ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

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ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ ΝΟΣ. ΑΛΕΞΑΝΔΡΑ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

«ΚΛΙΝΙΚΕΣ ΜΕΛΕΤΕΣ: ΣΧΕΔΙΑΣΜΟΣ ΚΑΙ ΕΚΤΕΛΕΣΗ»
MSc: "Clinical Trials: Design and Conduct"

Διευθυντής και Επιστημονικός Υπεύθυνος
Ευάγγελος Τέρπος, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ

Τίτλος ΜΔΕ

Luspatercept, ένας TGF-beta αναστολέας στη θεραπεία της αναιμίας σχετιζόμενης με Μυελοδυσπλαστικά Σύνδρομα, β-θαλασσαιμία & Μυελοΐνωση

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Όνομα: **Δέσποινα Τιμοθεάτου**, Χημικός

Αρ. μητρώου: 20170050

Επάγγελμα: Regional Medical Liaison

Επιβλέπων καθηγητής ΜΔΕ:

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ΑΘΗΝΑ 2019

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<u>Τα Μέλη της Εξεταστικής Επιτροπής</u>

Επιβλέπων: Ευάγγελος Τέρπος, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ
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ΑΘΗΝΑ 2019

Luspatercept, a TGF-beta inhibitor in the treatment of β-thalassemia, Myelodysplastic Syndromes & Myelofibrosis related anemia

Abstract

β-thalassemia syndromes are a group of hereditary disorders characterized by ineffective erythropoiesis due to a genetic deficiency in the synthesis of the beta chains of hemoglobin and is often accompanied with severe anemia and need for red blood cell transfusion dependence (RBC-TD).

On the other hand, Lower Risk Myelodysplastic Syndromes (MDS) are characterized by cytopenia of one or more lineages and ineffective hematopoiesis due to an increased susceptibility of clonal myeloid progenitors to apoptosis and a defective differentiation and maturation of erythroblasts despite a hypercellular bone marrow.

Myelofibrosis is a disorder of the spongy tissue of the bone marrow. Eventually, the bone marrow is replaced by fibrous tissue and the stem cells cannot produce enough blood cells.

There is an unmet need in treating β- thalassemia, as the optimal strategy is to balance regular transfusions with iron overload and manage a broad spectrum of complications affecting many organ systems. The same is true for Lower Risk Myelodysplastic Syndromes which are symptomatically managed, while the main treatment goal is to correct anemia and reduce the potential of

progression to Higher-Risk Myelodysplastic Syndromes or transformation in Acute Myelogenous Leukemia.

Luspatercept, also known as ACE-536, is a fusion protein that consists of a modified activin receptor IIB (ActRIIB), a member of the Transforming Growth Factor-β (TGF-β) superfamily, and the Fc domain of human immunoglobulin G (IgG1), showing promising efficacy in the treatment of hematologic disorders, such as Lower Risk Myelodysplastic Syndromes, β- thalassemia and Myelofibrosis.

In this review, we will try to present all aspects of the potential use of Luspatercet in the treatment of these haematological malignancies.

Development of Luspatercept in the Treatment of Ineffective Erythropoiesis of β-Thalassemia, Myelodysplastic Syndromes & Myelofibrosis

Luspatercept is a modified activing receptor type IIB ligand trap and it is currently studied in diseases characterized by ineffective erythropoiesis, like β-thalassemia, MDS and MF. Development of Luspatercept begun after the incidental observation that another ligand trap, activing receptor type IIA, Sotatercept, when administered to healthy postmenopausal women for their osteoporosis, improved their anemia. Preclinical data in animal models demonstrated that Luspatercept, improves late-stage erythroid maturation potentially through the GDF11-ActRIIB-Smad2/3–dependent pathway, under both steady-state and stress conditions, regardless of EPO levels. Following initial findings, phase I, II & III were designed in β-thalassemia & MDS, and lately, a phase II, with or without JAK inhibitor in primary MF.

Introduction

Normal erythropoiesis

Erythropoiesis (Valent et al., 2018) is the perpetual process of mature red blood cells, RBCs, being produced in the bone marrow from progenitor Haematopoietic Stem Cells into the peripheral blood. This process can be

adjusted and regulated substantially, in physiological and in stress conditions, to ensure that the appropriate oxygen supply reaches the peripheral tissues.

