Novel and Emerging Therapies Pharmaceutical approaches In NTDT &TDT

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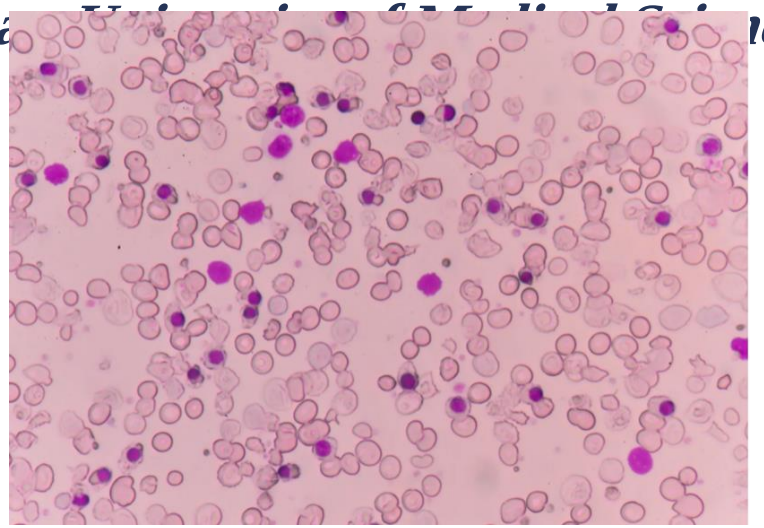
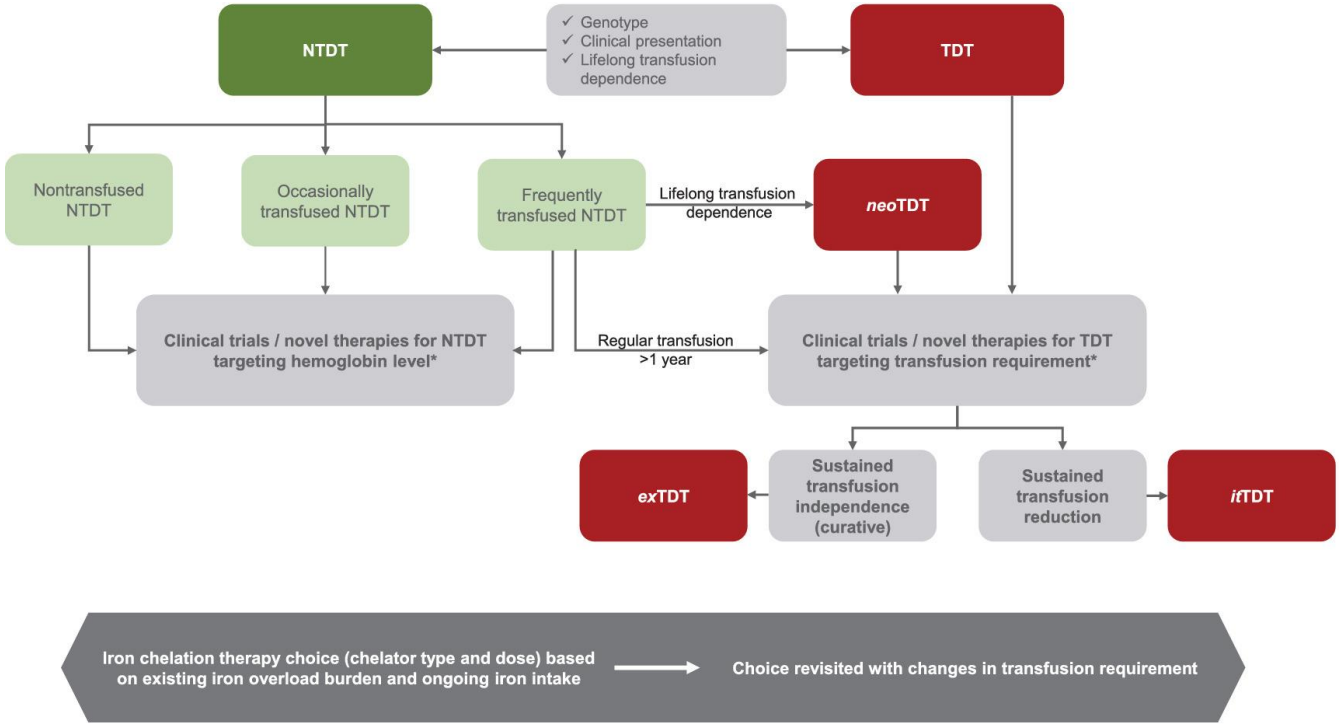


Introduction

- Management of patients withβ-thalassemia is undergoing a swift evolution
- Number of novel agents recently receiving marketing approval or entering clinical development
- Address several persisting unmet needs in this patient population, several questions remain on how such advances should be optimally integrated into standard of care

Revisiting the non-transfusion-dependent (NTDT) vs. transfusion-dependent (TDT) thalassemia classification 10 years later



