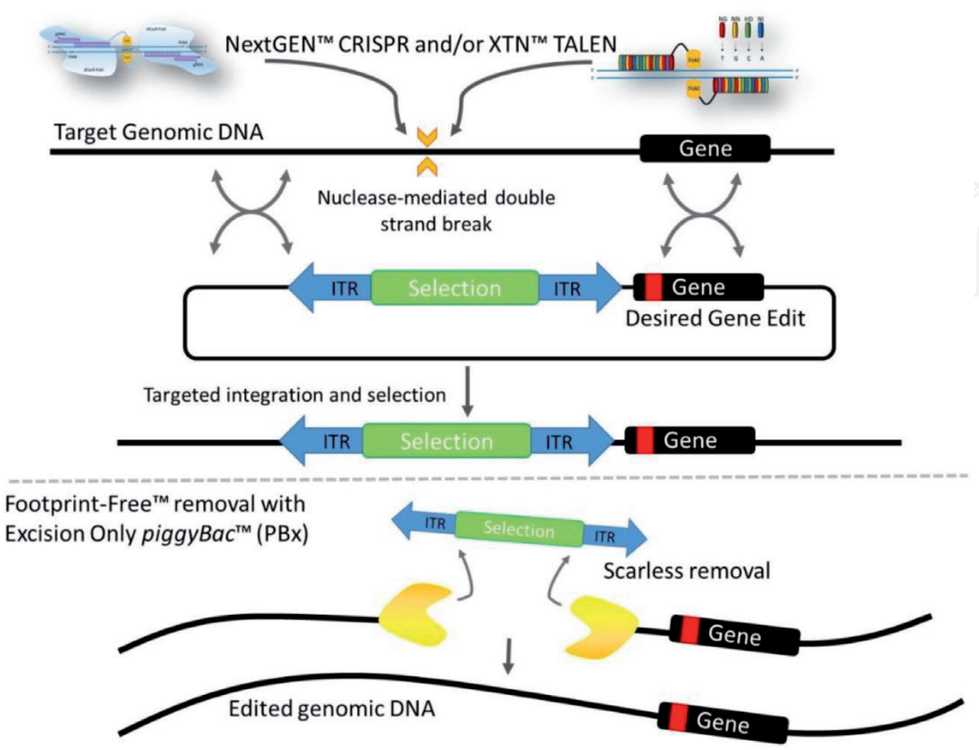

Figure 3.
Gene editing of beta thalassemia [7].

TGF-β-like molecules or inhibitors of JAK2, Foxo3 activators, HRI-Eif2ap stimulators, Prx-2 activators, HO-1 inhibitors, Hsp-70 chaperone induction and pyruvate kinase activation. These modulators could soon revolutionize the treatment of β-thalassemia and related disorders [10].

Activins, members of TGF-β family signaling, are crucial regulators of hematopoiesis and modulate various cellular responses such as differentiation, proliferation, migration, and apoptosis. They were observed to ameliorate hematologic parameters and improve hematopoiesis in preclinical and early clinical studies [11].

JAK2 plays an important role in the progression and worsening of ineffective erythropoiesis. Drugs inhibiting JAK2 activity could reverse ineffective erythropoiesis and splenomegaly [12].

1.2.4 Manipulating iron metabolism

Hepcidin is the central regulator of iron homeostasis. Hepcidin inhibitors, e.g., ERFE inhibitors are now extensively studied as a possible future treatment of iron overload. Induction of iron restriction by means of transferrin infusions, minihepcidins, or manipulation of the hepcidin pathway prevents iron overload, redistributes iron from parenchymal cells to macrophage stores, and partially controls anemia in β-thalassemic mice [13].

1.2.5 New formulation of iron chelators

Patients with transfusional iron overload usually require iron chelation therapy (ICT) to help decrease the iron burden and to prevent and/or delay long-term complications associated with iron deposition in tissues [14]. There are currently three available iron chelators.

1.2.5.1 Deferoxamine

DFO is a hexadentate iron chelator that binds iron in 1:1 complexes. It is given subcutaneously using a pump or intravenously as it is not absorbed orally. The dose ranges from 20 to 50 mg/kg/day. Though it is an effective drug, limited compliance was reported due to the inconvenience of parenteral administration as well as infectious complications [14].

1.2.5.2 Deferiprone

DFP was the first oral iron chelator to be used for transfusional iron overload. DFP is a bidentate iron chelator that forms 3:1 complexes. The dose ranges from 75 to 100 mg/kg/day divided over three doses. Treatment with DFP was associated with lower myocardial iron deposition compared to deferoxamine. The most common adverse effects of DFP include GIT disturbances, elevated liver enzymes and arthropathy. The most serious adverse event was neutropenia that was recovered after temporary discontinuation of treatment [14].