Erythropoiesis is a sequential multi-step process driving differentiation and regulate proliferation of erythroid cells· Erythroid progenitors differentiate morphologically into temporally stages of erythroid cells, characterized by changes in surface protein expression, in cell size, in progressive haemoglobinization, and nuclear condensation leading to reticulocytes and mature red blood cells. This process is regulated by combined effects of microenvironment and growth factors that promote survival, proliferation, and/or differentiation of erythroid progenitors and nuclear factors that regulate transcription of genes involved in survival and establishment of the erythroid phenotype (Ribeil et al., 2013).

Key molecules implicated in erythropoiesis

Cytokines, mainly **erythropoietin**, regulators of iron metabolism and a whole regulatory network is involved and can adjust itself to the physiological needs such as the oxygen concentration in altitude or pregnancy, as well as to pathological conditions such as blood loss. Erythropoietin, produced and secreted by the kidney, signals through the erythropoietin receptor (EPOR) in immature erythroid cells and induces phosphorylation and activation of the tyrosine kinase Janus kinase 2 (**JAK2**). In turn, the phosphorylated form of JAK2 activates signal transducer and activator of transcription 5 (STAD 5),

which modifies the expression of genes involved in proliferation, differentiation and survival of erythroid precursors (Ginzburg and Rivella, 2011).

The process of erythropoiesis is also known to be regulated by members of the Transforming Growth Factor-beta (**TGF-β**) superfamily, which includes a large number of proteins, like Bone Morphogenetic Proteins (BMPs), Activins and Growth Differentiation Factors (GDFs) (Valent et al., 2018).

The **BMP**s, were originally isolated for their ability to induce bone formation in vivo by promoting osteoblast differentiation. Like other members of the TGF-β superfamily, the cell-surface receptors are heterodimers and interact with combinations of type I and type II receptor dimers, like activin receptor II A and B (ActRIIA and ActRIIB), to produce multiple possible signaling complexes leading to the activation of one of two competing sets of **Smad** transcription factors (Rider et al., 2010).

Growth differentiation factor 11 (**GDF11**) is a member of TGF-β/ BMP superfamily that is involved in normal physiological processes, like embryonic development and erythropoiesis, but also, in the pathophysiology of aging, cardiovascular disease, diabetes mellitus, and cancer. GDF11 is found to play a critical role in the pathology of β-thalassemia, myelodysplastic syndrome (MDS), and erythropoietin-resistant anemia in hemodialysis (HD) patients (Zhang et al., 2017). In the normal process of erythropoiesis, GDF11 is mainly expressed in early, immature erythroid progenitors and is necessary for their survival and inhibition of terminal differentiation (Paulson RF., 2014). In anemia, GDF11 promotes proliferation of erythroid precursors and inhibits late-stage erythroid maturation (Suragani et al., 2014).

Activin together with other TGFβ superfamily members signaling through Smads 2 and 3 were found to sustain embryonic stem cell pluripotency and self-renewal and that by blocking activin signaling, embryonic stem cells resulted in differentiation (Yin Xia, 2009).

GDF11 binds to both ActRIIA and ActRIIB and phosphorylates Smad and non-Smad intracellular signaling proteins (Zhang et al., 2017).

It has been demonstrated that GDF11-ActRIIB-Smad2/3 signaling is a key regulatory mechanism in proliferating erythroid precursors that controls their late-stage maturation under both steady-state and stress conditions (Rochette et al., 2015).

Another key pathway in erythropoiesis, is through GATA binding protein 1 (**GATA-1**), the master transcriptional regulator of erythropoiesis, that is essential for differentiation, maturation and globin chain synthesis. GATA-1 is protected in the nucleus during terminal erythroid differentiation by heat shock protein 70 (**HSP70**), a supervisor protein that enables the refolding of proteins denatured by cytoplasmic stress to prevent their aggregation (Arlet et al., 2016).

