Review

Beta-Thalassemia: A Pharmacological Drug-Based Treatment

Shrabonti Biswas [1], Shanita Zaman Smrity [1], Md. Shimul Bhuia [1], Fatema Akter Sonia [1], Mst. Asma Aktar [1], Raihan Chowdhury [1], Tawhida Islam [1], Muhammad Torequl Islam [1], Gabriel Gonçalves Alencar [2], Cicera Laura Roque Paulo [2], Ana Pavla Almeida Diniz Gurgel [3] and Henrique Douglas Melo Coutinho [2,*]

[1] Department of Pharmacy, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj 8100, Bangladesh; shrabontibiswas8@gmail.com (S.B.); dmt.islam@bsmrstu.edu.bd (M.T.I.)
[2] Laboratory of Microbiology and Molecular Biology, Department of Biological Chemistry, Regional University of Cariri, Crato 63105-000, Brazil
[3] Department of Engineering and Environment, Federal University of Paraiba—UFPB, João Pessoa 58051-900, Brazil
* Correspondence: hdmcoutinho@gmail.com

Abstract: This review was performed to determine the potential of drugs that can remove or decrease the requirements for blood transfusion among beta (β)-thalassemia patients. A comprehensive literature search was conducted to identify clinical trials and studies using PubMed Central, Google Scholar, PubMed, and ScienceDirect archived articles published from 1996 to November 2023. According to this review, clinical trials for a number of drugs, including luspatercept, sotatercept, mitapivat, etavopivat, hydroxyurea, rapamycin, decitabine, thalidomide, and quercetin, have been performed as part of efforts to improve the cure strategy for β-thalassemia. Of these drugs, luspatercept and sotatercept have exhibited particularly promising results and have been granted US Food and Drug Administration (FDA) approval for use in β-thalassemia patients. The mode of action for the drugs luspatercept and sotatercept involves the stimulation of hemoglobin (Hb) production or enhancement of its functionality, thereby decreasing reliance on blood transfusions and enhancing the overall quality of life. In this way, drugs like luspatercept and sotatercept present an opportunity to notably decrease the necessity for blood transfusions in β-thalassemia patients, improving their standard of living and overall prognosis. However, more research is needed to evaluate the effectiveness and safety of these drugs in the long run.

Keywords: non-transfusion thalassemia; β-thalassemia; drug therapy; blood factors

1. Introduction

Thalassemia is a hereditary blood disease. The causes of thalassemia are mutations in the DNA of cells that transport oxygen throughout the body. Hemoglobin (Hb) qualifies red blood cells to convey oxygen. Thus, in thalassemia, Hb production lessens. There are a few factors that increase the risk of thalassemia, such as a family history of thalassemia, certain ancestries, and nutritional factors [1]. There are mainly two types of thalassemia: these are alpha (α)- and beta (β)-thalassemia. The number of genes absent from the four genes for α-globin or two genes for β-globin determines the severity of the thalassemia [2]. A- and β-thalassemias are usually autosomal recessive, with the first dominant hereditary incidents identified in an Irish family. Both parents must be carriers of the disease, and if a hemoglobinopathy trait is carried by both, the chance of an affected child is 25% for each pregnancy [3].

Hereditary Hb problems present an increasing global public health challenge. Almost 320,000 infants are born each year with Hb disease. About 80% of these births occur in developing countries. Most traditional estimates suggest that at least 5.2% of the global populace (more than 360 million people) has a notable Hb type, and over 100 million suffer from β-thalassemia, with a worldwide prevalence of 1.5%. Compound heterozygous

or homozygous conditions, among definite versions, may result in clinically apparent hemoglobinopathies [4]. The main symptoms of thalassemia result from the lack of or defective synthesis of β-globin chains [5]. Other symptoms include iron overload, infection, fatigue, slow growth, pale or yellowish skin, enlarged spleen, heart problems, facial bone deformities, dark urine, abdominal swelling, poor feeding, fussiness, weakness, etc. [6,7].

There are two kinds of treatment strategies for α- and β-thalassemia: transfusional and non-transfusional. Transfusional therapy involves regular and lifelong blood transfusions, which can prevent severe bone deformities, heart failure, and endocrinopathies related to thalassemia. The need for RBC transfusions continues throughout the life of the patient until a specific curative method, along with allogeneic stem cell transplantation, is achieved. Iron chelation therapy is also needed to manage the iron overload that results from ongoing transfusion therapy [8]. Non-transfusional therapy, meanwhile, includes drug and gene therapy options. Drug therapy aims to enhance levels of Hb and decrease blood transfusion burdens, such as the toxicities of iron chelation therapy and regular blood intake, finding blood donors, etc. [9]. Gene therapy involves harvesting the patient's own stem cells and genetically modifying them to express Hb properly before transplanting them back into the patient. Genetic therapy (bone marrow, cord blood, and peripheral blood) is a promising option to achieve a definitive cure for thalassemia in the future [10].

In this review, the drugs that can induce erythropoiesis and increase Hb in the blood of thalassemic patients will be discussed. The main topics are how they work, the studies that have been performed to date, and their potential to lift the transfusion burden.

2. Methodology

2.1. Search Strategy

A literature review on this topic was carried out to locate and report all previously published research on the drugs available in non-transfusion-dependent β-thalassemia. The search for this literature was performed using PubMed, Science Direct, PubMed Central, Google Scholar, and Elsevier archive-recognized articles published from 1966 to November 2023.

2.2. Inclusion Criteria

To evaluate the drugs that can replace blood transfusion in thalassemia, we reviewed the reports of original research projects with internal and external factor controls. More than 57 articles were selected to make up the data for this review.

2.3. Exclusion Criteria

Studies in the absence of internal/external factor controls, review articles, and those involving transfusion-dependent drugs were excluded from this review.

3. Pathophysiology of Thalassemia

Alpha-thalassemias come into being in a recessive fashion (Mendelian) through the *HBA1* and *HBA2* genes, along with a number of the (four) alleles present in diploid cells. The severity of the disease is determined by the number of alpha-globin alleles that are influenced, with decreased α-globin production resulting in an excess of β or γ chains. Thalassemia is widespread in Southeast Asia, people of African descent, the Middle East, and China [3,11].

Genetic mutations in the β-globin gene complex cause β-thalassemia, developing into a total absence of or an intense reduction in β-globin production. The disease is characterized by inefficient erythropoiesis, hemolysis, and hypersplenism, with clinical severity depending on the nature and presence of mutations in both alleles of the *HBB* gene. Mutated alleles are classified as β+ or βo based on partial or complete loss of protein function [12,13].

4. Current Treatment Strategies

4.1. Transfusional Therapy

To manage a toddler's condition, a blood transfusion every three weeks to maintain their Hb level is required [8]. Recurrent transfusions increase the risk of RBC alloimmunization, so taking the blood of a donor from the same ethnic class is recommended. The requirement for transfusions is lifelong, but stem cell transplantation or genetic therapy may offer a cure in the future. Until then, regular transfusions are necessary to maintain the toddler's health. Repeated transfusions lead to the accumulation of excess iron in various organs, causing damage and dysfunction. To manage this potentially fatal side effect, iron chelation is essential to remove the iron buildup [14].

4.2. Non-Transfusional Therapy

The two options here are drug therapy and gene therapy. Treatment with thalassemia blood transfusions throughout the lifespan results in iron overload, which requires management over a long period with iron chelation therapy. In this context, drug therapy aims to enhance Hb levels and decrease blood transfusion problems, with various options available including hydroxyurea (HU), sotatercept, luspatercept, quercetin, thalidomide, decitabine, and rapamycin [9]. Alternatively, in gene therapy, a patient's stem cells (bone marrow, peripheral blood, cord blood) are harvested. Genetic modification of Hb expression is performed via gene addition, knockdown, and gene editing. Then, cells are transplanted back into the patient [10]. In this way, bone marrow transplantation offers a potentially curative treatment.

4.3. Drugs Replacing Blood Transfusion Process in Thalassemia

In this review, details of drugs that can replace blood transfusion in thalassemia are provided. We focus on discussing seven promising drugs: luspatercept, sotatercept, mitapivat, etavopivat, HU, rapamycin, decitabine, thalidomide, and quercetin (Figure 1). The status of different phases of natural compounds in the treatment of β-thalassemia is shown in Table 1.
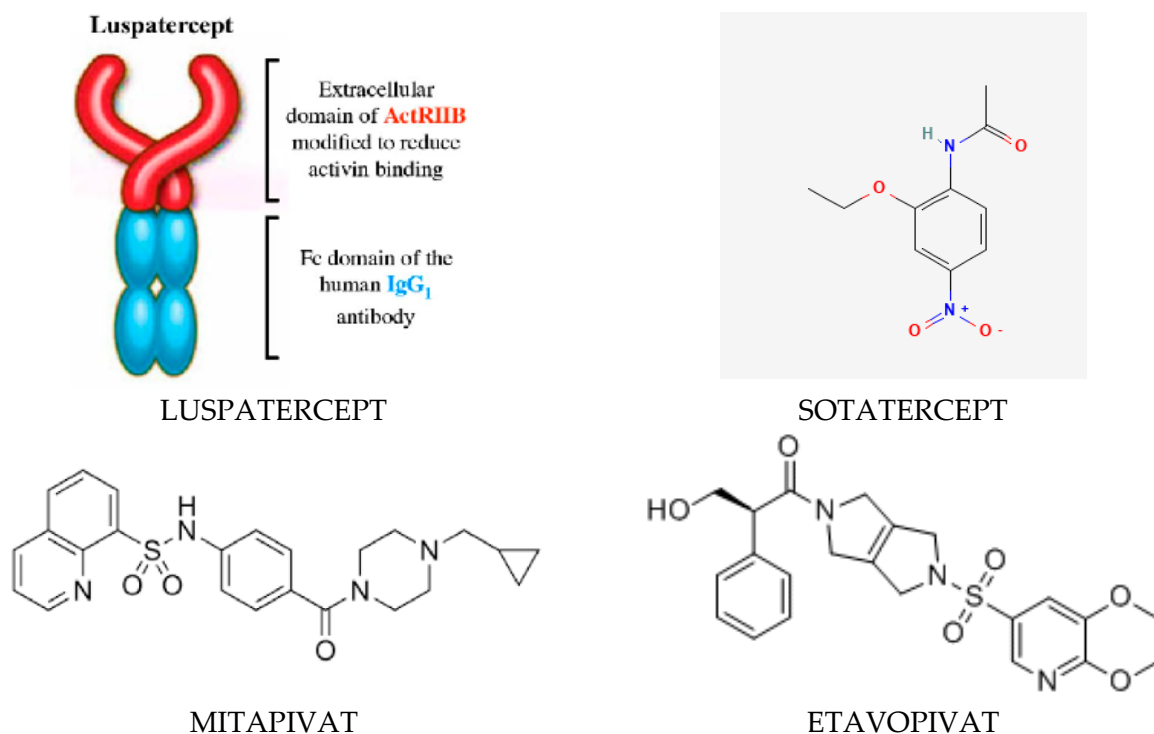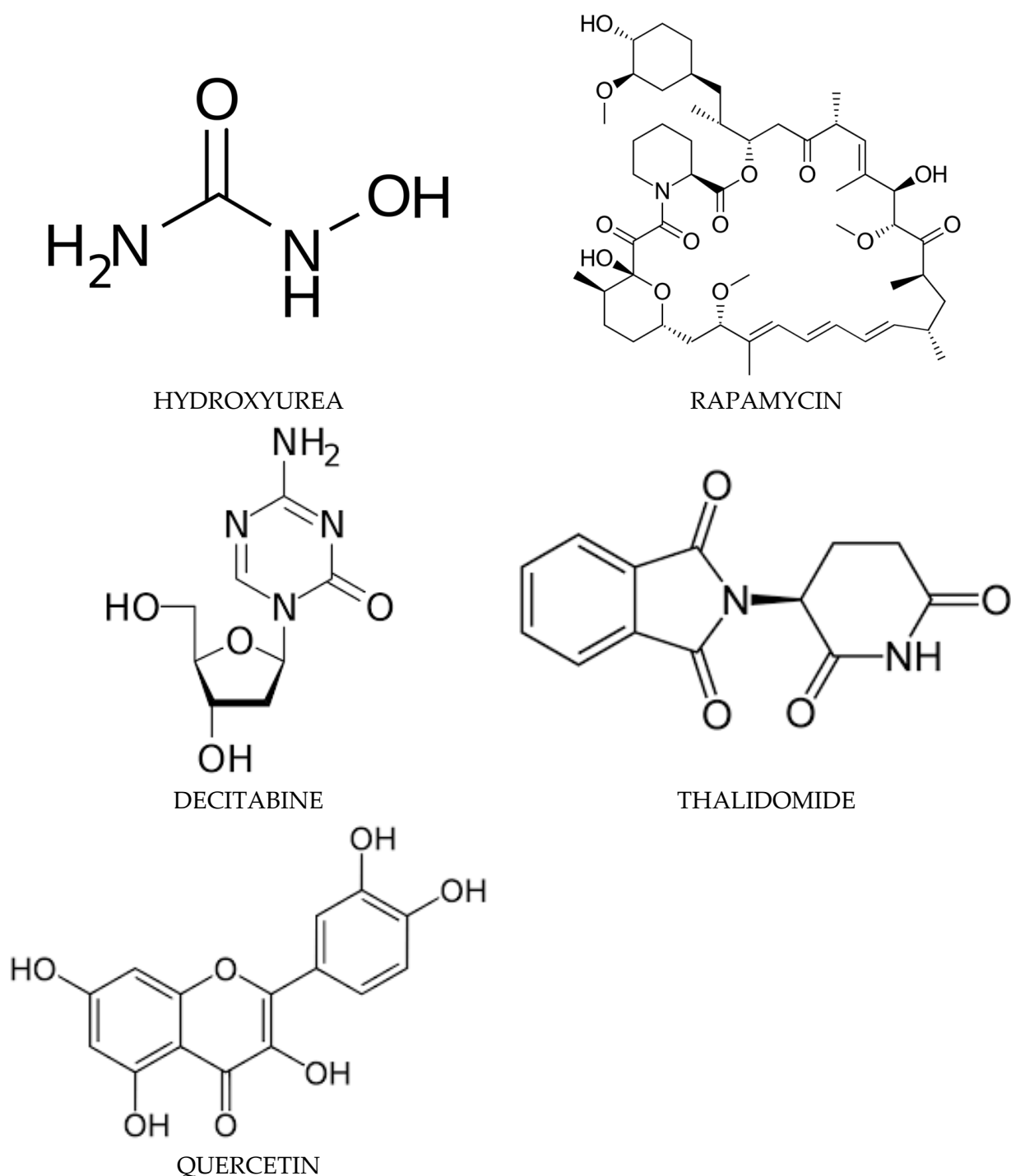