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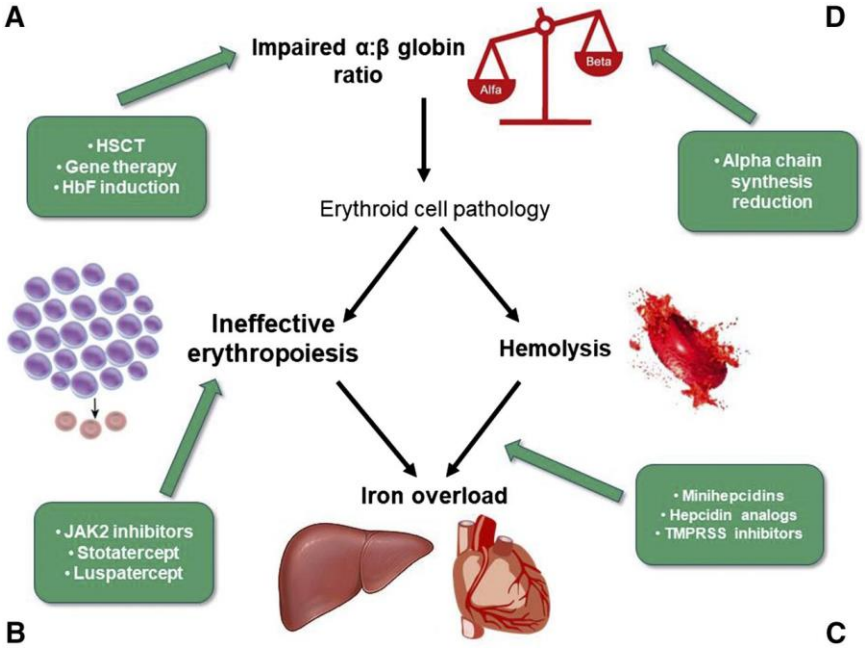
Introduction

- These can be classified into three major categories based on their efforts to address different aspects of the underlying pathophysiology of β thalassemia:
  - ***Correction of the α/β globin chain imbalance***
  - ***Gene therapy***
  - ***Targeting in effective erythropoiesis, and iron dysregulation***

New therapeutic targets in transfusion-dependent and -independent thalassemia



M. Domenica Cappellini,Irene Motta, New therapeutic targets in transfusion-dependent and -independent thalassemia, Hematology Am Soc Hematol Educ Program, 2017, Figure 1.

Introduction

- Currently approved therapy for patients with non-trans-fusion-dependentβ-thalassemia (NTDT) is iron chelation (for patients≥10 years)
- Patients with NTDT accumulate iron from increased intestinal iron absorption and release from the reticuloendothelial system, signaled by low hepcidin levels attributed to ineffective erythropoiesis.

TARGETING INEFFECTIVE ERYTHROPOIESIS AND IRON DYSREGULATION

DISEASE-MODIFYING" (NON-CURATIVE) TREATMENTS

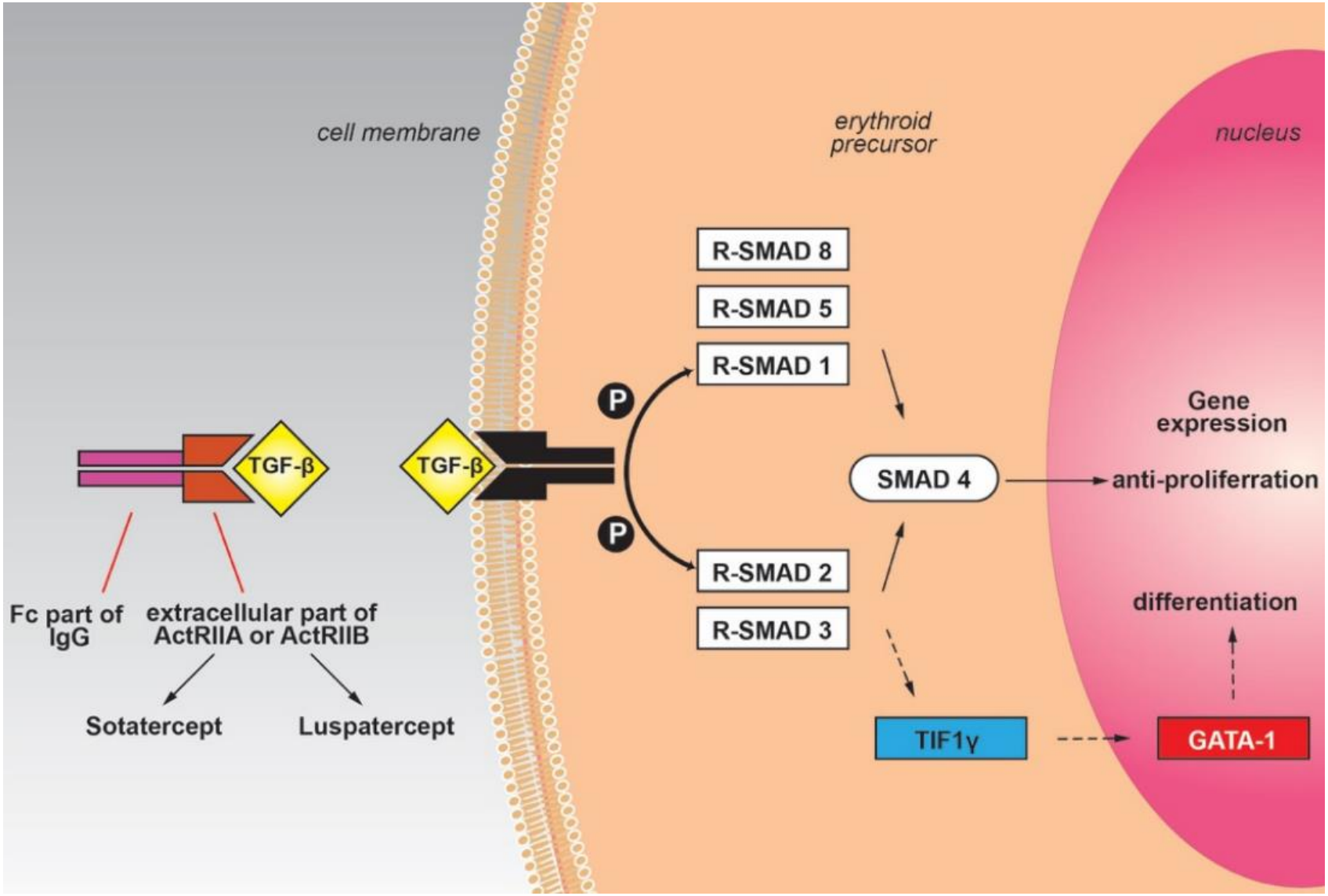
The TGF-β (Transforming Growth Factor-β) Superfamily Ligand Traps