Ineffective erythropoiesis in β-thalassemia and MDS

β-thalassemia is an inherited monogenic disorder, characterized by reduced or absent production of b-globin chain, resulting in a/b-chain unbalance and accumulation of highly toxic free a-globin–haem complexes (Capellini et al., 2017). Ineffective erythropoiesis in β-thalassemia leads to tissue hypoxia and

overexpression of erythropoietin to drive the expansion of early erythroid progenitors. Erythroid differentiation leads up to the polychromatophilic normoblast stage, then maturation process fails, and polychromatophilic erythroblasts are driven to apoptosis (Arlet et al., 2016).

Over time, the combination of tissue hypoxia, increased erythropoietin and ineffective erythropoiesis creates a vicious cycle that ultimately leads to a massive expansion of erythroblasts (Tanno and Miller, 2010).

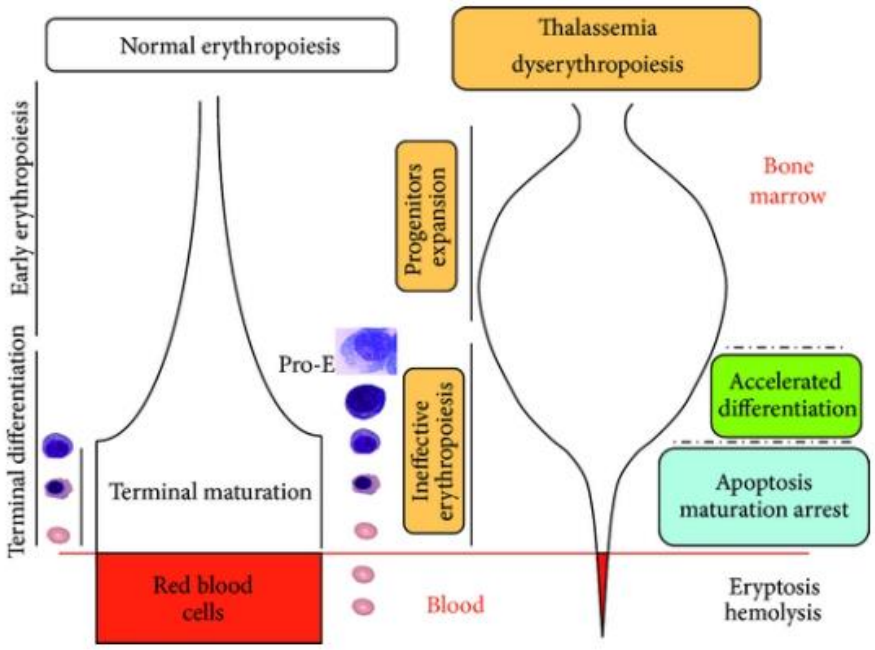


Figure 1. *Schematic representation of normal and ineffective erythropoiesis in β-thalassemia* (Ribeil et al., 2013). Erythropoiesis under normal circumstance, is the pathway producing mature RBCs from hematopoietic stem cells, with a regulated balance between the proliferation, differentiation and apoptosis of erythrocytes. Ineffective erythropoiesis in β-thalassemia is characterized by

expansion of early erythroid precursors and suboptimal production of mature erythrocytes

In parallel, α-globin chains isolate HSP70 in the cytoplasm, resulting in cleavage of GATA-1 by a protein known as caspase 3.This results in end-stage maturation arrest and apoptosis at the erythroblast stage (Arlet et al., 2016).

Additionally, as already mentioned, GDF11-ActRIIB-Smad2/3 dependent signaling is a key regulatory mechanism in proliferating erythroid precursors that controls their late-stage maturation under both steady-state and stress conditions. In thalassemic mice, expression of GDF11 is increased in splenic erythroblasts and inhibits erythroid maturation in mice (Suragani et al., 2014). Preclinical findings indicate that increased Smad2/3 phosphorylation was correlated with reduced GATA-1 protein levels, which suggests that phosphorylated Smad2/3 may negatively regulate terminal erythroid differentiation by decreasing GATA-1 (Martinez et al., 2015).