Figure 1. *Cont.*

Figure 1. Chemical structures of certain anti-thalassemia drugs. LUSPATERCEPT [15], SO-TATERCEPT [16], MITAPIVAT [17], ETAVOPIVAT [18], HYDROXYUREA [19], RAPAMYCIN [20], DECITABINE [21], THALIDOMIDE [22], QUERCETIN [23].

4.3.1. Luspatercept

Luspaterept is derived from human activin receptor type IIB (ActRIIB) (Figure 2A). It is a recombinant fusion protein, which can be obtained from immunoglobulin G. The FDA and the European Medicines Agency approved it, respectively, in 2019 and 2020 [15]. The drug is used to cure anemia in adults with β-thalassemia who need periodic transfusions of RBC. Luspatercept enhances erythroid maturation and reduces the transfusion burden by attaching to transforming growth factor β superfamily ligands. As a result, it

offers a promising treatment option for β-thalassemia-affected individuals who need RBC transfusions regularly [24].

A. Luspatercept ($C_{3350}H_{5070}N_{906}O_{1044}S_{38}$)

B. Antithalassemic effect of luspatercept
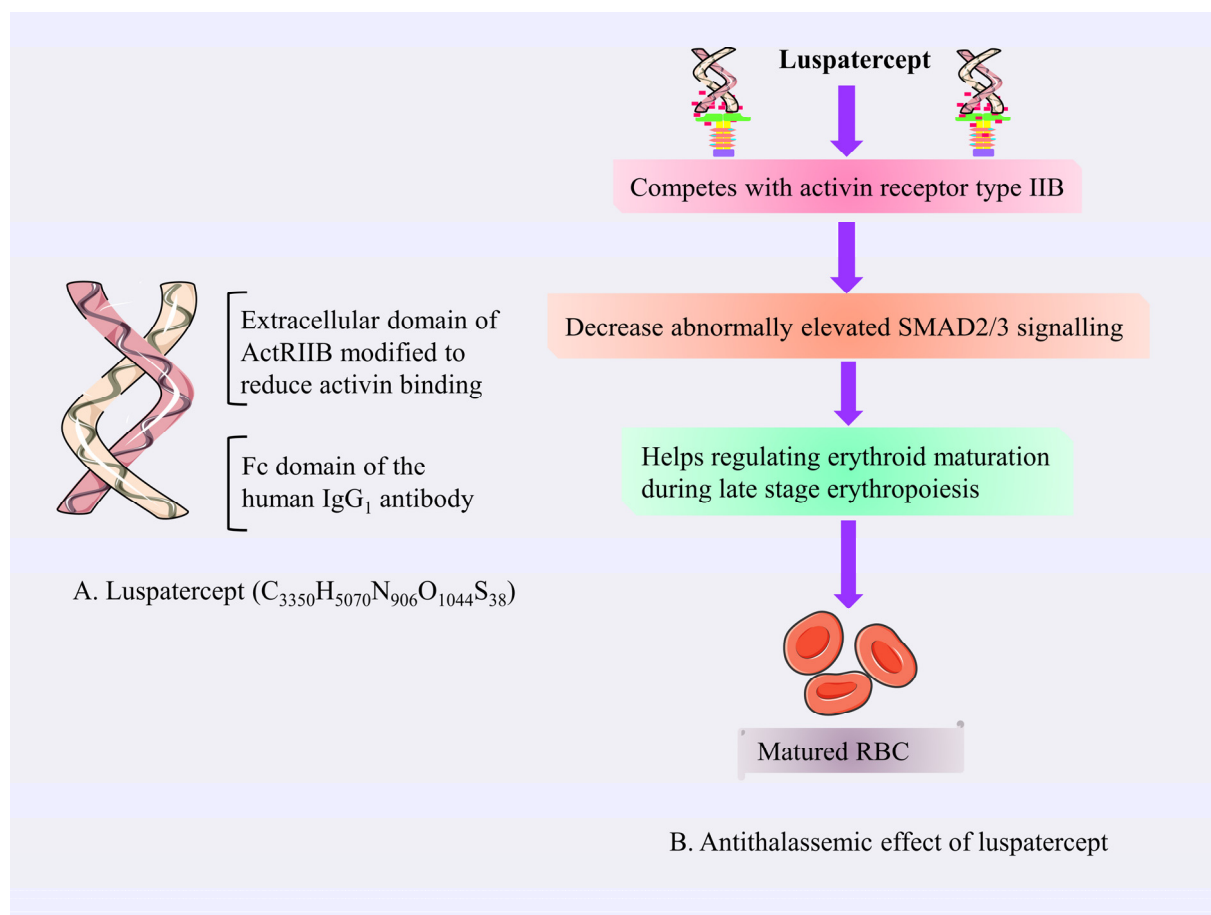
Figure 2. Structure (**A**) and mechanism of action of luspatercept (**B**).

Mechanism of Action of Luspatercept

Ineffective erythropoiesis is often caused by increased activity of tumor growth factor β (TGF-β) superfamily ligands during later-stage erythropoiesis, which inhibits the growth of erythroid precursors. The SMAD2/3 pathway—in particular, SMAD2/3 signaling—is involved in this inhibitory effect. TGF-β superfamily ligands activate specific receptors during maturation, including ActRIIB, which elevates the phosphorylation of SMAD2/3 protein, halting erythroid maturation and reducing the number of red blood cells. Luspatercept is a medication that promotes erythroid maturation by acting as a ligand trap and competing with ActRIIB to bind to TGF-β superfamily ligands. By decreasing abnormally elevated SMAD2/3 signaling, luspatercept helps to regulate erythroid maturation and improve red blood cell production [25]. The anti-thalassemic effect of luspatercept is shown in Figure 2B.

4.3.2. Sotatercept

Sotatercept (Figure 3A) is a first-in-class recombinant fusion protein, made of the type IIA human activin receptor (ActRIIA) extracellular domain linked to the human immunoglobulin G1 Fc domain, which enhances the secretion of fully grown erythrocytes into the bloodstream predominantly by interfering with later-stage erythropoiesis [16]. It is also called ACE-011. The cure with sotatercept results in enhanced RBC factors, including the level of Hb, as was shown in clinical data [26]. A murine ortholog of sotatercept, RAP-011, was successful in a β-thalassemia intermedia mouse model [27].
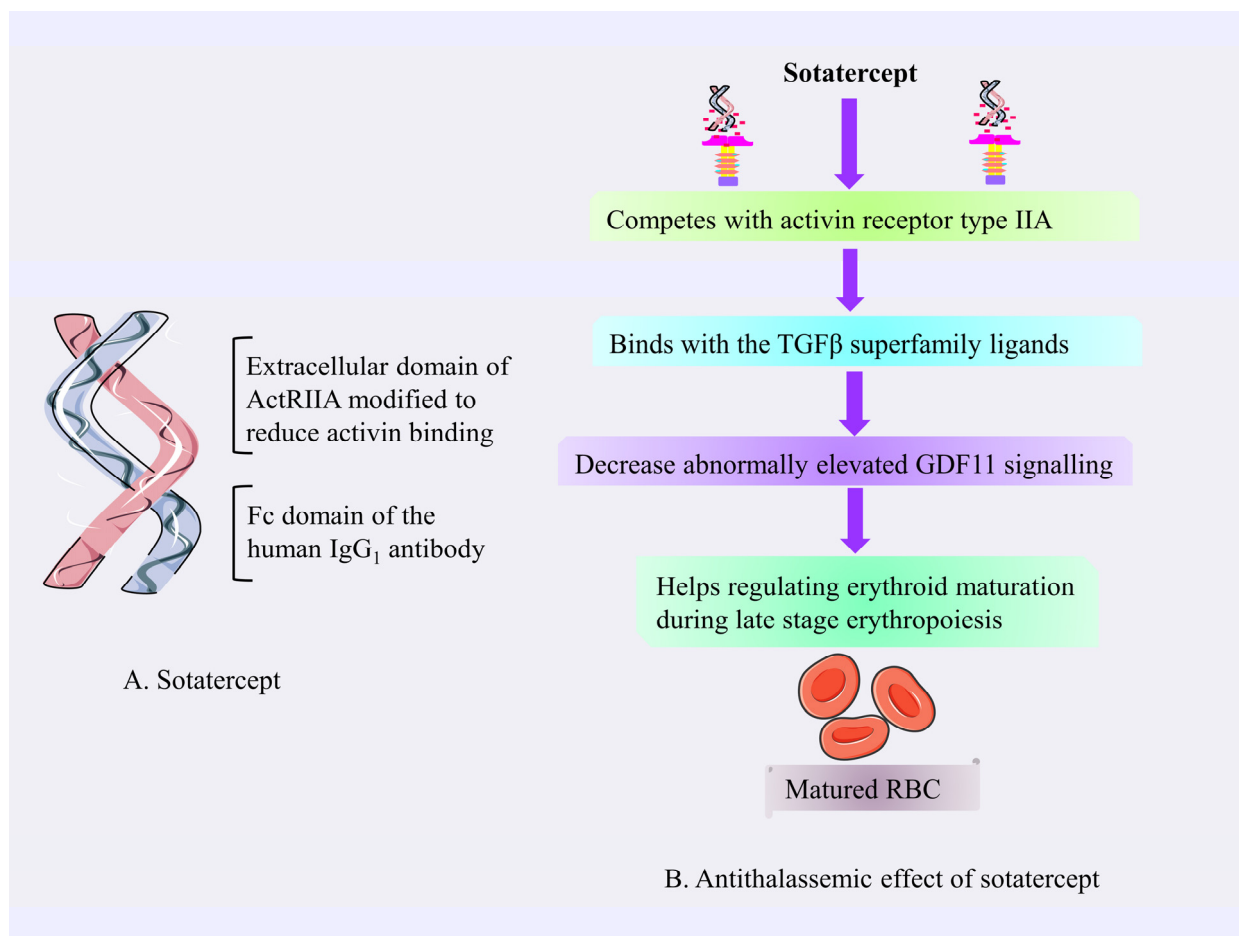
Figure 3. Structure (**A**) and mechanism of action of sotatercept (**B**).