- TGF-β signaling is significant for the regulation of essential cellular pathways, especially in the bone and hematopoietic tissue , and comprises four similar protein groups:
  - ***Activins***
  - ***TGF-β***
  - ***GDFs (growth and differentiating factors)***
  - ***BMP (bone morphogenetic proteins)***

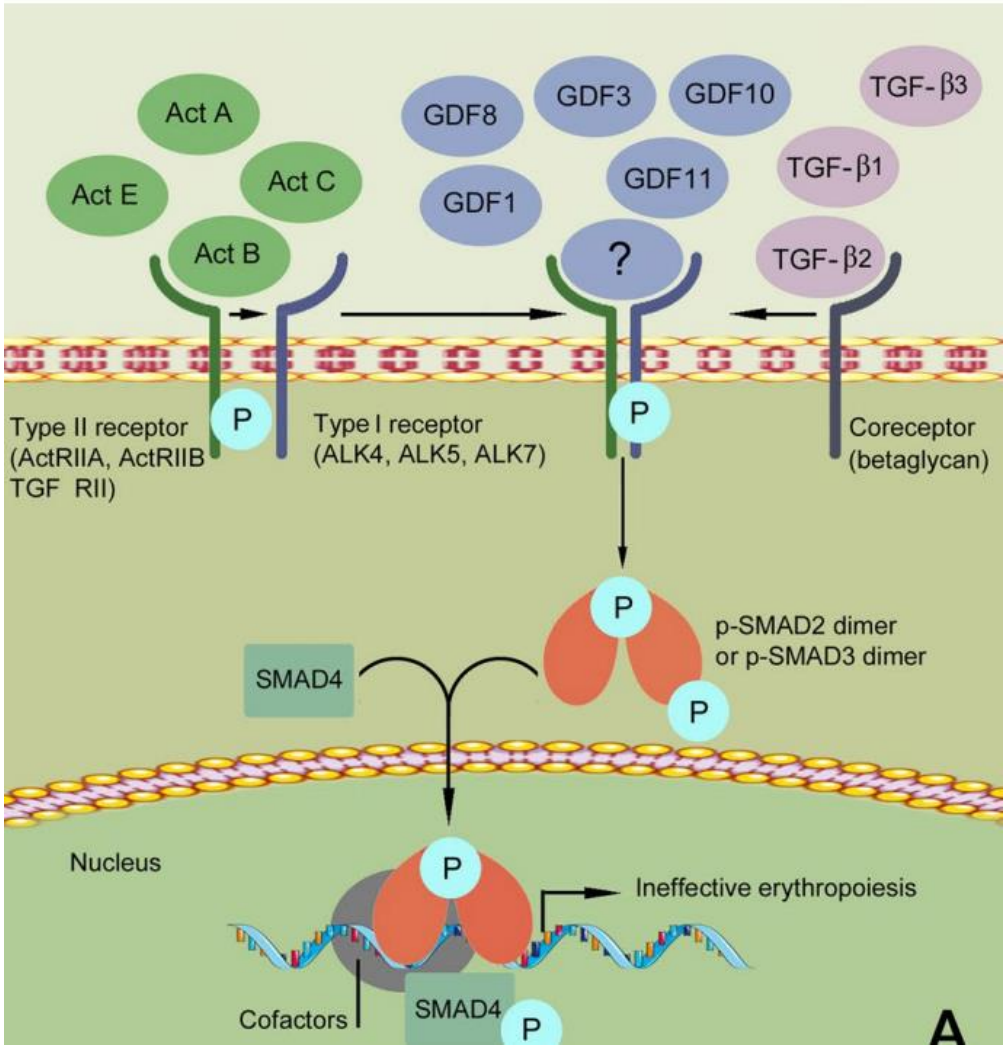
Cont'

- The effect of these proteins on the erythroid lineage can be either inductive (TGF-β, BMP4) or inhibitory (activin, GDFs)
- Their receptors are serine/threonine kinases that stimulate intracellular paths by recruiting the Sma and Mad related proteins (Smad).
- Smad proteins (Smad2/3 or Smad1/5/8) in the cytoplasm are phosphorylated and create an oligomeric combination with Smad4 (coSmad) and insert the nucleus to modify gene transcription
- Activins, GDF8, and GDF11 act through the Smad2/3 pathway, whereas BMPs and other GDFs act through the Smad1/5/8 pathway
- The Smad2/3 pathway also has the ability to bypass the Smad4 and to alternatively bind to TIF1γ (transcription intermediary factor 1γ), according to the stage of cellular differentiation
- TIF1γ stimulates the expression of the key erythroid transcription factor GATA-1 and promotes the differentiation of erythroid lineage

TGF-β signaling pathway and its ligand traps



SMAD2/3 signaling pathway



Sotatercept

- *Sotatercept or ACE-011 has been also shown to correct ineffective erythropoiesis by* acting as a ligand trap to inhibit negative regulators of late-stage erythropoiesis in the transforming growth factor β (TGF-β) superfamily.
- Luspatercept (ACE-536) is a recombinant fusion protein that binds to select transforming growth factor β superfamily ligands and enhances late-stage erythropoiesis
- A phase 2 study conducted on 16 TDT patients showed that the majority of TDT patients (66%) treated with higher doses of sotatercept (0.75-1.0 mg/kg) achieved reductions of ≥33% in red cell transfusion requirements.
- The increase in haemoglobin and reduction in red cell transfusion correlated with increased serum exposure to sotatercept (Cappellini et al.,2019).
- Sotatercept exhibited an overall good safety profile and was tolerated by most patients.
- Treatment discontinuation due to adverse events was rare, and the incidence of grade 3-4 adverse events was low.
- A decision, however, was made not to advance trials of sotatercept in β thalassaemia due to binding of sotatercept to activin A.