Translational studies investigating the inactivation of GDF11 in models of thalassemia, further highlight the important role of GDF in disease pathology, and the potential of GDF11 as a therapeutic target. GDF inactivation via a ligand trap in thalassemic mice decreases oxidative stress in erythroblasts and the amount of α-globin membrane precipitates in RBCs, resulting in increased terminal erythroid differentiation. GDF11 appears to block terminal erythroid maturation via an autocrine amplification loop involving reactive oxygen species (ROS) and α-globin precipitation, as well as promoting the accumulation of immature erythroblasts by inhibiting another pathway, the Fas-Fas ligand

(FasL) pathway. GDF11 inactivation via this pathway also corrected the abnormal ratio of immature/mature erythroblasts by inducing apoptosis of immature erythroblasts (Dussiot et al., 2014).

Clinically, β-thalassemia can be categorized in transfusion-dependent thalassemia (TD) and non–transfusion-dependent thalassemia (NTD) according to the severity of the phenotype. Aim of the current treatment approaches is to maintain hemoglobin levels through regular transfusions. Nevertheless, chronic transfusions lead to iron overload with multi-organ burden and therefor, iron chelation therapy is necessary but sometimes not enough. Splenectomy, is not preferred nowadays due to increased risk of thrombosis, while allogenic stem cell transplant is only addressed to young patients (18 years old) and promising gene therapy is only available in clinical trials (Capellini et al., 2017).

The National Registry for Haemoglobinopathies in Greece (NRHG), has recorded 4032 patients from 38 hemoglobinopathy units throughout Greece from January 2010 to December 2015. Among them, 2099 (52.06%) patients were diagnosed with thalassemia major (TM) and received regular transfusions, while 873 (21.65%) patients were sporadically or non-transfused patients with thalassemia intermedia or hemoglobinopathy “H” (TI or HH). The peak of patient distribution corresponds to the age group of 36–45 years among TM patients and 46–55 years among TI cases. From January 2010 to December 2015 among patients with thalassaemia, 28.1% of deaths were attributable to heart disease, whereas liver disorders [hepatocellular carcinoma (HCC) and liver failure] led to 23.4% of the total number of deaths (Voskaridou et al., 2019).

Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal hematopoietic disorders characterized by ineffective hematopoiesis, bone marrow failure, and morphologic dysplasia in hematopoietic cells of one or more cell lineages, and with a risk of transformation to acute myeloid leukemia (AML). Classification of MDS was last updated in 2016 by the International Prognostic Scoring System-Revised (IPSS-R) taking into consideration blood and bone marrow blast proportion, cytopenias, positive ring sideroblast, karyotype and molecular genetic findings (Fenaux et al. 2014).

Prognostic characteristics	Points						
	0	0.5	1	1.5	2	3	4
Cytogenetic risk category[a]	Very good		Good		Intermediate	Poor	Very poor
Blasts in bone marrow, %	≤2		>2%–5%		5%–10%	>10%	
Haemoglobin, g/dl	≥10		8–<10	<8			
Platelet count, $\times 10^9$/l	≥100	50–<100	<50				
Absolute neutrophil count, $\times 10^9$/l	≥0.8	<0.8					

IPSS-R risk group	Score	Median overall survival, years	Median time to 25% AML evolution, years
Very low	≤1.5	8.8	NR
Low	>1.5–3	5.3	9.4
Intermediate	>3–4.5	3.0	2.5
High	>4.5–6	1.6	1.7
Very high	> 6	0.8	0.7

[a]Very good: –Y and del(11q) as single abnormalities; good: normal, del(5q), del(12p) and del(20q) as single abnormalities, double abnormalities including del(5q); intermediate: del(7q), +8, +19, i(17q) and any other single abnormalities, any other double abnormalities; poor: −7 and inv(3)/t(3q)/del(3q) as single abnormalities, double abnormalities including −7/del(7q), complex (3 abnormalities); very poor; >3 abnormalities.

Figure 2. *Revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndromes* (Fenaux et al. 2014)

The IPSS-R revised version further stratifies patients into 5 risk groups. “Lower-risk” MDS are subdivided into Very Low, Low, and Intermediate up to 3.5 points, with different outcomes in terms of AML evolution and survival (Uwe Platzbecker, 2019).

Lower risk MDS, usually presented with anemia at diagnosis, are thought to have low risk of transformation to AML and prolonged survival. Almost 75% of IPSS lower risk MDS patients, especially those having ringed sideroblasts (MDS-RS) with SF3B1 mutation (90% frequency in MDS-RS) experience limited risk of progression to AML (Fenaux et al., 2019).