Mechanism of Action of Sotatercept

Sotatercept is a ligand trap that prohibits TGF-β superfamily members, including ActRB and GDF-11. In β-thalassemia, overexpressed GDF-11 causes maturation arrest of late erythroid precursors and defective erythropoiesis. In preclinical studies, it was found that taking an ActRIIA ligand trap reduced GDF-11 amount, decreased the levels of reactive oxidative stress, and advanced terminal maturation in infant erythroblasts. Sotatercept was also shown to increase Hb levels and RBC count when administered subcutaneously in normal postmenopausal women. These findings suggest that sotatercept may offer a future treatment option for β-thalassemia patients [28]. The anti-thalassemic effect of sotatercept is shown in Figure 3B.
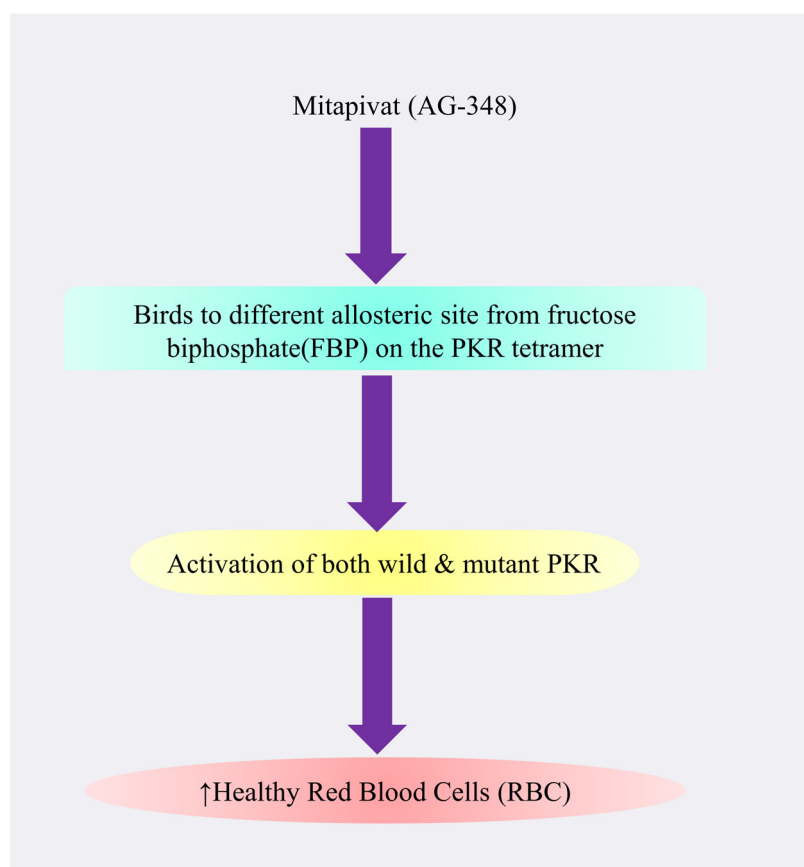
4.3.3. Mitapivat

A new, first-in-class, oral, small-molecule, allosteric activator of the pyruvate kinase enzyme, called mitapivat (AG-348), has been demonstrated to dramatically upregulate erythrocyte pyruvate kinase (PKR), both in the wild-type and in a variety of mutant forms. This results in an increase in ATP synthesis and a decrease in 2,3-diphosphoglycerate levels. Given its mechanism, pyruvate kinase deficiency (PKD), sickle cell disease, and thalassemias are among the hereditary hemolytic anemias for which mitapivat has been studied in clinical trials [17]. In 2022, mitapivat became the first drug to be approved by the FDA and EHA to treat hemolytic anemia caused by PKD. This significant advancement was the result of ongoing research aimed at developing new therapies [29].

Mechanism of Action of Mitapivat

The tetramer 4 erythrocyte pyruvate kinase (PKR) is physiologically activated by fructose bisphosphate in an allosteric process. Mitapivat of the PKR tetramer binds to an allosteric site that is different from that of FBP, allowing for the activation of the enzyme in both its wild-type and mutant forms. The latter allows activation even in many mutant PKR enzymes that are not induced by FBP. Through this mechanism, it may prove beneficial in the treatment of hemolytic anemias characterized by elevated erythrocyte energy demands and pyruvate-kinase-deficient states (PKD specifically). Mitapivat has been classified as an orphan drug by the FDA and the European Medicines Agency (EMA). Numerous clinical trials have evaluated the effectiveness of mitapivat in treating sickle cell disease, thalassemia, and PKD, and more will soon begin [17]. Figure 4 illustrates the mechanism of action of mitapivat.

Figure 4. Anti-thalassemic effect of mitapivat.
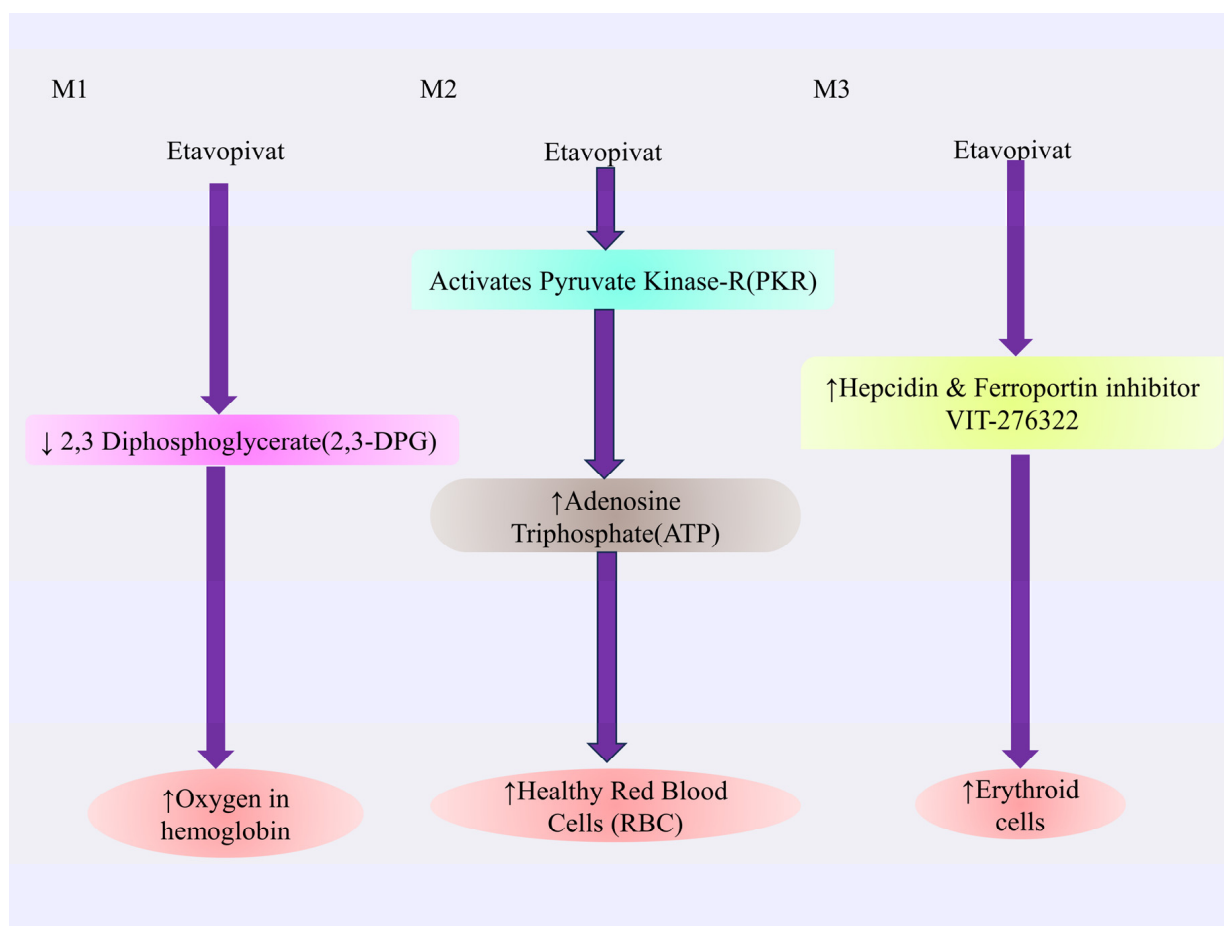
4.3.4. Etavopivat

Etavopivat (FT-4202) is a small-molecule activator of erythrocyte pyruvate kinase (PKR) that is being developed orally as a potential treatment for hemoglobinopathies such as sickle cell disease (SCD). When compared to a PKR protein-binding compound and its analogs, FT-4202 demonstrated comparable interactions with the adjacent amino acids, and the good agreement of its activities with those of the compound and its analogs was demonstrated through biochemical and cellular analyses [18].

Mechanism of Action of Etavopivat

Minimization of 2,3-diphosphoglycerate (2,3-DPG) enhances the oxygen affinity of Hb, which, in turn, reduces sickle hemoglobin polymerization and sickling. The activation of PKR is proposed to improve sickling of SCD red blood cells (RBCs) through multiple mechanisms. Furthermore, PKR activation promotes the production of adenosine triphosphate

through glycolytic flux, preserving the integrity of the membrane and the deformability of red blood cells [30].

Ferroportin inhibitor (VIT-276322), small interfering ribonucleic acid (SLN124), antisense oligonucleotide (TMPRSS6-LRx), mitapivat, and etabapivat are all in varying phases of clinical development, with luspatercept and mitapivat leading the way with phase II trial final data. In animal models, all of these medications have been shown to improve ineffective erythropoiesis or lengthen the lifespan of erythroid cells [31]. Figure 5 depicts the mechanism of action of etavopivat.

Figure 5. Anti-thalassemic effect of Etavopivat (M1: Mode 1, M2: Mode 2, M3: Mode 3).

4.3.5. Hydroxyurea

Chemically, hydroxyurea (HU) is an antimetabolite and a monohydroxyl-substituted urea (hydroxycarbamate). It is similar to other anti-cancer drugs that are antimetabolites [19], and it is a drug capable of producing fetal Hb. For this reason, HU is readily used to treat non-transfusion-dependent β-thalassemia patients. Hydroxycarbamide has been in therapeutic use in the United States since it was approved in 1967, and today, it appears on the essential medicines list of the World Health Organization (WHO). Hydroxycarbamide is available as a generic medication [32].
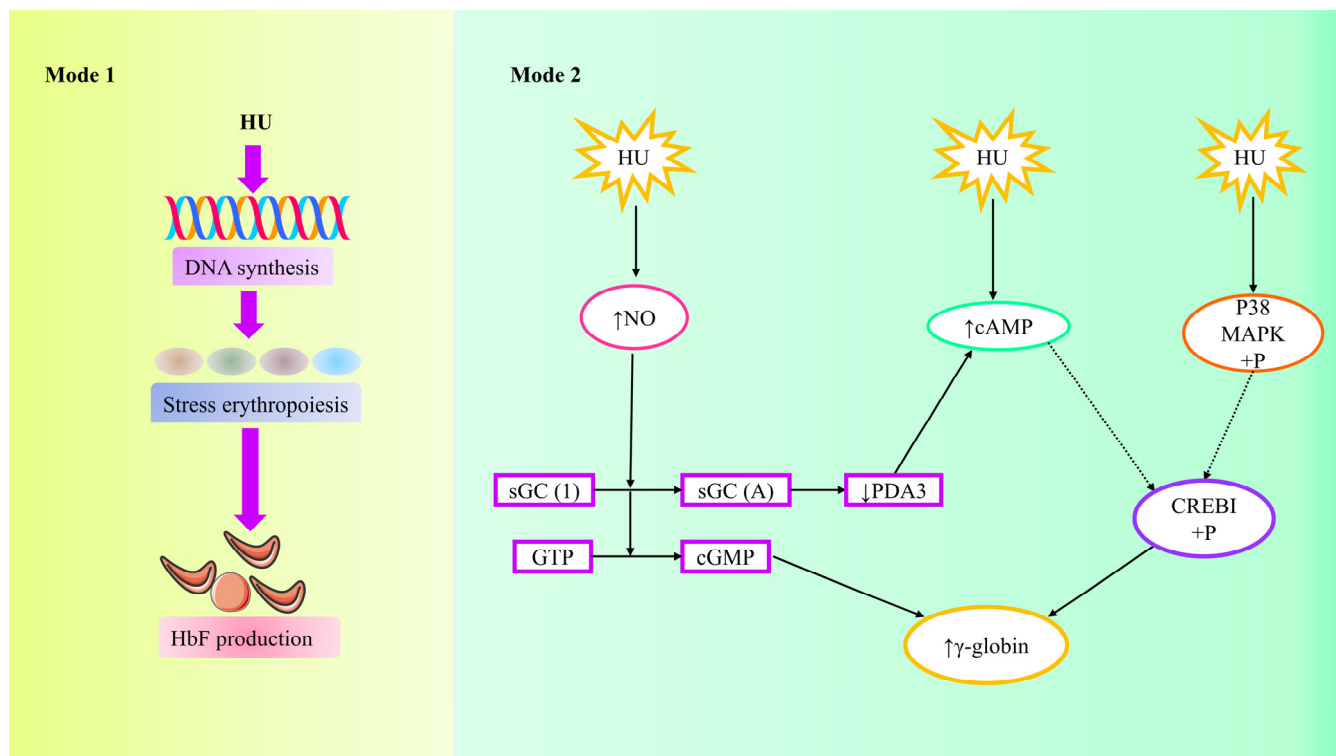
Mechanism of Action of Hydroxyurea (HU)

HU increases the production of HbF, which is the main carrier of oxygen. To explain HbF induction, two mechanisms have been proposed.