Luspatercept

- Preliminary data with sotatercept led to the initiation of similar trials in TDT patients using luspatercept.
- *Luspatercept or ACE-536 is a recombinant fusion protein that* binds to specific ligands of the TGF-β superfamily and enhances erythroid maturation.
- It is the most recently approved therapy (FDA and EMA) for the management of TDT.
- Pre-clinical data on murine models showed that treatment with RAP-536 reduced α globin chain aggregation and haemolysis, while increasing erythrocyte life span and improving iron overload (Suragani et al., 2014b).
- Additionally, RAP-536 increased haemoglobin concentration and red cell count (RBC), and reduced comorbidities associated with β thalassemia, such as decreased bone mineral density and splenomegaly (Suragani et al., 2014a).
- In the phase 1 study, 32 healthy volunteers were randomized 3:1 to receive 2 doses of luspatercept (0.0625–0.25 mg/kg) or placebo subcutaneously every 2 weeks (ClinicalTrials.gov number NCT01432717).
- Luspatercept was well-tolerated and dose-dependent and increases in haemoglobin concentration
- and RBC were observed after the first dose (Attie et al., 2014).

Luspatercept

- A phase 2, open-label,nonrandomized, uncontrolled, dose-finding study was then conduced to evaluate the effects of luspatercept in β thalassaemia patients.
- The study enrolled 33 NTDT patients and 31 TDT patients (ClinicalTrials.gov number NCT01749540) (Piga et al., 2019).
- Luspatercept was administered subcutaneously every 21 days (0.2–1.25 mg/kg) in dose escalation and expansion cohorts.
- The primary endpoint of mean increase in haemoglobin concentration from baseline of ≥ 15 g/l for ≥2 weeks (in the absence of red cell transfusions) was achieved by 58% (95% confidence interval [CI] 39.1 to 75.5) of NTDT patients receiving the higher dose range of Luspatercept (0.6–1.25 mg/kg).
- In TDT patients, the primary endpoint of a transfusion-burden reduction of ≥ 20% over any 12 weeks vs baseline was achieved by 81% (95% CI 63.6, 92.8) of patients receiving the higher dose range of luspatercept.
- These findings,including the achievement of secondary endpoints, prompted a randomized Phase 3 clinical trial (BELIEVE Trial) to assess efficacy and safety.