Anemia in lower risk MDS is managed symptomatically by red blood cell (RBD) transfusions or by Erythropoietin Stimulating Agents (ESAs), except for TD patients with isolated del5q, where lenalidomide is the only EU approved targeted treatment (EMA Revlimid®, SPC 2018). RBC transfusions however can only achieve an average Hemoglobin (Hb) level below 9 g/dL and are associated with fatigue, lower quality of life, infections, cardio vascular mortality and iron overload needing iron chelation (Fenaux et al., 2019).

As far as ESAs are concerned, in a phase 3 randomized, placebo-controlled study assessing the efficacy and safety in anemic patients with low-risk MDS, epoetin-α significantly improved anemia outcomes in low-risk MDS (Fenaux et al., 2018). Eprex® is now indicated in EU for the treatment of symptomatic anemia (Hb ≤10 g/dL) in adults with low- or intermediate-1-risk primary myelodysplastic syndromes (MDS) who have low serum erythropoietin (<200 mU/mL) (EMA Eprex® , SPC 2018). Similarly, the use of darbepoetin alfa, the glycosylated form of EPO, to treat anemia in patients with lower-risk myelodysplastic syndromes (MDS) was evaluated in a phase 3 trial and appeared to significantly reduce transfusions and increase rates of erythroid response (Platzbecker et al., 2017).

Still, about 1/3 patients are primary resistant to ESAs or lose response over time after 2 years of treatment (Kelaidi et al., 2013), whereas patients primary

resistant to ESAs, have a higher risk of progression to AML than those experiencing secondary failure, although this is not translated into a significant OS difference (Park et al., 2017). It is of note, that approximately 70% of the patient relapsing after initial response to ESAs, are not associated with progression to higher-risk MDS but simply to loss of sensitivity to ESAs (Park S., 2008).

That means, that there is an unmet need of efficiently treating anemia of lower risk MDS patients.

Ligand Traps of Activing Receptor II in Ineffective Erythropoiesis

It has been previously demonstrated that dysregulated signaling by activin receptors is involved in anemia from hematologic malignancies such as MDS, cancer-related osteolysis, and metastatic bone disease, as well as in carcinogenesis. Activin A is present in osteoblasts, osteoclasts and bone marrow cells, and is found to promote osteoclast development in vitro (Fields et al., 2013).

Sotatercept (ACE-011) is a chimeric protein consisting of an extracellular domain of ActRIIA and the Fc domain of human immunoglobulin G1 (IgG1) (Figure 3.). Early preclinical studies evaluated its murine analogue, RAP-011, as it was thought that targeting activin receptor signaling and potentially other TGF-β superfamily members, could result in therapeutic intervention in anemia

and osteoporosis-related bone loss (Raje and Vallet, 2010). In cynomolgus monkeys, RAP-011 increased bone volume by decreasing bone resorption and increasing bone formation, leading to enhanced mechanical strength and bone quality (Lotinin et al., 2012).

Unexpectedly, Sotatercept demonstrated notable erythroid responses when administered in healthy subjects aiming to increase bone density. In a single-dose, randomized, double-blind, and placebo-controlled study in postmenopausal women, ACE-011 managed to increase bone formation and decrease bone resorption but what was coincidental, was the escalated growth in the number of RBCs, reticulocytes and hematocrit & Hb. This observation was not reported as adverse events· on the contrary, authors identified that this could be beneficial to patients with ineffective erythropoiesis (Ruckle et al., 2009).

Luspatercept (ACE-536) is a fusion protein that consists of a modified human Activin IIB Receptor (ActRIIB) extracellular domain and the Fc domain of human immunoglobulin G1 (IgG1) and although they seem similar with Sotatercept, their potential to trap especially activing A, differs (Fenaux et al., 2019).