Mode 1

According to Figure 6, by inhibiting DNA synthesis, HU stresses erythropoiesis. The induction of HbF production is led by stressed erythropoiesis [33].

Figure 6. Anti-thalassemic effects I and II of hydroxyurea.

Mode 2

In another pathway (Figure 6), γ-globin may be induced by promoting nitric oxide (NO). Once taken orally, solute carrier transporters facilitate the uptake of HU rapidly from the intestine to blood cells. To produce NO, HU can then react with heme. The NO that is produced can nitrosylate the soluble guanylate cyclases, causing their activation and resulting in the production of cyclic guanosine monophosphate (cGMP). Then, cGMP can induce γ-globin [34].

4.3.6. Rapamycin

Rapamycin, a lipophilic macrolide that is also called sirolimus, was first discovered as an antifungal entity produced by *Streptomyces hygroscopicus* from an Easter Island soil sample (also known as Rapa Nui) [20]. Sirolimus was isolated from *Streptomyces hygroscopicus* and found to consist of a 29-membered ring including a cyclic acetal, cyclic ketone, ether, secondary alcohol, organic heterotricyclic compound, antibiotic antifungal drug, and macrolide lactam ring containing 4 *trans* double bonds, of which three are conjugated. It can act as an agent that is immunosuppressive, antibacterial, antineoplastic, and anti-coronaviral, as well as being a mammalian target of rapamycin (mTOR) inhibitor and a geroprotector [35].
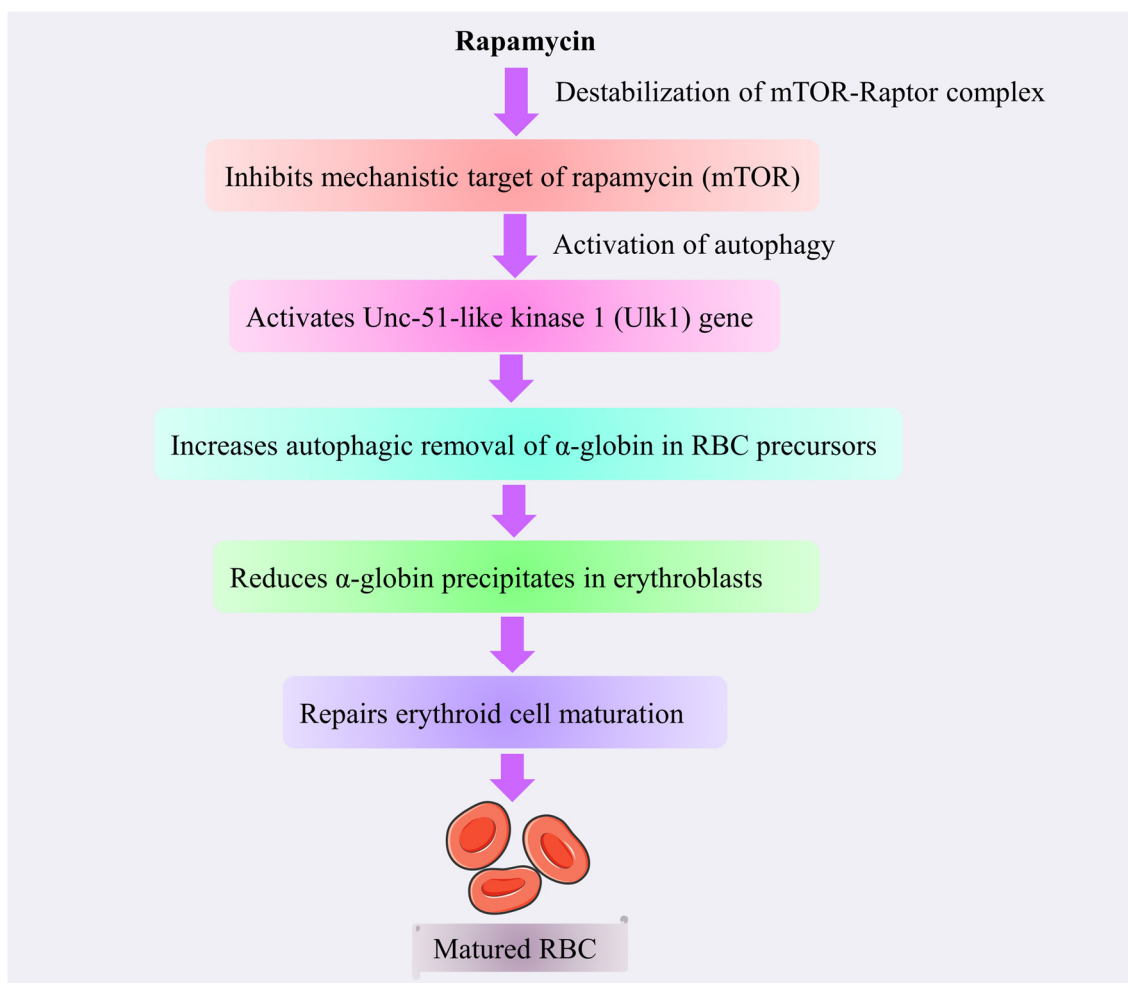
Mechanism of Action of Rapamycin

By phosphorylating ULK1 (encoded by *ULK1* gene), mTORC1 inhibits autophagy. Inhibition of mTORC1 can improve thalassemia by enhancing autophagic removal of free globin [36]. The anti-thalassemic effect of rapamycin is shown in Figure 7.

4.3.7. Decitabine

Decitabine is a cytosine analog and an intravenously administered antineoplastic agent used in the therapy of myelodysplastic syndromes and thalassemia [21]. It is a chemotherapy drug that reduces the growth of cancer cells. The treatment is quite well-tolerated and effective at increasing the percentage of HbF in patients with HbE β-thalassemia. When

decitabine was utilized, there was a reduction in transfusion need among the transfusion-dependent thalassemic (TDT) group, and an improvement in total Hb was found in the non-transfusion-dependent thalassemic (NTDT) group [37].
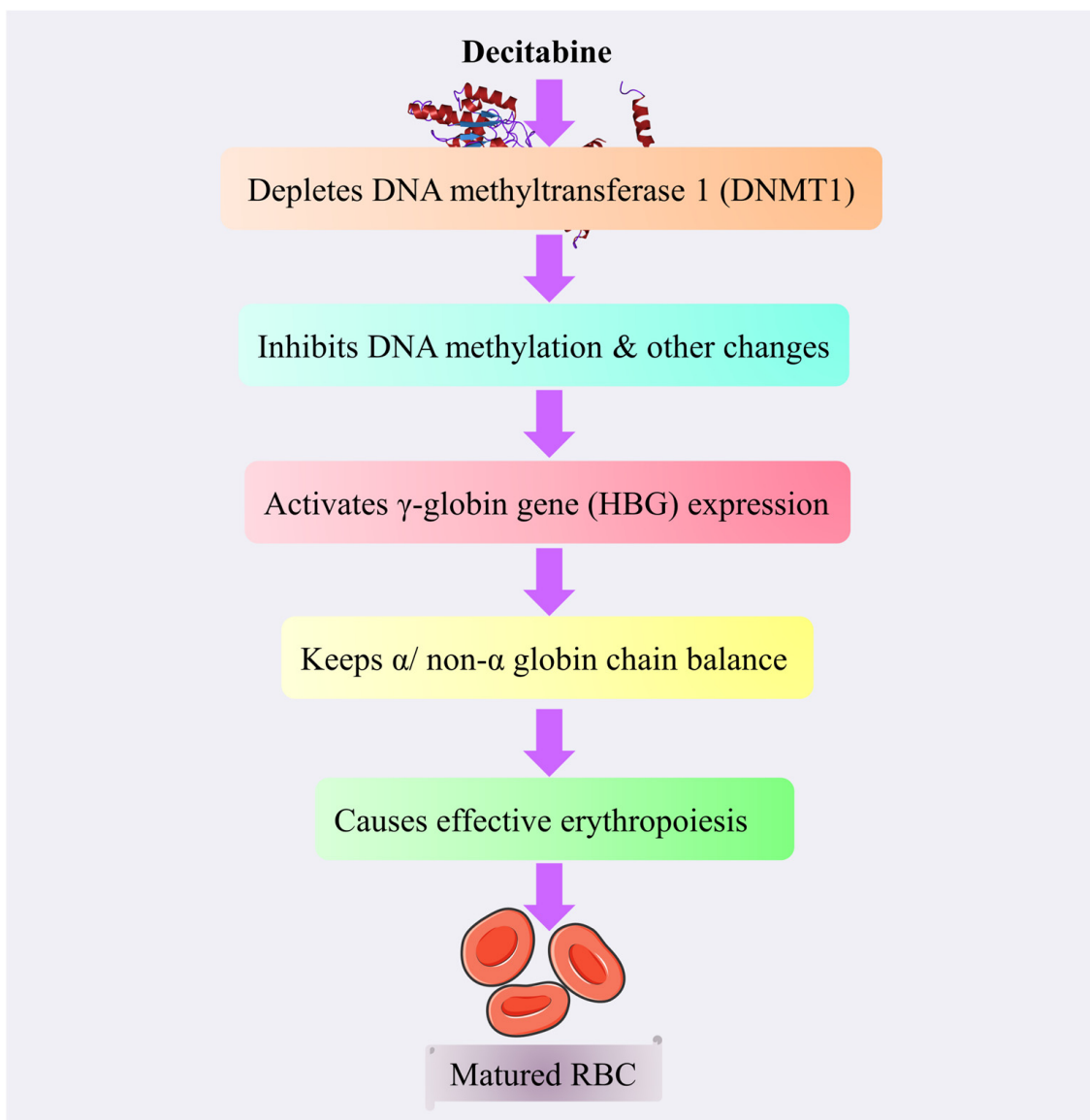

Figure 7. Anti-thalassemic effect of rapamycin.

Mechanism of Action of Decitabine

By depleting DNA methyltransferase-1 (DNMT1), decitabine can inhibit DNA methylation and other changes. Thus, it activates β-globin gene (HBG) expression. *HB* expression maintains a β/non-β chain balance, which results in effective erythropoiesis. Thus, matured RBC forms [38]. The anti-thalassemic effect of decitabine is shown in Figure 8.

Toxicity

One study [39] found that during the course of treatment, no patients experienced adverse effects following the drug's administration or any instances of nausea or vomiting. Antiemetics were not reported to have been used. All patients saw a drop in their white blood cell count, and two experienced absolute neutropenia (absolute neutrophil count less than 500), which resolved in three days. No patient had an infection or fever. Six patients had an increase in hemoglobin levels of at least 1 g/dL. Throughout the course of treatment, platelet counts remained constant; by the end of the cycle, they had increased by an average of 220% [39]. According to another study [40], decitabine's sugar moiety is unaltered, in contrast to certain other cytosine analogs, such as gemcitabine or cytarabine. As a result, decitabine can deplete DNMT1 without significantly harming DNA or causing cytotoxicity at low concentrations, both in vitro and in vivo [26,27,40].
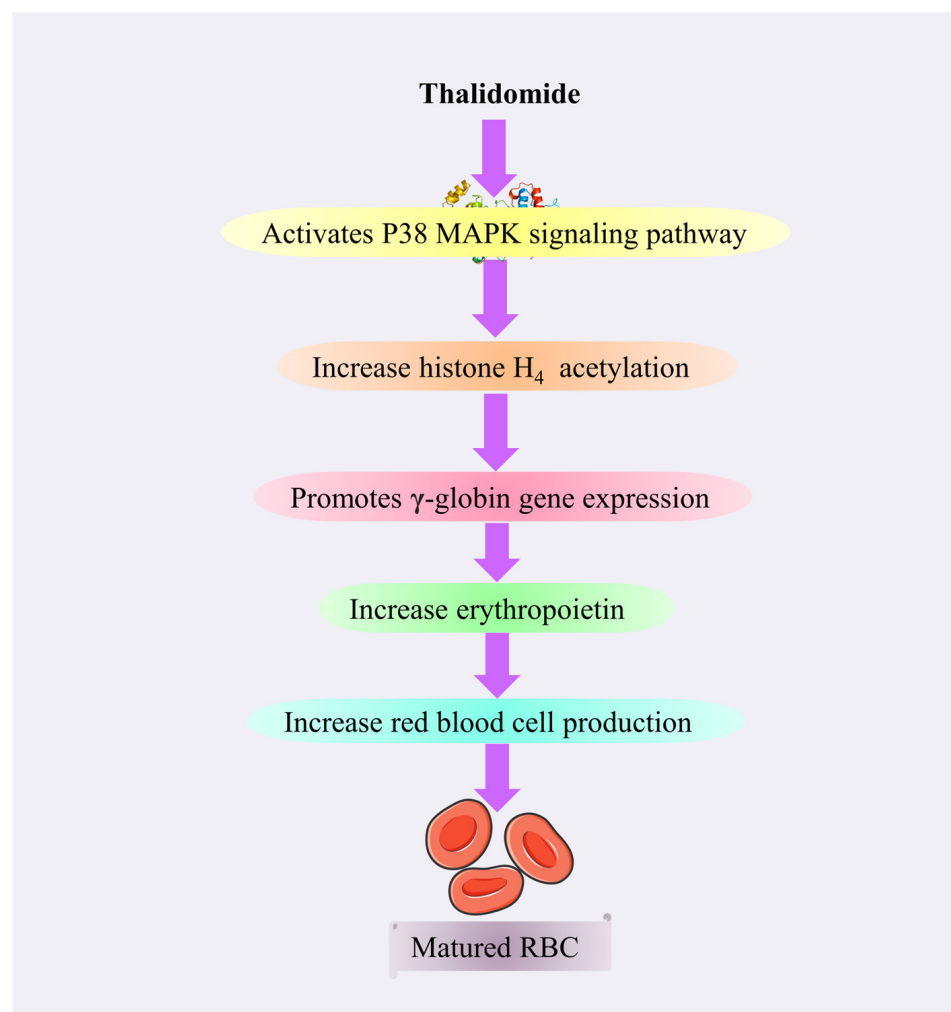
Figure 8. Anti-thalassemic effect of decitabine.