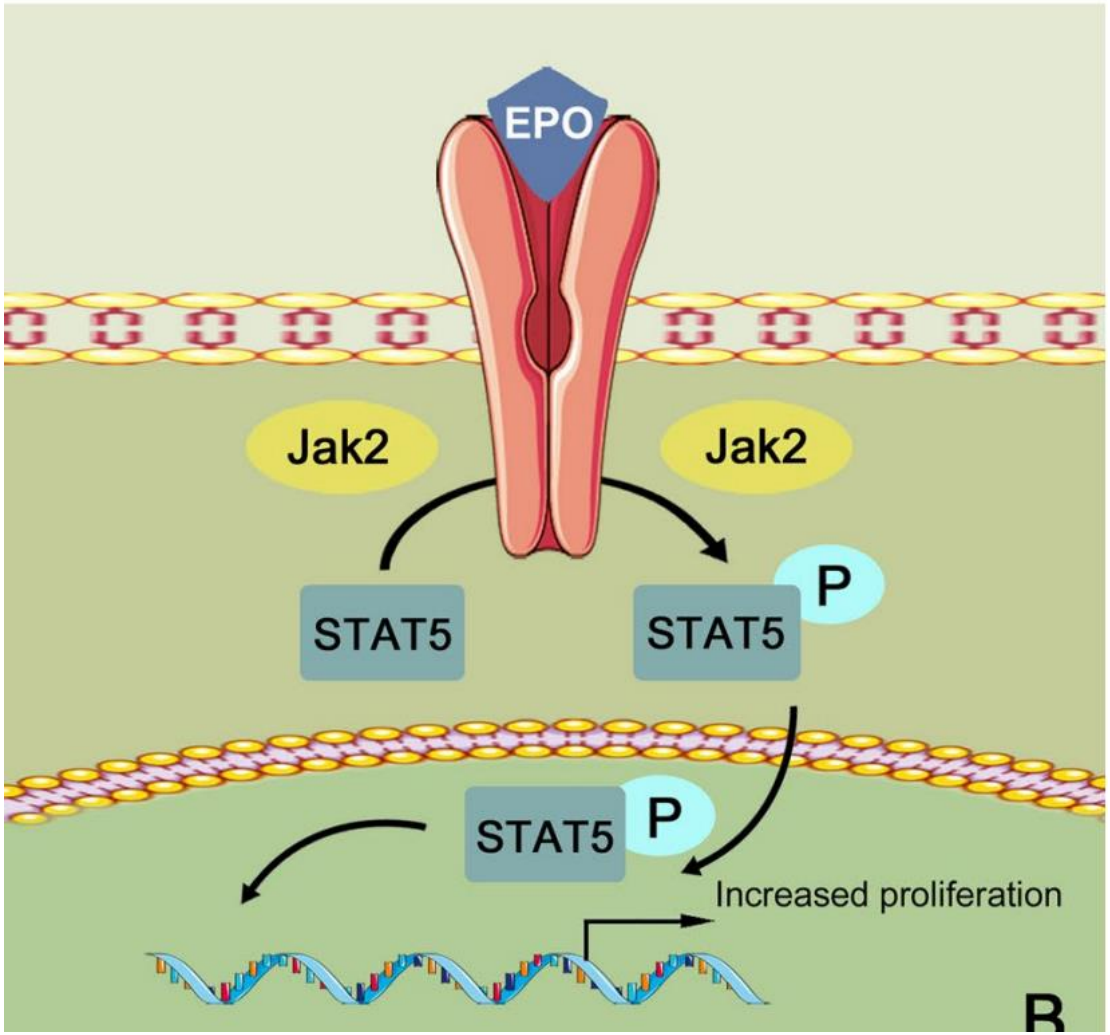
Luspatercept

- The approval of Luspatercept was based on the results the BELIEVE trial, a phase 3,randomised, double-blind, placebo-controlled trial which showed that subcutaneous administration of luspatercept (n=224) at doses of 1-1.25 mg/kg led to a reduction in the transfusion burden of at least 33% from baseline during weeks 13 through 24.
- Moreover, a reduction of at least 2 red-cell units over this 12-week interval was significantly greater in the luspatercept group than in the placebo group (21.4% vs. 4.5%) (Cappellini et al., 2020b).
- During any 12-week interval, the percentage of patients who had a reduction in transfusion burden of at least 33% was greater in the luspatercept group than in the placebo group (70.5% vs. 29.5%), as was the percentage of those who had a reduction of at least 50% (40.2% vs. 6.3%).
- Parallel reductions in serum ferritin levels were also observed.
- Adverse events more commonly seen in the luspatercept group compared to placebo included bone pain, arthralgia,dizziness, hypertension and hyperuricaemia.
- Data on the long-term use of luspatercept, its real-life application and its use in the paediatric population are awaited.

Luspatercept

- The effects of luspatercept on iron loading over time and the impact of baseline iron parameters on response to luspatercept was also evaluated and showed that luspatercept treatment resulted in clinically meaningful and maintained reductions in serum ferritin levels (Porter et al., 2019).
- Baseline iron overload did not seem to affect response rates with luspatercept.
- Treatment with luspatercept resulted in clinically meaningful reductions in red cell transfusion burden regardless of baseline serum ferritin level.
- There was a trend for decrease in liver iron concentration (LIC) with longer follow up at 96 weeks; among responders, the decrease was more pronounced compared with non-responders (Porter et al., 2019).
- In a sub-analysis on long-term efficacy and safety of luspatercept, it was shown that luspatercept treatment was associated with prolonged periods of clinically meaningful reductions in transfusion burden, including in patients who crossed over from the placebo arm (Taher et al., 2020).
- The safety profile of crossover patients was consistent with that reported in the luspatercept arm.
- Another sub-analysis study explored the association between β globin genotype and response to luspatercept in adult patients with β thalassaemia in the BELIEVE trial (Cappellini et al., 2020a).
- It was found that although response rates were lower in patients with the most severe disease (β0/β0), clinically meaningful reductions in transfusion burden were observed across all genotypes (Cappellini et al., 2020a).

JAK2/STAT5 signaling pathway



Ruxolitinib

- Many preclinical studies have provided evidence on the role of ruxolitinib (JAK1/JAK2 inhibitor) as a potential target to improve ineffective erythropoiesis
- The inhibition of JAK2 in TDT and non-transfusion-dependent thalassaemia (NTDT) mouse models was shown to not only improve ineffective erythropoiesis but also to decrease splenomegaly (Casu et al., 2018).
- A phase 2a study assessed the efficacy and safety of ruxolitinib in TDT patients with spleen enlargement.
- Ruxolitinib was overall well tolerated in this study population, and the safety profile was consistent with the previous reports.
- However, because the major purpose of reducing spleen size in patients with TDT is to improve pre-transfusion haemoglobin and related reduction in transfusion needs where ruxolitinib had shown a limited effect, no further studies were conducted.

Mitapivat

- Mitapivat (AG-348) is an oral, small-molecule, allosteric activator of the red blood cell (RBC)-specific form of pyruvate kinase (PK-R).
- Adenosine triphosphate (ATP) supply appears to be insufficient in thalassemic RBCs to maintain RBC membrane fitness and clearance of globin precipitates.
- Inβ-thalassemia mouse models, mitapivat increased ATP levels, reduced markers of ineffective erythropoiesis, and improved anemia, RBC survival, and indices of iron overload
- An open-label, phase two trial (NCT03692052) in adults with NTDT and a baseline hemoglobin of ≤10 g/dL is evaluating the efficacy of mitapivat in improving hemoglobin level (by ≥1.0 g/dL) as well as markers of hemolysis and ineffective erythropoiesis.
- Interim data showed response in eight of nine patients following 12 weeks oftherapy