1.2.5.3 Deferasirox

DFX is another iron chelator. It is a tridentate that forms 2:1 complexes. The dose ranges from 20 to 40 mg/kg/day. DFX is a long acting iron chelator. It is given once daily which is convenient to most patients. It lacks the DFP's potentially life-threatening adverse effect of agranulocytosis. Patient Compliance and adherence to long-term chelation therapy in patients with transfusion-dependent β-thalassemia

is challenging and critical to prevent iron overload-related complications. Thanks to oral iron chelators formulations that allow better compliance and improve patients and parents' adherence to the drugs. Once-daily deferasirox dispersible tablets (DT) have proven long-term efficacy and safety in patients ≥2 years old with chronic transfusional iron overload. However, barriers to optimal adherence remain, including palatability, preparation time, and requirements for fasting state. A new film-coated tablet (FCT) formulation was developed, swallowed once daily (whole/crushed) with/without a light meal [15]. Key differences between deferasirox dispersible and film coated tablets are listed in **Table 2**.

1.2.5.4 Combined iron chelators

Combined DFO and DFP chelation therapy was introduced to manage iron overload in patients with suboptimal chelation with maximum DFP doses. A shuttle mechanism was proposed to explain the synergistic effect of DFP and DFO. DFP enters cells due to its low molecular weight and removes iron, and then passes it on to DFO to be excreted in urine and stool. Other combinations like DFX & DFO and DFX & DFP were used to maximize efficacy, improve compliance and minimize the side effects [14].

1.2.6 Fetal hemoglobin induction

The main pathophysiological determinant of the severity of β-thalassemia syndromes is the extent of α/non-α globin chain imbalance. Thus in β-thalassemia, pharmacologically induced increase in γ-globin chains would be expected to decrease globin chain imbalance with consequent amelioration of clinical manifestation. Increased production of the fetal γ-globin can bind the excess α-chains to produce fetal hemoglobin and hence improve α/β-globin chain imbalance leading to more effective erythropoiesis. This partly explains the more favorable phenotype in some patients with β-thalassemia intermedia and hemoglobin E/β-thalassemia compared with transfusion-dependent β-thalassemia major [16].

Several pharmacologic compounds including: 5-azacytidine, decitabine, hydroxyurea (HU), butyrate (short-chain fatty acids), erythropoietin and short chain fatty acid derivatives (SCFAD) as fetal hemoglobin-inducing agents had encouraging results in clinical trials [16].

1.2.7 Haploidentical hematopoietic stem cell transplantation

Haploidentical transplant approach have a crucial clinical significance in patients with beta thalassemia major as it provides a graft source to almost all patients who do not have an HLA-matched donors. Haploidentical HSCT is always available and parents of children are highly motivated. Haploidentical means 50% identity and the problem is the subsequent high risk of graft versus host disease (GVHD) caused by donor T cells. Successful haploidentical HSCT depends on effective removal of donor T cells [17].

Methods of T-Cell depletion [17]:

1. CD34+ positive selection with immunomagnetic separation which leads to enrichment of stem cells;

2. CD3 depletion with immunomagnetic separation which leads to specific reduction of T cells; and

3. Addition of Alemtuzumab (anti-CD52 antibody) to the transplant bag.

	Dispersible tablet [EXJADE]	Film-coated tablet [JADENU]
Dosage forms and strengths	• White round tablet ○ 125 mg ○ 250 mg ○ 500 mg	• Film-coated blue oval tablet ○ 90 mg (light blue) ○ 180 mg (medium blue) ○ 360 mg (dark blue)
Administration	• Once daily on an empty stomach ≥30 minutes before a meal • Stir to disperse in water, orange juice or apple juice ○ 3.5 oz. liquid for <1 g ○ 7.0 oz. liquid for ≥1 g • Consume the suspension • Resuspend residue in small amount of liquid and consume immediately • Tablets should not be chewed or swallowed whole	• Once daily on an empty stomach or with a light meal (<7% fat; ~250 calories) • Swallow whole or crush and mix with soft foods such as yogurt or apple sauce immediately before use
Starting dose	• Transfusion-dependent iron overload: 20 mg/kg/day • NTDT: 10 mg/kg/day	• Transfusion-dependent iron overload: 14 mg/kg/day • NTDT: 7 mg/kg/day
Titration increments	• Transfusion-dependent iron overload: 5–10 mg/kg/day • NTDT: 5–10 mg/kg/day	• Transfusion-dependent iron overload: 3.5–7 mg/kg/day • NTDT: 3.5–7 mg/kg/day
Maximum dose	• Transfusion-dependent iron overload: 40 mg/kg/day • NTDT: 20 mg/kg/day	• Transfusion-dependent iron overload: 28 mg/kg/day • NTDT: 14 mg/kg/day

Table 2.
Deferasirox formulations: Key differences.

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