4.3.8. Thalidomide

Thalidomide is a compound derived from glutamic acid [22]. It belongs to the piperi-done and phthalimide families and is otherwise known as 2-(2,6-dioxopiperidin-3-yl)-1H-isoindole-1,3(2H)-dione. It is an isoindole-1,3(2H)-dione, where the hydrogen joined to the nitrogen is displaced by a 2,6-dioxopiperidin-3-yl group. Thalidomide is classified as an immunomodulatory drug, and it has been used to produce HbF in the treatment of thalassemia intermedia, as demonstrated by [41]. Although its exact mode of action is not fully understood, thalidomide leads to significantly reduced transfusion requirements in TI patients. However, as [42] note, thalidomide has side effects including drowsiness, confusion, anxiety, depression, and difficulty sleeping.

Mechanism of Action of Thalidomide

Thalidomide produces anti-tumor, anti-inflammatory, and anti-angiogenic results. It activates the p38 mitogen-activated protein kinase (MAPK) signaling pathway, which increases histone H4 acetylation. This increases the gene expression of globin and increases erythropoietin, which results in matured RBCs [43]. The anti-thalassemic effect of thalidomide is shown in Figure 9.

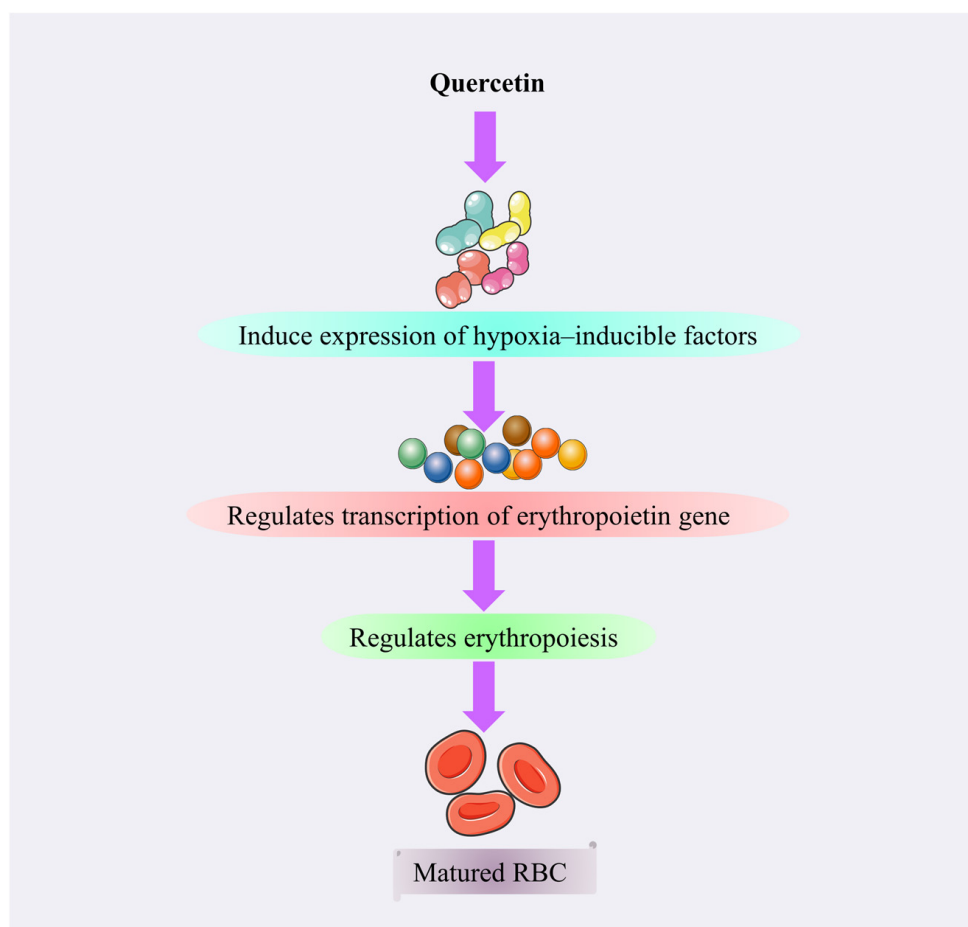**Figure 9.** Anti-thalassemic effect of thalidomide.

Toxicity

Yang and their group found out that ten patients experienced mild side effects of thalidomide. The most common site of toxicity was the gastrointestinal tract (5 out of 62 people), followed by a rash (2 out of 62 people) and menstrual problems (2 out of 62 people). However, these side effects were only temporary and subsided with symptomatic treatment or a brief stoppage of the medication. One patient experienced intermittent numbness in both lower limbs due to peripheral neurotoxicity during long-term follow-up, but the patients did not experience any bone marrow suppression or hematological toxicity during the course of treatment. Additionally, thalidomide did not adversely affect the function of the liver or kidneys, nor did it cause any appreciable alterations in the levels of creatinine or alanine aminotransferase [44]. A study by Li and their group gathered retrospective data on toxicity from laboratory test results and historical records. Lethargy, arthralgia, edema, headaches, nausea, vomiting, dizziness, peripheral neuropathy (numbness in the extremities), constipation, abdominal distension, chest tightness, diarrhea, and skin rash were among the adverse events that were noted in their research and suspected of being related. Additionally, the counts of platelets, white blood cells, absolute neutrophils, alanine aminotransferases, aspartate and total bilirubin, creatine, γ-glutamyl transpeptidase, and blood urea nitrogen were noted. The Common Terminology Criteria for Adverse Events (CTCAE) v5.0 was modified to grade adverse events and toxicity; if grade III or IV adverse reactions occur, the off-label medicine committee recommends reducing the dosage of thalidomide or ceasing its use [45].

4.3.9. Quercetin

Quercetin, a pentahydroxyflavone, has five hydroxy groups placed at the 3-, 3′-, 4′-, 5-, and 7-positions [23]. Quercetin can be found as a pigment in many plants, herbs, and foods such as onions, red wine, apples, green tea, and berries. Accordingly, by increasing fruit and vegetable consumption, cancer incidence can be reduced. Furthermore, a few years ago, it was discovered that the flavonoid quercetin can sensitize several thalassemic cell lines [46].

Mechanism of Action of Quercetin

Quercetin induces hypoxia-inducible factor expression, which regulates transcription of the erythropoietin gene. Thus, it regulates erythtropoiesis, which results in matured RBCs [47]. The anti-thalassemic effect of quercetin is shown in Figure 10.

Figure 10. Anti-thalassemic effect of quercetin.

4.3.10. Betibeglogene Autotemcel (Zynteglo)

A significant milestone was reached on 17 August 2022, when the FDA recognized Zynteglo (manufacturer: Bluebird Bio, Massachusetts, US), also known as betibeglogene autotemcel (Beti-cel), a one-time cell-mediated gene therapy treatment [48]. Patients suffering from transfusion-dependent β-thalassemia are being investigated for their suitability for treatment with gene therapy using Beti-cel. This drug's significant repair of globin chain disparity might prove to be an effective therapy for prolonged anemia, erythropoiesis, and dysfunction of hemoglobin synthesis [49].

Mechanism of Action of Betibeglogene Autotemcel (Zynteglo)

For the treatment of transfusion-dependent β-thalassemia, autologous CD34+ hematopoietic stem cells and precursor cells transduced with the BB305 lentiviral vector, which encode the β-globin gene, are employed in Beti-cel gene therapy [48]. CD34+ cells that carry a deficient, self-inactivating BB305 lentiviral vector, Beti-cel, deliver β-globin regulatory components and active copies of a mutated β-globin gene (β^{A-T87Q}) with an amino acid substitution into hematopoietic stem cells [50]. By introducing self-inactivating lentiviral vectors, Beti-cel incorporates a gene construct containing the β^{A-T87Q} gene and other components necessary for expressing the gene, and the harvested cells undergo transduction ex vivo. After that, the patient receives these modified cells, which are engrafted and improve regular blood supply (Figure 11) [51].
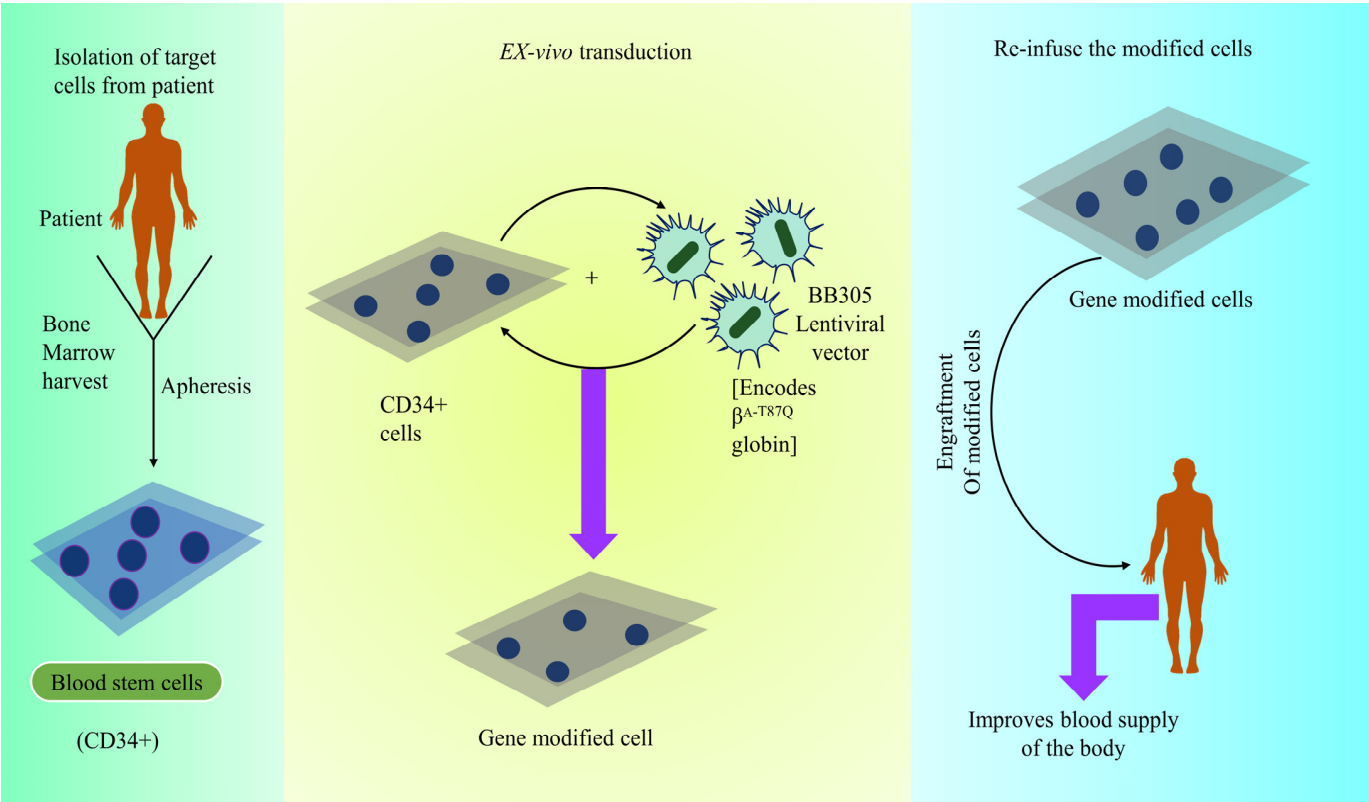

Figure 11. Anti-thalassemic effect of betibeglogene autotemcel.