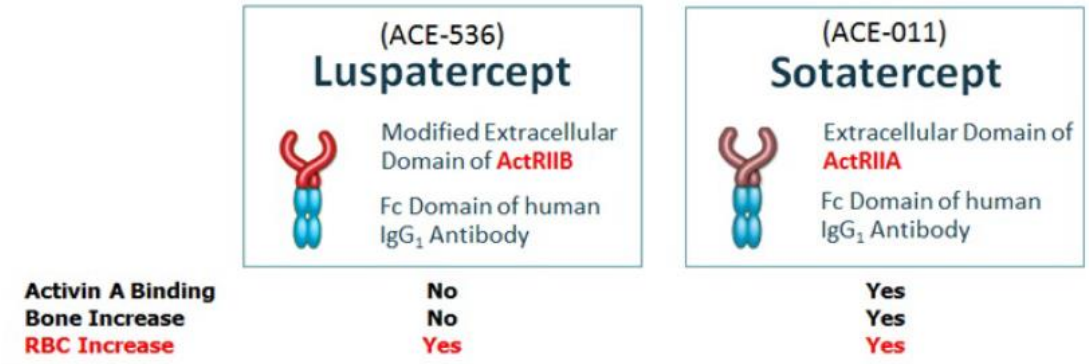


Figure 3. *Structure of Luspatercept and Sotatercept (Fenaux et al., 2019) showing the basic similarities and differences*

Luspatercept is known as a 'ligand trap' due to its ability to bind and 'trap' ligands such as GDF11. Luspatercept binds GDF11 and inhibits GDF11-ActRIIB-Smad2/3 dependent signaling, promoting late-stage maturation of erythroid precursors (Suragani et al., 2014).

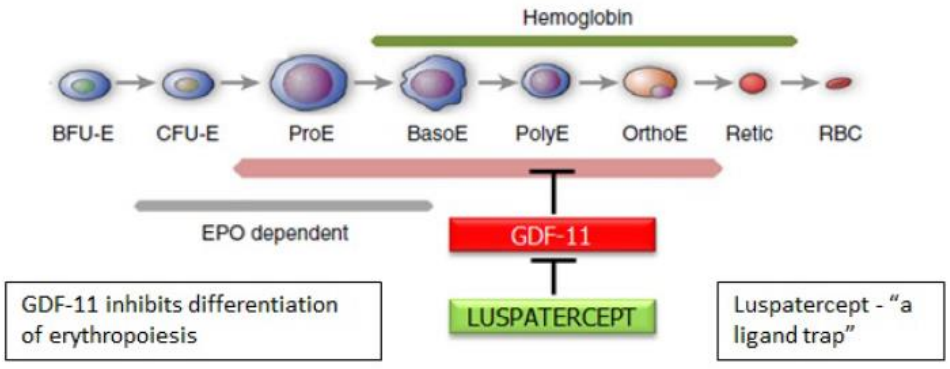


Figure 4. *Hypothetical mechanism of action of Luspatercept (Fenaux et al., 2019)*

Luspatercept and its mode of action in β-thalassemia & MDS

Preclinical studies in physiological conditions, in MDS & β-thalassemic mice

Suragani et al., investigated the role of ActRIIB ligand trap, Luspatercept (ACE-056) or its mouse version, RAP-536, in the regulation of late-stage erythropoiesis (Suragani et al., 2014).

RBC parameters in basal physiological conditions: administration of ACE-536 increased RBC parameters (RBC count, hemoglobin and hematocrit) in a dose-dependent manner in mice, rats and monkeys under basal conditions. Absolute reticulocyte numbers in peripheral blood increased during treatment and then declined to baseline levels coincident with rising RBC numbers.

Therapeutic effects in acute or chronic anemia: Compared to vehicle, RAP-536 treatment led to a more rapid recovery of RBC index in a rat model of acute anemia caused by blood loss, a rat model of chemotherapy-induced anemia and significantly ($P < 0.001$) increased RBC indices in a mouse model of chronic kidney disease. Treatment with ACE-536 did not affect kidney size or function.

Promotion of maturation: To identify specific erythropoietic precursors affected by ACE-536, numbers of early-stage progenitors was assessed using erythroid burst-forming unit (BFU-E) and erythroid colony-forming unit (CFU-E) tests, as well as of maturing erythroblasts, in both bone marrow and spleen of mice. ACE-536 treatment led to reduced BFU-Es and CFU-Es in bone marrow

compared to vehicle ($P < 0.05$) and was associated with a similar trend in the spleen ($P = 0.08$). These effects of ACE-536 on erythroid progenitors are in contrast with the well-characterized proliferative effects of EPO, as RAP-536 promoted the maturation of developing erythroblasts. The number of basophilic erythroblasts was decreased and the numbers of poly- and orthochromatophilic erythroblasts and reticulocytes was concurrently increased in bone marrow and spleen as compared with cell numbers in vehicle-treated mice.