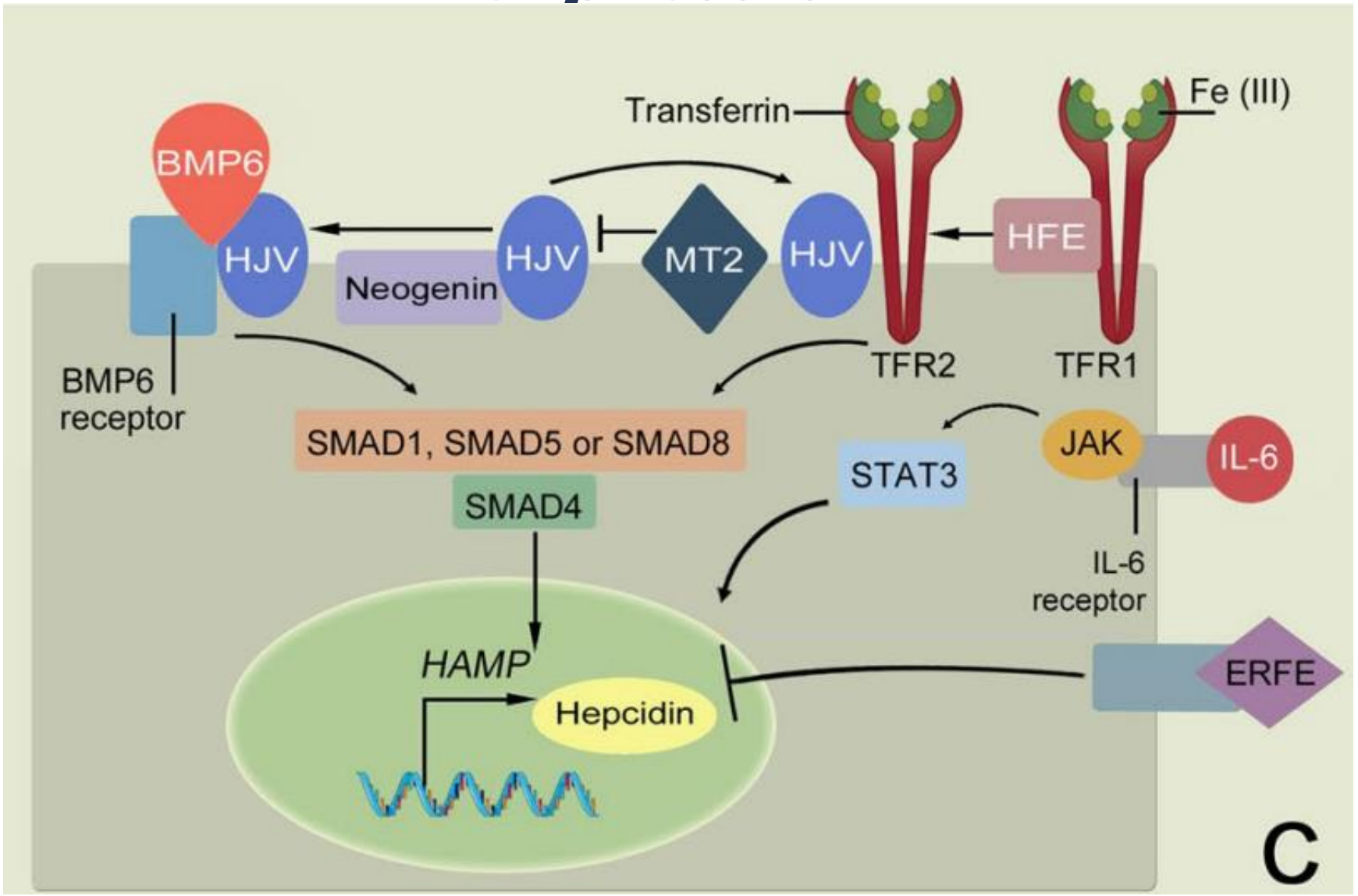
VIT-2763

- VIT-2763 is an oral ferroportin inhibitor which restricted iron availability, ameliorated anemia, and reversed the dysregulated iron homeostasis inβ-thalassemia mouse models
- VITHAL(NCT04364269) is a randomized, double-blind, placebo-controlled , phase two trial evaluating the efficacy of VIT-2763 in improving hemoglobin and iron indices in NTDT patients aged ≥12 years with abase line hemoglobin ≤11 g/dL

Phosphodiesterase 9 Inhibition

- Alteration of intracellular cyclic guanosine monophosphate (cGMP) is a novel therapeutic objective for sickle cell disease and thalassemia.
- The cGMP-dependent pathway is significant for the production of HbF and has multiple roles in vascular biology.
- As phosphodiesterase (PDE) 9 selectively degrades cGMP in erythropoietic cells, the use of inhibitors of PDE9 can result in increased cGMP levels and the reactivation of HbF
- IMR-687 is a novel agent that has been developed for the inhibition of PDE 9
- Oral use of IMR-687 in sickle cell disease patients has been recently completed (NCT03401112) and has shown to stimulate HbF production and to improve Hb levels and hemolysis indices
- A similar phase 2 study has been launched in order to evaluate the safety and tolerability of IMR-687 given once daily for 36 weeks in TDT and NTDT adult thalassemic patients (NCT04411082)

Regulation of hepcidin expression



Iron Metabolism Manipulation

- Hepcidin, a protein produced from the hepatocytes, is the key controller of iron metabolism.
- Hepcidin degrades ferroportin, the main iron exporter in intestinal cells; macrophages of the reticuloendothelial system; and the hepatocytes
- β-thalassemia,hepcidin production is inhibited through the action of ERFE, contributing to increased intestinal iron absorption and tissue deposition
- Restoration of hepcidin levels could improve iron overload and ineffective erythropoiesis
- Hepcidin production is mainly regulated by matriptase-2 (MT-2), a transmembrane serine protease, which is encoded by the TMPRSS6 gene
- MT-2 inhibits hepcidin activation by cleaving membrane hemojuvelin
- The use of minihepcidins, which act as hepcidin agonists, has been proven to improve ineffective erythropoiesis and splenomegaly in a TDT mouse model
- Another approach is to restrict the availability of iron, targeting both the iron overload and the ineffective erythropoiesis,with the use of ferroportin inhibitors (VIT-2763).
- These agents could be beneficial in NTDT.