Table 1. Statuses of different phases of natural compounds in the treatment of β-thalassemia.

Drug	Concentration/Dose and Route of Administration	Test system/Model	Results	Reference
Luspatercept	0.2–1.25 mg/kg per body weight; subcutaneously	Clinical trial	A significant proportion of individuals experienced a positive change in their Hb levels or reduced need for blood transfusions after using luspatercept	[52]
Luspatercept	1 mg/kg once every 3 weeks; starting dose (s.c.) enhanced to 1.25 mg/kg in individuals who did not experience a reduction in RBC transfusion problems after ≥2 treatment cycles (6 weeks)	Clinical trial (approved)	Patients may experience a decrease in their need for blood transfusions through treatment with luspatercept	[53]
Luspatercept	0.6–1.25 mg/kg	Phase II clinical trial	Increased Hb level	[54]
Luspatercept	1–1.25 mg/kg	Randomized, phase III, double-blind	Enhanced Hb production	[55]

Table 1. *Cont.*

Drug	Concentration/Dose and Route of Administration	Test system/Model	Results	Reference
Sotatercept	0.1, 0.3, 0.5, 0.75, and 1.0 mg/kg used to evaluate the effective and safe dose; subcutaneous injection $\leq$ 22 months	Clinical trial	In patients with transfusion-dependent β-thalassemia, the result was evaluated as a reduction of at least 20% in the need for blood transfusions that lasted for 24 weeks. For non-transfusion-dependent β-thalassemia patients, the response was evaluated as a sustained increase in the Hb levels of no less than 1.0 g/dL for 12 weeks	[28]
HU	10 mg/kg per day as beginning dose, which was increased by 5 mg/kg (per day) at 4-weekly intervals to a maximum of 20 mg/kg per day or until myelotoxicity appeared	Clinical trial	Out of the total number of patients, which was 26, 70.2% responded positively to the HU therapy. Among the patients, 45.9% presented a significant response, while 24.3% presented a less significant response	[56]
HU	16 mg/kg/day	Clinical trial	The study found that HU was a safe treatment option for patients with α, β-thalassemia major, and it led to a decrease in the need for blood transfusions	[57]
HU	14 mg/kg/day	Clinical trial	The analysis of gamma-globin expression in the groups under study revealed a notable increase in gamma-globin gene expression	[58]
HU	15 mg/kg	Clinical trial	To obtain a more comprehensive representation of the exact mechanism, a study with a larger number of participants and a longer duration of HU treatment is required	[59]
HU	10–20 mg/kg once; 6 months	Clinical trial	65.93% responded well	[57]
Rapamycin	On the 4th or 5th day of phase II, rapamycin (manufactured by Sigma/Aldrich, Milwaukee, WI, USA) was introduced; the cells were then collected and examined on day 12	In vitro	The majority of the samples (six out of seven analyzed) showed an increase in the total amount of Hb per cell (measured in pg/cell)	[56]
Rapamycin	A concentration of 100 nanomolar rapamycin was administered to the culture	In vitro	Rapamycin enhanced the expression of α-globin mRNA in 60.0% of sickle cell disease samples and 56.0% of b-TI samples examined	[57]
Rapamycin	4 mg/kg once for 30 days	In vivo	Rapamycin decreased by approximately 50% the amount of insoluble α-globin observed in the red blood cells of Hbb^{Th3+} mice ($p < 0.001$). However, this reduction was not observed in $Hbb^{Th3+}/Ulk1^{-/-}$ mice	[36]
Rapamycin	16 and 24 ng/mL	Clinical trial	The main goal was to observe an increase in the fraction of HbF in blood samples taken at day 360 compared to baseline (day 0)	[60]
Decitabine (2-deoxy 5-azacytidine)	0.15–0.30 mg/kg administered on 5 days of the week for 2 weeks	A dose-escalating phase I/II study	Before treatment, the mean ratio was 3.19% with a standard deviation of 1.43%. Following treatment, this ratio increased to 13.66%, with a standard deviation of 4.35%	[39]
Decitabine	0.2 mg/kg administered 2 times each week for 12 weeks (s.c.)	Clinical trial	The total Hb level increased significantly from 7.88 ± 0.88 g/dL to 9.04 ± 0.77 g/dL, and there was also a significant rise in the absolute HbF level from 3.64 ± 1.13 g/dL to 4.29 ± 1.13 g/dL	[40]

Table 1. *Cont.*

Drug	Concentration/Dose and Route of Administration	Test system/Model	Results	Reference
Decitabine	0.2 mg/kg of 5-aza-2′-deoxycytidine (decitabine) was administered subcutaneously on two consecutive days each week for a minimum of 12 weeks	Phase II	There was a substantial increase in Hb level, observed as soon as week 2	[61]
Thalidomide	50 mg each night (p.o.)	Clinical trial	After the treatment period, there was an increase of 2.5 ± 1.8 g/dL in both Hb and HbF levels	[62]
Thalidomide	Starting dose of 50 mg/d, or a daily dose of 100 mg/d was administered to individuals requiring blood transfusions at least twice a month (2016–2019)	Clinical trial	Thalidomide appears to be a highly promising cure option for individuals with β-thalassemia, with the potential to substantially enhance Hb level, thereby reducing the need for blood transfusions	[44]
Thalidomide	2–10 mg/kg at any time throughout the therapy; at first, a dose of 3 mg/kg (balanced to closest 50 mg) was utilized	Clinical trial	Thalidomide treatment produced a marked response in over 75% of victims with symptomatic β-thalassemia, regardless of whether they had TDT or NTDT	[23]
Thalidomide	2.5–3.6 mg/kg/d	Clinical trial	Safely enhanced Hb production	[45]
Quercetin	500 mg/day quercetin tablet	Double-blinded, randomized clinical trial	Suppressed inflammation	[63]
Quercetin	500 mg/day	Clinical trial	The use of quercetin may improve iron levels in individuals with thalassemia major, but its impact on inflammation is not clear	[41]
Mitapivat	(p.o.)	Clinical trial	Twenty-six patients (50%, mean maximum Hb increase of 3 g/dL, 4 g/dL range, with a good safety profile) had an Hb increase of more than 1 g/dL from baseline	[64]
Mitapivat	More often when dosed over 700 mg (p.o.)	Phase I	Positive pharmacokinetics with minimal variability of dose-dependent alterations in blood glycolytic intermediates (increased ATP, decreased 2,3-DPG) consistent with the activation of glycolysis	[65]
Mitapivat	50 mg twice a day	Phase II	An increase in Hb was observed at weeks 12 and 72, with a median (Q1, Q3) of 1.3 g/dL (0.60, 1.90) and 1.2 g/dL (−0.03, 2.2)	[66]
Mitapivat	16 patients received 100 mg of BID mitapivat and one patient received 50 mg	Phase III	Continuous improvements in hemolysis, inefficient erythropoiesis, and hemoglobin	[67]
Mitapivat	50 mg twice a day	Phase III	Hemolysis markers and ineffective erythropoiesis were improved during the core period	[68]
Mitapivat	20 mg twice a day	Phase II	A rise in hemoglobin from the starting point and a corresponding fall in hemolysis-related indicators	[69]
Mitapivat	5–50 mg two times a day	Phase III	Of the 27 patients enrolled, 20 finished the study; with 22% achieving a transfusion-free response and 37% reporting a decrease in their transfusion burden, the primary endpoint was met	[70]
Etavopivat	Once daily	Preclinical and phase I clinical trial	Proof of concept without significant adverse events was demonstrated in controls treated for up to 14 days with 7-day follow-up	[71]
Etavopivat	Less than or equal to 400 mg once daily	Phase I	Pharmacokinetic/pharmacodynamic analysis results showed that 400 mg taken once daily was the maximal dose at which PKR activation was achieved	[72]

Table 1. *Cont.*

Drug	Concentration/Dose and Route of Administration	Test system/Model	Results	Reference
Betibeglogene Autotemcel	Patients received autologous CD34+ cells transduced with BB305 lentiviral vector after receiving busulfan, a single-agent, pharmacokinetically adjusted treatment; myeloablation	Phases II and IV	Patients with TDT could maintain and stabilize their response following β-cell gene therapy. Hematologic parameters and the iron burden were improved as a result of sustained levels of HbA^{T87Q} and efficient iron reduction with phlebotomy and/or iron chelation	[73]
Betibeglogene autotemcel	In patients with transfusion-dependent β-thalassemia who were adult or pediatric and had a non-β°/β° genotype, Beti-cel was used. Patients received Beti-cel intravenously and underwent myeloablation with busulfan (dosages modified based on pharmacokinetic analysis). A median follow-up of 29.5 months was observed for the 23 patients who were enrolled and received treatment	Phase IV	Higher HbA^{T87Q} levels were obtained in the study compared to the phase I–II studies, which were attributed to improvements in hematopoietic stem-cell transduction. According to these findings, a single Beti-cel infusion may be therapeutic for the majority of patients with transfusion-dependent β-thalassemia and a non-β°/β° genotype, as it can lead to near-normal hemoglobin levels and transfusion independence	[51]
Betibeglogene autotemcel	Gene therapy using lentiviral technology and ex vivo autologous hematopoietic stem cells using betibeglogene autotemcel	Phase III	41 patients received Beti-cel treatment and were cured	[50]

p.o.: oral administration, s.c.: subcutaneous administration.

5. Discussion

Table 1 demonstrates the anti-thalassemic effects of various drugs. For luspatercept, in patients with β-thalassemia, the dose level ranges from 0.2 to 1.25 mg/kg [52], but the recommended starting dose is 1 mg/kg once every 3 weeks. The dose is increased to 1.25 mg/kg in patients who do not achieve a reduction in RBC transfusion burden after ≥2 treatment cycles (6 weeks) [53]. After clinical trials, it was found that luspatercept can produce healthy hemoglobin cells in patients with β-thalassemia and can reduce transfusional burden [52]. It is the first officially approved drug for β-thalassemia, having been approved in 2019. It is working quite well, but as it is a new drug, post-marketing drug safety must be proven.

Metapivat is a recently approved drug for pyruvate kinase disease. Most of the studies performed in this case were for sickle cell disease and very few were for β-thalassemia. Table 1 depicts that patients were treated with different doses, including 20 and 50 mg twice a day, and the highest dose used was 700 mg. This drug worked best at a dose close to 700 mg per day. At this dose, almost all patients with pyruvate kinase disease were treated [64–68].

Etavopivat is another pyruvate-kinase-disease-treating drug. It is still in the final phases of trials, and vast research is being conducted. Very few data have been published in this case. Table 1 shows that when trialed, it worked best at a dose of 400 mg per day, and most patients with pyruvate kinase disease were treated [71,72].

To find a safe and effective dose for sotatarcept, patients were treated with the drug at a concentration of 0.1, 0.3, 0.5, 0.75, or 1.0 mg/kg. Every three weeks, subcutaneous injections of the doses were given. Patients received care for at least 22 months. For transfusion-dependent β-thalassemia patients, a response was defined as a sustained reduction of at least 20% in transfusion burden over a 24-week period, and for non-transfusion-dependent β-thalassemia patients, an increase in hemoglobin level of at least 1.0 g/dL over a 12-week period [28]. Sotatercept is still an investigational drug, mainly used in pulmonary arterial

hypertension. Very few studies have so far been performed on β-thalassemia, and vast studies are needed in this area to establish its use for the condition.

The starting dose of HU is 10 mg/kg and the maximum dose is 20 mg/kg [56], meaning the mean dose is 14–16 mg/kg. In trials, HU was found to be safe and effective in β-thalassemia [57–59]. It could cure thalassemia intermedia and reduce the transfusion burden, but it could not cure major thalassemia.

For rapamycin, the dose starts at 2 mg/day, but the effective dose is 4 mg/kg per day [36,52,74,75]. In one trial, rapamycin treatment reduced insoluble globin by about 50% in the RBCs of HbbTh3/+ mice. In a human trial, it was found that it increased the HbF fraction in peripheral blood [60]. It is already established as a safe drug for humans, having been approved in 1999 by the FDA, and it has potential in β-thalassemia. However, a vast amount of research is needed in this area.

For decitabine, doses ranged from 0.15 to 0.30 mg/kg [39], and the effective dose was 0.2 mg/kg subcutaneously, 2 times per week for 12 weeks [40,61]. Significant hemoglobin increments were documented as early as week 2 [61]. Decitabine is already established as a safe drug for the human body, and it has potential against β-thalassemia, but very few studies have been performed in this area, leaving considerable research still needed. In regard to its toxicity, few adverse effects were found, but every patient saw a drop in their white blood cell count, and two experienced absolute neutropenia [40,61].