Effects on erythropoiesis independent of EPO: To determine whether ACE-536–induced erythropoiesis is EPO dependent, mice were treated with ACE-536 or a neutralizing monoclonal antibody against EPO (EPO mAb). By day 4, ACE-536 treatment increased RBC count, hemoglobin and hematocrit ($P < 0.001$), whereas EPO mAb treatment decreased these parameters ($P < 0.001$). These results demonstrate that the stimulatory effect of ACE-536 on RBC parameters is largely EPO independent, but EPO was necessary for the continued supply of early-stage progenitors.

Synergistic effect: Co-treatment of mice with ACE-536 and EPO resulted in robust increases in RBC parameters at 72 h that were greater than the sum of the agents' separate effects. Flow cytometric analysis of splenic erythroblasts revealed that co-treatment with ACE-536 and EPO significantly increased maturation of basophilic erythroblasts compared to EPO alone. The greater-than-additive effect of combining ACE-536 and EPO treatment is probably due to the increased availability of early progenitors induced by EPO.

Signaling pathways responsible for the action of ACE-536: ACE-536 bound preferentially to GDF11, GDF8 and activin B (Smad2/3-activating ligands),

while additional analysis revealed that ACE-536 potently inhibited Smad2/3 signaling induced by GDF11 and GDF8.

Evolvement of GDF11-ActRIIB-Smad2/3 pathway in erythropoiesis: Erythroid precursors respond to exogenous GDF11 treatment with Smad2/3 activation participating in a cell-autonomous signaling, that regulates terminal erythroid differentiation. ACE-536 or RAP-536 is able to inhibit GDF11-induced signaling and negatively regulate late-stage erythroid differentiation. Together, these results support a model in which GDF11-Smad2/3 pathway in erythroid progenitors is upregulated upon acute EPO stimulation to promote erythroid expansion and then balanced accordingly, to allow early precursors undergo maturation in response to an acute demand for RBCs. RAP-536 showed ability to enhance the acute response to EPO or to accelerate recovery from acute blood loss and this is consistent with this model.

GDF11 role in MDS: On the basis of these findings, circulating GDF11 concentrations were investigated in a limited sample of humans with the heterogeneous disorder MDS and found these subjects to have elevated levels in comparison with age-matched control subjects, consistent with the erythroid hyperplasia and ineffective erythropoiesis that characterizes this syndrome. Inhibition of Smad2/3 signaling with RAP-536 in MDS mice, ameliorated anemia, erythroid hyperplasia and ineffective erythropoiesis regardless of disease severity.

To conclude, Suragani et al., through this initial evaluation, under both steady-state and stress conditions, demonstrated that Lupsatercept, a modified ActRIIB ligand trap, revealed GDF11-ActRIIB-Smad2/3–dependent signaling

as a key regulatory mechanism in proliferating erythroid precursors that controls late-stage maturation.

Those findings, were confirmed by Martinez et al. (Martinez et al., 2015), investigating murine ACE-536 (RAP-536) in β-thalassemic erythroblasts. Results depicted significant upregulation of target genes of multiple transcriptional regulators including GATA-1, a master transcriptional regulator of terminal erythroid differentiation. Additionally, preliminary results in differentiating mouse erythroleukemic (MEL) cells showed increased Smad2/3 phosphorylation that is correlated with reduced GATA-1 protein levels suggesting that pSmad2/3 may negatively regulate terminal erythroid differentiation by decreasing GATA-1 availability. Those data provided a potential mechanistic role for Luspatercept treatment in β-thalassemia, by transcriptionally upregulating genes that promote erythroid differentiation and processing of unpaired α-globins. By inhibiting Smad2/3 signaling, Luspatercept relieves the block of terminal erythroid maturation and decreases ineffective erythropoiesis in diseases such as β-thalassemia and MDS.