TMPRSS6

- Anti-sense oligonucleotides (ASO) downregulating TMPRSS6, ametalloprotease which plays a key role in hepcidin expression, stimulated hepcidin, reduced iron burden, and improved ineffective erythropoiesis and RBC survival in β-thalassemia mouse models
- TMPRSS6-LRx is a generation 2+ ligand-conjugated ASO subcutaneous drug (given every 4 weeks) that is now being evaluated in a randomized, open-label, phase two trial (NCT04059406) in adults with NTDT and baseline hemoglobin ≤10 g/dL
- The main endpoints include increasing hemoglobin level (by≥1.0 g/dL) and decreasing LIC

Novel Agents

- All novel agents primarily aim to ameliorate anemia and iron over-load in patients with NTDT, and final data from ongoing and subsequent registration trials are awaited

Clinical Trials

Clinical trials with new drugs for β-thalassemias

Drug trial name	Trial no.	Phase	Purpose	Disease	No. of patients	Status
Luspatercept	NCT01749540/Extension NCT02268409	2	Open-label, ascending dose study to evaluate the effects	TDT and NTDT	64 (30 TDT, 34 NTDT)	Completed/active, not recruiting
Luspatercept BELIEVE Study	NCT02604433	3	Double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety plus BSC vs placebo plus BSC	TDT	300	Active, not recruiting
Sotatercept (ACE-011)	NCT01571635	2A	Dose-finding study to determine safety and tolerability	TDT and NTDT	46	Active, not recruiting
Ruxolitinib TRUTH Study	NCT02049450	2	Study of efficacy and safety	TDT	30	Completed

Table 1. Clinical trials of novel treatment in β-thalassemia.

Treatment Modality	Mechanism	Route	Phase	ClinicalTrials.gov (8 May 2021)	Status	Institution/ Developer
Luspatercept	Ligand trap TGF beta superfamily	Subcutaneous	2	NCT01749540	Completed	Acceleron Pharma, Celgene Corporation
			2, extension study	NCT02268409	Completed	
			2	NCT03342404	Open	
			2	NCT04143724	Not yet recruiting	
			3	NCT02604433	Completed	
Mitapivat	Pyruvate kinase activation	Oral	2	NCT03692052	Open	Agios Pharmaceuticals
TMPRSS6-LRx	Matriptase-2 inhibition, hepcidin activation	Subcutaneous	2	NCT04059406	Open	Ionis Pharmaceuticals
SLN124	Matriptase-2 inhibition, hepcidin activation	Subcutaneous	2	NCT04718844	Open	Silence Therapeutics plc
PTG-300	Hepcidin analog	Subcutaneous	2	NCT04054921	Completed	Protagonist Therapeutics, Inc.
VIT-2763	Ferroportin inhibition	Oral	2	NCT04364269	Open	Vifor (International) Inc.
IMR-687	Phosphodiesterase 9 inhibition, HbF stimulation	Oral	2	NCT04411082	Completed	Imara, Inc.

New therapeutic approaches in preclinical development for thalassemias

Drug	Mechanisms	Material
Minihepcidins	Hepcidin upregulation	Th3/+ mice
TMPRSS6-LRx	TMPRSS6 disruption	Th3/+ mice, monkeys
Exogenous transferrin	TfR1 downregulation	Th3/+ mice
ERFE downregulation	Hepcidin upregulation	Th3/+ mice
IOX1	Selective silencing of α-globin	Erythroid cultures

Summary and Recommendations

- Luspatercept, now known as Reblozyl®, after official authorisation by EMA (European Medicines Agency) and FDA (Food and Drug Administration), seems to be quite promising, with significant improvement in haemoglobin levels and reduction in transfusion requirements.
- Moreover, this reduction in transfusion burden decreases ongoing iron intake and, thus, the iron-chelation therapy requirements.
- The reduction in the serum ferritin level observed with luspatercept also suggests favourable early effects on iron balance.
- This could be due to improved iron utilisation (by reducing ineffective erythropoiesis and promoting red cell production), reduced transfusional iron intake augmenting the efficiency of iron-chelation therapy, or both (Suragani et al., 2014b, 2014a).
- Patients with TDT living in resource poor areas with limited access to regular and safe blood transfusions as well as those patients who were previously transfusion independent and are currently under transfusion are likely to be the ones to benefit considerably from this drug.

Summary and Recommendations

- In conclusion, several novel therapeutic approaches are currently under development for TDT patients, with the aim of improving outcomes in thalassaemia care and cure and overall quality of life.
- Once the efficacy and safety of all these novel therapies are established, long-term, head-to-head, and comparison trials are necessary to determine the optimal management of TDT patients, which is becoming more and more personalised.
- Moreover, the optimal use of these novel therapies on their own or in combination with other conventional therapeutic modalities also warrants future clinical studies.
- Healthcare systems all over the world, with the support of treating physicians, health academia, health economists, industry, other relevant stakeholders and importantly the patients, will need to work very closely to identify those tools and solutions that will make these novel therapies accessible and available, as far as possible, to patients.

Reblozyl

Basic recommendations regarding the use of Reblozyl®

• Reblozyl® can be considered for:

Patients who require regular red blood cell transfusions

≥18 years of age

• The recommended starting dose is 1 mg/kg once every 3 weeks by subcutaneous injection

• If the pre-dose haemoglobin level is ≥ 115 g/l and is not influenced by recent transfusion, consider delaying dosing of Reblozyl® until the level is ≤ 110 g/l

• Before administration of Reblozyl® haemoglobin level, and liver function tests including alanine transferase and aspartate transferase levels should be monitored to ensure proper dosing and metabolism of the medication.

• If a TDT patient does not achieve a reduction in red cell transfusion burden after at least 2 consecutive doses (6 weeks) at the 1 mg/kg starting dose, increase the Reblozyl® dose to 1.25 mg.

• If a patient experienced a response followed by a lack of or lost response to Reblozyl®, consider initiating a search for causative factors

• Reblozyl® should be discontinued if a patient does not experience a decrease in transfusion burden after 9 weeks of treatment (administration of 3 doses)

Complications

- *Thrombosis/Thromboembolism*
- In adult patients with beta thalassemia, thromboembolic events (TEE) were reported in 8/223 (3.6%) REBLOZYL-treated patients.
- Reported TEEs included deep vein thrombosis, pulmonary embolus, portal vein thrombosis, and ischemic strokes.
- Patients with known risk factors for thromboembolism, e.g. splenectomy or concomitant use of hormone replacement therapy, may be at further increased risk of thromboembolic conditions.
- Consider thromboprophylaxis in patients with beta thalassemia at increased risk of TEE.
- Monitor patients receiving REBLOZYL for signs and symptoms of thromboembolic events and institute treatment promptly.