Thalidomide was prescribed at a dose of 50 mg/day [43,44,62]. It can significantly improve Hb levels, minimizing the need for blood transfusions [44,62]. However, pregnant women cannot use this drug. Furthermore, the most common site of toxicity was found to be the gastrointestinal tract. Other side effects were menstrual problems, lethargy, arthralgia, edema, headaches, nausea, vomiting, dizziness, peripheral neuropathy (numbness in the extremities), constipation, abdominal distension, chest tightness, diarrhea, and skin rash [44,45]. Although it has potential as a drug for thalassemia, its toxicity is a major concern.

Quercetin was prescribed at a dose of 500 mg/day. It suppressed inflammation [63] and could reduce β-thalassemia to a lesser extent, failing to totally cure β-thalassemia. Nonetheless, using quercetin is a good option for thalassemic patients.

In a study by Locatelli, Whitney, and their group, patients received autologous CD34+ cells transduced with a BB305 lentiviral vector after receiving busulfan, a single-agent, pharmacokinetically adjusted treatment (myeloablation). Improvements in hematopoietic stem cell transduction led to higher HbA^{T87Q} levels when compared to phase I and II studies. These results indicated that a single Beti-cel infusion can result in near-normal hemoglobin levels and transfusion independence, suggesting it has therapeutic potential for most patients with a non-β°/β° genotype and transfusion-dependent β-thalassemia [51,73].

6. Conclusions

In summary, this review identified three FDA-approved drugs for enhancing healthy hemoglobin production: luspatercept, mitapivat, and betibeglogene autotemcel. When comparing these drugs, attention should be paid to their mechanisms. Luspatercept works by reducing SMAD2/3 signaling. On the other hand, mitapivat works by activating pyruvate kinase and enhancing ATP production. This is helpful when a definite patient's hemoglobin level is low in PKD. Another FDA-approved drug, betibeglogene autotemcel, offers a kind of gene therapy for thalassemia patients. It works by modifying β-globin genes that are changed in β-thalassemia. On the whole, betibeglogene autotemcel has the greatest efficacy against transfusion-dependent β-thalassemia as it modifies the gene. However, when selecting among these three drugs, the one that will be most effective for a particular patient depends on the problem faced by the patient. If a particular patient has low hemoglobin due to pyruvate kinase disease, then mitapivat will be best, and the same goes for luspatercept in patients with SMAD2/3 signaling problems. Another drug, etavopivat, is also working well in PKD, but more research is needed. Beyond those already mentioned, rapamycin and sotatercept are the most promising, but research on their use

in thalassemia is limited, with plenty more required in this regard. HU, meanwhile, has been used in β-thalassemia for many years, but iron chelation therapy is needed with this drug, which is quite painful for patients. Then, there is quercetin, a natural food product that has some kind of beneficial effect against β-thalassemia, though it cannot totally cure the disease. Finally, among the other two drugs, decitabine seems to be effective and has very minor side effects, but huge research efforts are required to confirm its potential, while thalidomide has unavoidable side effects but is not carcinogenic. In sum, the three FDA-approved drugs stand out among all the drugs described here, but great research efforts should be devoted to exploring the other potential drugs, as they may offer treatment solutions for patients with different causes of β-thalassemia.

References

1. Ali, S.; Saffiullah, M.F. Awareness of parents regarding beta thalassemia major disease. *Khyber Med. Univ. J.* **2015**, *7*, 72–75.
2. Rund, D.; Rachmilewitz, E. Beta-thalassemia. *N. Engl. J. Med.* **2005**, *353*, 1135–1146. [CrossRef] [PubMed]
3. Cao, A.; Moi, P.; Galanello, R. Recent advances in β-thalassemias. *Pediatr. Rep.* **2011**, *3*, e17. [CrossRef]
4. Williams, T.N.; Weatherall, D.J. World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harb. Perspect. Med.* **2012**, *2*, a011692. [CrossRef]
5. Fucharoen, S.; Winichagoon, P. Haemoglobinopathies in southeast Asia. *Indian J. Med. Res.* **2011**, *134*, 498–506. [PubMed]
6. Brancaleoni, V.; Di Pierro, E.; Motta, I.; Cappellini, M.D. Laboratory diagnosis of thalassemia. *Int. J. Lab. Hematol.* **2016**, *38* (Suppl. S1), 32–40. [CrossRef]
7. Muncie, H.L., Jr.; Campbell, J. Alpha and beta thalassemia. *Am. Fam. Physician* **2009**, *80*, 339–344. [PubMed]
8. Galanello, R.; Origa, R. Beta-thalassemia. *Orphanet. J. Rare Dis.* **2010**, *5*, 11. [CrossRef]
9. Pinto, V.M.; Forni, G.L. Management of Iron Overload in Beta-Thalassemia Patients: Clinical Practice Update Based on Case Series. *Int. J. Mol. Sci.* **2020**, *21*, 8771. [CrossRef]
10. Canver, M.C.; Orkin, S.H. Customizing the genome as therapy for the β-hemoglobinopathies. *Blood* **2016**, *127*, 2536–2545. [CrossRef]
11. Farashi, S.; Harteveld, C.L. Molecular basis of α-thalassemia. *Blood Cells Mol. Dis.* **2018**, *70*, 43–53. [CrossRef]
12. Origa, R.; Galanello, R. Pathophysiology of beta thalassaemia. *Pediatr. Endocrinol. Rev.* **2011**, *8* (Suppl. S2), 263–270. [PubMed]
13. Mettananda, S.; Gibbons, R.J.; Higgs, D.R. α-Globin as a molecular target in the treatment of β-thalassemia. *Blood J. Am. Soc. Hematol.* **2015**, *125*, 3694–3701. [CrossRef]
14. Poggiali, E.; Cassinerio, E.; Zanaboni, L.; Cappellini, M.D. An update on iron chelation therapy. *Blood Transfus.* **2012**, *10*, 411–422. [CrossRef]
15. Kubasch, A.S.; Fenaux, P.; Platzbecker, U. Development of luspatercept to treat ineffective erythropoiesis. *Blood Adv.* **2021**, *5*, 1565–1575. [CrossRef] [PubMed]
16. Carrancio, S.; Markovics, J.; Wong, P.; Leisten, J.; Castiglioni, P.; Groza, M.C.; Raymon, H.K.; Heise, C.; Daniel, T.; Chopra, R.; et al. An activin receptor IIA ligand trap promotes erythropoiesis resulting in a rapid induction of red blood cells and haemoglobin. *Br. J. Haematol.* **2014**, *165*, 870–882. [CrossRef] [PubMed]
17. Al-Samkari, H.; van Beers, E.J. Mitapivat, a novel pyruvate kinase activator, for the treatment of hereditary hemolytic anemias. *Ther. Adv. Hematol.* **2021**, *12*, 20406207211066070. [CrossRef] [PubMed]
18. Zhao, S.; Wang, J.; Wang, Z.; Li, J.; Zhang, L.; Hu, X.; Zhang, X.; Tang, J.; Zhang, J.; Li, S. Double-bonded azacycles used as bioisosteric moieties in drug discovery. *Tetrahedron Lett.* **2023**, *129*, 154719. [CrossRef]
19. Larsen, I.K.; Jerslev, B. Crystal and Molecular Structure of Hydroxyurea. *Acta Chem. Scand.* **1966**, *20*, 983. [CrossRef]
20. Li, J.; Kim, S.G.; Blenis, J. Rapamycin: One drug, many effects. *Cell Metab.* **2014**, *19*, 373–379. [CrossRef]

21. Neupane, Y.R.; Sabir, M.D.; Ahmad, N.; Ali, M.; Kohli, K. Lipid drug conjugate nanoparticle as a novel lipid nanocarrier for the oral delivery of decitabine: Ex vivo gut permeation studies. *Nanotechnology* **2013**, *24*, 415102. [CrossRef]
22. Melchert, M.; List, A. The thalidomide saga. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 1489–1499. [CrossRef]
23. Materska, M. Quercetin and its derivatives: Chemical structure and bioactivity—A review. *Pol. J. Food Nutr. Sci.* **2008**, *58*, 407–413.
24. Cappellini, M.D.; Viprakasit, V.; Taher, A.T.; Georgiev, P.; Kuo, K.H.M.; Coates, T.; Voskaridou, E.; Liew, H.K.; Pazgal-Kobrowski, I.; Forni, G.L.; et al. A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent β-Thalassemia. *N. Engl. J. Med.* **2020**, *382*, 1219–1231. [CrossRef]
25. Cappellini, M.D.; Taher, A.T. The use of luspatercept for thalassemia in adults. *Blood Adv.* **2021**, *5*, 326–333. [CrossRef]
26. Abdulkadyrov, K.M.; Salogub, G.N.; Khuazheva, N.K.; Sherman, M.L.; Laadem, A.; Barger, R.; Knight, R.; Srinivasan, S.; Terpos, E. Sotatercept in patients with osteolytic lesions of multiple myeloma. *Br. J. Haematol.* **2014**, *165*, 814–823. [CrossRef]
27. Cappellini, M.-D.; Porter, J.; Origa, R.; Forni, G.L.; Laadem, A.; Galacteros, F.; Miteva, D.; Sung, V.; Chopra, R.; Arlet, J.-B. A phase 2a, open-label, dose-finding study to determine the safety and tolerability of sotatercept (ACE-011) in adults with beta (β)-thalassemia: Interim results. *Blood* **2013**, *122*, 3448. [CrossRef]
28. Cappellini, M.D.; Porter, J.; Origa, R.; Forni, G.L.; Voskaridou, E.; Galactéros, F.; Taher, A.T.; Arlet, J.B.; Ribeil, J.A.; Garbowski, M.; et al. Sotatercept, a novel transforming growth factor β ligand trap, improves anemia in β-thalassemia: A phase II, open-label, dose-finding study. *Haematologica* **2019**, *104*, 477–484. [CrossRef]
29. Iqbal, A.; Habiba, U.; Waseem, R.; Islam, Z. Pyruvate kinase activator: A major breakthrough in the world of Hematology. *Ann. Med. Surg.* **2022**, *82*, 104631. [CrossRef]
30. Schroeder, P.; Fulzele, K.; Forsyth, S.; Ribadeneira, M.D.; Guichard, S.; Wilker, E.; Marshall, C.G.; Drake, A.; Fessler, R.; Konstantinidis, D.G.; et al. Etavopivat, a Pyruvate Kinase Activator in Red Blood Cells, for the Treatment of Sickle Cell Disease. *J. Pharmacol. Exp. Ther.* **2022**, *380*, 210–219. [CrossRef]
31. Musallam, K.M.; Taher, A.T.; Cappellini, M.D.; Hermine, O.; Kuo, K.H.M.; Sheth, S.; Viprakasit, V.; Porter, J.B. Untreated Anemia in Nontransfusion-dependent β-thalassemia: Time to Sound the Alarm. *Hemasphere* **2022**, *6*, e806. [CrossRef]
32. McGann, P.T.; Ware, R.E. Hydroxyurea therapy for sickle cell anemia. *Expert Opin. Drug Saf.* **2015**, *14*, 1749–1758. [CrossRef]
33. Koren, A.; Levin, C.; Dgany, O.; Kransnov, T.; Elhasid, R.; Zalman, L.; Palmor, H.; Tamary, H. Response to hydroxyurea therapy in beta-thalassemia. *Am. J. Hematol.* **2008**, *83*, 366–370. [CrossRef]
34. Banan, M. Hydroxyurea treatment in β-thalassemia patients: To respond or not to respond? *Ann. Hematol.* **2013**, *92*, 289–299. [CrossRef]
35. Abraham, R.T.; Wiederrecht, G.J. Immunopharmacology of rapamycin. *Annu. Rev. Immunol.* **1996**, *14*, 483–510. [CrossRef]
36. Lechauve, C.; Keith, J.; Khandros, E.; Fowler, S.; Mayberry, K.; Freiwan, A.; Thom, C.S.; Delbini, P.; Romero, E.B.; Zhang, J.; et al. The autophagy-activating kinase ULK1 mediates clearance of free α-globin in β-thalassemia. *Sci. Transl. Med.* **2019**, *11*, eaav4881. [CrossRef]
37. Brus, J.; Czernek, J.; Kobera, L.; Urbanova, M.; Abbrent, S.; Husak, M. Predicting the crystal structure of decitabine by powder NMR crystallography: Influence of long-range molecular packing symmetry on NMR parameters. *Cryst. Growth Des.* **2016**, *16*, 7102–7111. [CrossRef]
38. Lal, A.; Vichinsky, E. The role of fetal hemoglobin-enhancing agents in thalassemia. *Semin. Hematol.* **2004**, *41*, 17–22. [CrossRef]
39. Koshy, M.; Dorn, L.; Bressler, L.; Molokie, R.; Lavelle, D.; Talischy, N.; Hoffman, R.; van Overveld, W.; DeSimone, J. 2-deoxy 5-azacytidine and fetal hemoglobin induction in sickle cell anemia. *Blood* **2000**, *96*, 2379–2384. [CrossRef]
40. Olivieri, N.F.; Saunthararajah, Y.; Thayalasuthan, V.; Kwiatkowski, J.; Ware, R.E.; Kuypers, F.A.; Kim, H.Y.; Trachtenberg, F.L.; Vichinsky, E.P. A pilot study of subcutaneous decitabine in β-thalassemia intermedia. *Blood* **2011**, *118*, 2708–2711. [CrossRef]
41. Mehta, P.; Yadav, N.; Soni, P.; Thekuddan, S.F.; Singh, R.; Khushoo, V.; Mirgh, S.P.; Agrawal, N.; Ahmed, R.; Kapoor, J. Experience with Low Dose Thalidomide in Transfusion Dependent Beta Thalassaemia in a Resource Limited Setting. *Blood* **2019**, *134*, 963. [CrossRef]
42. Tseng, S.; Pak, G.; Washenik, K.; Pomeranz, M.K.; Shupack, J.L. Rediscovering thalidomide: A review of its mechanism of action, side effects, and potential uses. *J. Am. Acad. Dermatol.* **1996**, *35*, 969–979. [CrossRef]
43. Yassin, A.K. Promising Response to Thalidomide in Symptomatic β-Thalassemia. *Indian J. Hematol. Blood Transfus.* **2020**, *36*, 337–341. [CrossRef]
44. Yang, K.; Wu, Y.; Zhou, Y.; Long, B.; Lu, Q.; Zhou, T.; Wang, L.; Geng, Z.; Yin, X. Thalidomide for Patients with β-Thalassemia: A Multicenter Experience. *Mediterr. J. Hematol. Infect. Dis.* **2020**, *12*, e2020021. [CrossRef]
45. Li, X.; Hu, S.; Liu, Y.; Huang, J.; Hong, W.; Xu, L.; Xu, H.; Fang, J. Efficacy of Thalidomide Treatment in Children With Transfusion Dependent β-Thalassemia: A Retrospective Clinical Study. *Front. Pharmacol.* **2021**, *12*, 722502. [CrossRef]
46. Sajadi Hezaveh, Z.; Azarkeivan, A.; Janani, L.; Hosseini, S.; Shidfar, F. The effect of quercetin on iron overload and inflammation in β-thalassemia major patients: A double-blind randomized clinical trial. *Complement. Ther. Med.* **2019**, *46*, 24–28. [CrossRef]
47. Nishimura, K.; Matsumoto, R.; Yonezawa, Y.; Nakagawa, H. Effect of quercetin on cell protection via erythropoietin and cell injury of HepG2 cells. *Arch. Biochem. Biophys.* **2017**, *636*, 11–16. [CrossRef]
48. Asghar, A.A.; Khabir, Y.; Hashmi, M.R. Zynteglo: Betibeglogene autotemcel—An innovative therapy for β- thalassemia patients. *Ann. Med. Surg.* **2022**, *82*, 104624. [CrossRef]