Complications

- *Hypertension*
- Hypertension was reported in 10.7% (61/571) of REBLOZYL-treated patients.
- Across clinical studies, the incidence of grade 3-4 hypertension ranged from 1.8% to 8.6%.
- In adult patients with beta thalassemia with normal baseline blood pressure, 13 (6.2%) patients developed systolic blood pressure (SBP) ≥130 mm Hg and 33 (16.6%) patients developed diastolic blood pressure (DBP) ≥80 mm Hg.
- In adult patients with MDS with normal baseline blood pressure, 26 (29.9%) patients developed SBP ≥130 mm Hg and 23 (16.4%) patients developed DBP ≥80 mm Hg.
- Monitor blood pressure prior to each administration.
- Manage new-onset hypertension or exacerbations of preexisting hypertension using anti-hypertensive agents.

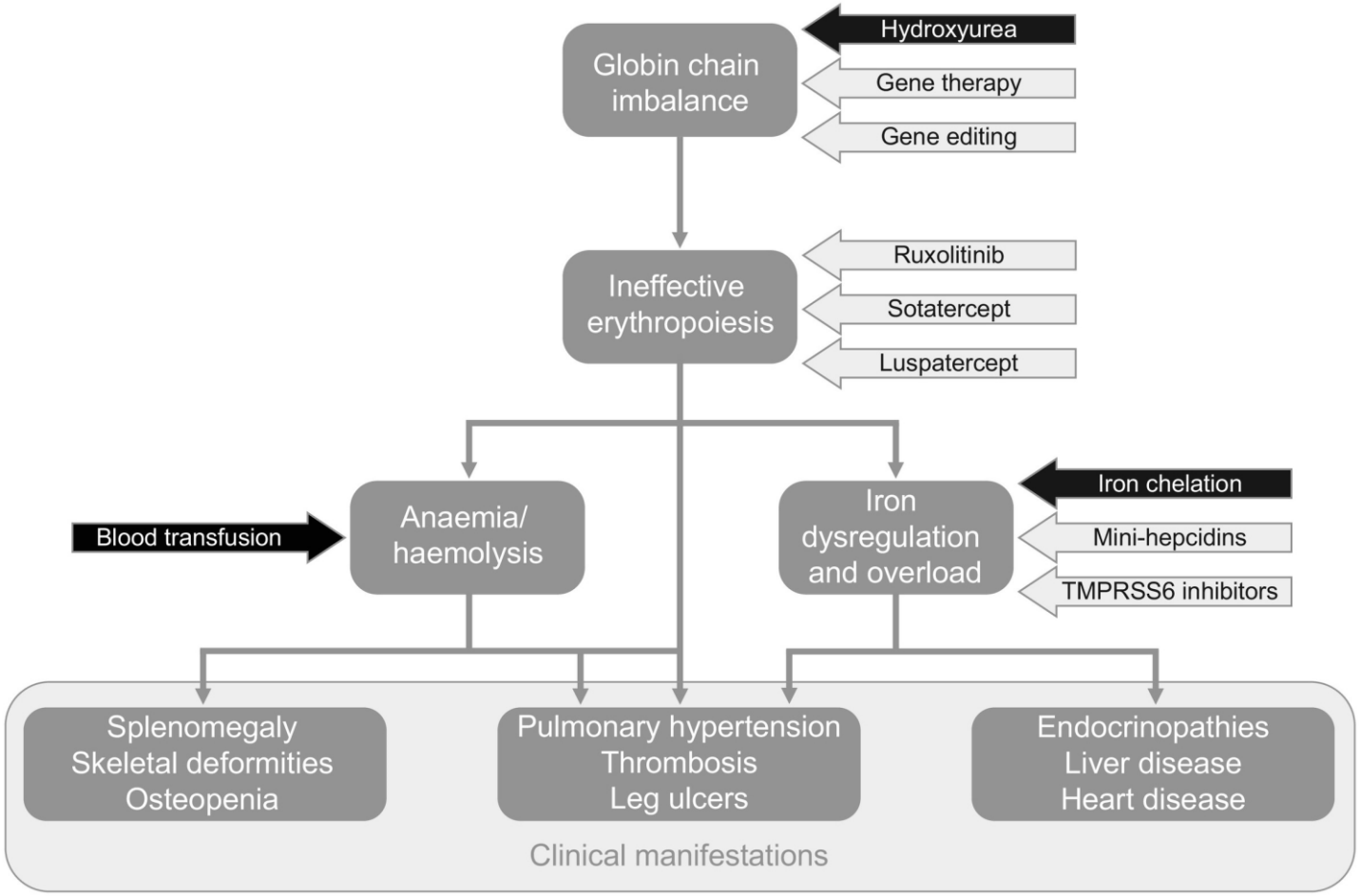
Embryo-Fetal Toxicity

- Based on findings from animal reproductive studies, Reblozyl® may cause fetal harm when administered to a pregnant woman.

Pediatric Use

- Safety and effectiveness in pediatric patients have not been established.
- Based on findings in juvenile animals, REBLOZYL is not recommended for use in pediatric patients

Conclusion


Globin chain imbalance
Hydroxyurea
Gene therapy
Gene editing
Ineffective erythropoiesis
Ruxolitinib
Sotatercept
Luspatercept
Anaemia/ haemolysis
Blood transfusion
Iron dysregulation and overload
Iron chelation
Mini-hepcidins
TMPRSS6 inhibitors
Splenomegaly
Skeletal deformities
Osteopenia
Pulmonary hypertension
Thrombosis
Leg ulcers
Endocrinopathies
Liver disease
Heart disease
Clinical manifestations

Thank You
Any Questions