49. Taher, A.T.; Bou-Fakhredin, R.; Kattamis, A.; Viprakasit, V.; Cappellini, M.D. Improving outcomes and quality of life for patients with transfusion-dependent β-thalassemia: Recommendations for best clinical practice and the use of novel treatment strategies. *Expert Rev. Hematol.* **2021**, *14*, 897–909. [CrossRef]

50. Whitney, D.; Shestopalov, I.; Fincker, M.; d'Anjou, M.; Kral, K.; Gayron, M.; Pierciey, F.J.; Colvin, R.A. Drug Product Attributes Predict Clinical Efficacy in betibeglogene autotemcel Gene Therapy for β-thalassemia. *Mol. Ther. Methods Clin. Dev.* **2023**, *31*, 101155. [CrossRef]

51. Locatelli, F.; Thompson, A.A.; Kwiatkowski, J.L.; Porter, J.B.; Thrasher, A.J.; Hongeng, S.; Sauer, M.G.; Thuret, I.; Lal, A.; Algeri, M.; et al. Betibeglogene Autotemcel Gene Therapy for Non-β(0)/β(0) Genotype β-Thalassemia. *N. Engl. J. Med.* **2022**, *386*, 415–427. [CrossRef]

52. Piga, A.; Perrotta, S.; Gamberini, M.R.; Voskaridou, E.; Melpignano, A.; Filosa, A.; Caruso, V.; Pietrangelo, A.; Longo, F.; Tartaglione, I.; et al. Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with β-thalassemia. *Blood* **2019**, *133*, 1279–1289. [CrossRef]

53. Markham, A. Luspatercept: First Approval. *Drugs* **2020**, *80*, 85–90. [CrossRef]

54. Piga, A.; Longo, F.; Gamberini, M.R.; Voskaridou, E.; Ricchi, P.; Caruso, V.; Pietrangelo, A.; Zhang, X.; Shetty, J.K.; Attie, K.M.; et al. Long-term safety and erythroid response with luspatercept treatment in patients with β-thalassemia. *Ther. Adv. Hematol.* **2022**, *13*, 20406207221134404. [CrossRef] [PubMed]

55. Aydinok, Y. Highlights on the Luspatercept Treatment in Thalassemia. *Thalass. Rep.* **2023**, *13*, 77–84. [CrossRef]

56. Dixit, A.; Chatterjee, T.C.; Mishra, P.; Choudhry, D.R.; Mahapatra, M.; Tyagi, S.; Kabra, M.; Saxena, R.; Choudhry, V.P. Hydroxyurea in thalassemia intermedia—A promising therapy. *Ann. Hematol.* **2005**, *84*, 441–446. [CrossRef]

57. Ansari, S.H.; Shamsi, T.S.; Ashraf, M.; Perveen, K.; Farzana, T.; Bohray, M.; Erum, S.; Mehboob, T. Efficacy of hydroxyurea in providing transfusion independence in β-thalassemia. *J. Pediatr. Hematol. Oncol.* **2011**, *33*, 339–343. [CrossRef]

58. Bordbar, M.R.; Silavizadeh, S.; Haghpanah, S.; Kamfiroozi, R.; Bardestani, M.; Karimi, M. Hydroxyurea Treatment in Transfusion-Dependent β-Thalassemia Patients. *Iran. Red Crescent Med. J.* **2014**, *16*, e18028. [CrossRef]

59. Zohaib, M.; Ansari, S.H.; Shamsi, T.S.; Zubarev, R.A.; Zarina, S. Pharmacoproteomics Profiling of Plasma From β-Thalassemia Patients in Response to Hydroxyurea Treatment. *J. Clin. Pharmacol.* **2019**, *59*, 98–106. [CrossRef]

60. Gamberini, M.R.; Prosdocimi, M.; Gambari, R. Sirolimus for treatment of β-thalassemia: From pre-clinical studies to the design of clinical trials. *Health Educ.* **2021**, *4*, 3.

61. Kalantri, S.A.; Ray, R.; Chattopadhyay, A.; Bhattacharjee, S.; Biswas, A.; Bhattacharyya, M. Efficacy of decitabine as hemoglobin F inducer in HbE/β-thalassemia. *Ann. Hematol.* **2018**, *97*, 1689–1694. [CrossRef]

62. Li, Y.; Ren, Q.; Zhou, Y.; Li, P.; Lin, W.; Yin, X. Thalidomide has a significant effect in patients with thalassemia intermedia. *Hematology* **2018**, *23*, 50–54. [CrossRef] [PubMed]

63. Spagnuolo, C.; Russo, M.; Bilotto, S.; Tedesco, I.; Laratta, B.; Russo, G.L. Dietary polyphenols in cancer prevention: The example of the flavonoid quercetin in leukemia. *Ann. N. Y. Acad. Sci.* **2012**, *1259*, 95–103. [CrossRef] [PubMed]

64. Fattizzo, B.; Motta, I. Rise of the planet of rare anemias: An update on emerging treatment strategies. *Front. Med.* **2022**, *9*, 1097426. [CrossRef] [PubMed]

65. Yang, H.; Merica, E.; Chen, Y.; Cohen, M.; Goldwater, R.; Kosinski, P.A.; Kung, C.; Yuan, Z.J.; Silverman, L.; Goldwasser, M.; et al. Phase 1 Single- and Multiple-Ascending-Dose Randomized Studies of the Safety, Pharmacokinetics, and Pharmacodynamics of AG-348, a First-in-Class Allosteric Activator of Pyruvate Kinase R, in Healthy Volunteers. *Clin. Pharmacol. Drug Dev.* **2019**, *8*, 246–259. [CrossRef] [PubMed]

66. Kuo, K.H.; Layton, D.M.; Lal, A.; Al-Samkari, H.; Kosinski, P.A.; Tong, B.; Estepp, J.H.; Uhlig, K.; Vichinsky, E.P. Mitapivat Improves Markers of Erythropoietic Activity in Long-Term Study of Adults with Alpha-or Beta-Non-Transfusion-Dependent Thalassemia. *Blood* **2022**, *140*, 2479–2480. [CrossRef]

67. Kuo, K.H.; Layton, D.M.; Lal, A.; Al-Samkari, H.; Bhatia, J.; Kosinski, P.A.; Tong, B.; Lynch, M.; Uhlig, K.; Vichinsky, E. Long-term efficacy and safety of the oral pyruvate kinase activator mitapivat in adults with non-transfusion-dependent alpha-or beta-thalassemia. *Blood* **2021**, *138*, 576. [CrossRef]

68. Kuo, K.H.M.; Layton, D.M.; Lal, A.; Al-Samkari, H.; Bhatia, J.; Kosinski, P.A.; Tong, B.; Lynch, M.; Uhlig, K.; Vichinsky, E.P. Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in adults with non-transfusion dependent α-thalassaemia or β-thalassaemia: An open-label, multicentre, phase 2 study. *Lancet* **2022**, *400*, 493–501. [CrossRef] [PubMed]

69. van Dijk, M.J.; Rab, M.A.; Rijneveld, A.W.; Nur, E.; Bartels, M.; Jans, J.J.; van Solinge, W.W.; Schutgens, R.E.; van Wijk, R.; Van Beers, E.J. Safety and efficacy of mitapivat (AG-348), an oral activator of pyruvate kinase R, in subjects with sickle cell disease: A phase 2, open-label study (ESTIMATE). *Blood* **2021**, *138*, 2047. [CrossRef]

70. Luke, N.; Hillier, K.; Al-Samkari, H.; Grace, R.F. Updates and advances in pyruvate kinase deficiency. *Trends Mol. Med.* **2023**, *29*, 406–418. [CrossRef]

71. van Dijk, M.J.; de Wilde, J.R.A.; Bartels, M.; Kuo, K.H.M.; Glenthøj, A.; Rab, M.A.E.; van Beers, E.J.; van Wijk, R. Activation of pyruvate kinase as therapeutic option for rare hemolytic anemias: Shedding new light on an old enzyme. *Blood Rev.* **2023**, *61*, 101103. [CrossRef] [PubMed]

72. Bou-Fakhredin, R.; Kuo, K.H.M.; Taher, A.T. Emerging Therapies in β-Thalassemia. *Hematol. Oncol. Clin. N. Am.* **2023**, *37*, 449–462. [CrossRef] [PubMed]

73. Kwiatkowski, J.L.; Walters, M.C.; Hongeng, S.; Locatelli, F.; Rasko, J.E.; Cavazzana, M.; Chen, Y.; Colvin, R.A.; Thompson, A.A. Long-term efficacy and safety of betibeglogene autotemcel gene therapy for the treatment of transfusion-dependent β-thalassemia: Results in patients with up to 6 years of follow-up. *Blood* **2020**, *136*, 51–52. [CrossRef]

74. Fibach, E.; Bianchi, N.; Borgatti, M.; Zuccato, C.; Finotti, A.; Lampronti, I.; Prus, E.; Mischiati, C.; Gambari, R. Effects of rapamycin on accumulation of alpha-, beta- and gamma-globin mRNAs in erythroid precursor cells from beta-thalassaemia patients. *Eur. J. Haematol.* **2006**, *77*, 437–441. [CrossRef]

75. Pecoraro, A.; Troia, A.; Calzolari, R.; Scazzone, C.; Rigano, P.; Martorana, A.; Sacco, M.; Maggio, A.; Di Marzo, R. Efficacy of Rapamycin as Inducer of Hb F in Primary Erythroid Cultures from Sickle Cell Disease and β-Thalassemia Patients. *Hemoglobin* **2015**, *39*, 225–229. [CrossRef]