In parallel, effects of RAP-536 were also evaluated by Suragani et al. (Suragani et al., 2014), in an $Hbb^{th1/th1}$ mouse model of β-thalassemia intermedia, which is characterized by abortive maturation of erythroid precursors, ineffective erythropoiesis, and a hypochromic, microcytic anemia. Compared with vehicle, RAP-536 treatment significantly increased RBC number by 29% (P<. 001), hemoglobin concentration by 16% (P<. 001), and hematocrit by 19% (P<. 001) accompanied by a 33% reduction in reticulocytes (P<.01).

During ineffective erythropoiesis, elevated Smad2/3 activation occurs in erythroid precursors of β -thalassemic mice compared with wild-type mice, and

RAP-536 treatment can inhibit this activation and promote differentiation of terminal erythroid precursors in both $Hbb^{th1/th1}$ mice and wild-type mice.

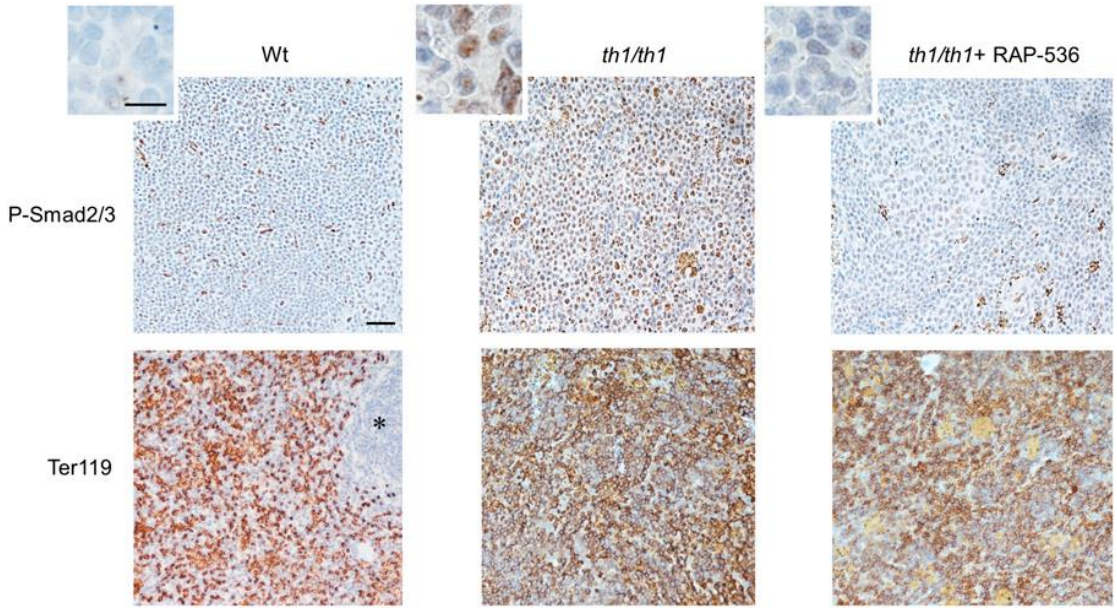


Figure 5. *RAP-536 inhibits Smad2/3 activation in* $Hbb^{th1/th1}$ *mice* (Suragani et al., 2014). (Upper) Representative phospho-Smad2/3 immunostaining (brown) in hematoxylincounterstained spleen sections from a wild-type mouse (Wt), a vehicle-treated Hbbth1/th1 mouse (th1/th1), and an Hbbth1/th1 mouse treated for 12 hours with a single dose of RAP-536 (30 mg/kg, intraperitoneally, n 5 3-4 mice per group). (Insets) Higher magnification. (Lower) Representative Ter119 immunostaining (brown) denoting red pulp in adjacent hematoxylincounterstained spleen sections. *White pulp. Images were obtained with a 203 objective or 1003 oil immersion objective. Bar 5 100 or 15 mm (insets).

Another aspect of anemia, the principal feedback mechanism of tissue hypoxia elevating expression of EPO to increase RBC production, was regulated

following RAP-536 treatment, as it reduced serum EPO levels in β -thalassemic mice by ~60% and splenomegaly by 36% compared with vehicle, likely due to alleviation of hypoxia by increased numbers of functional RBCs.

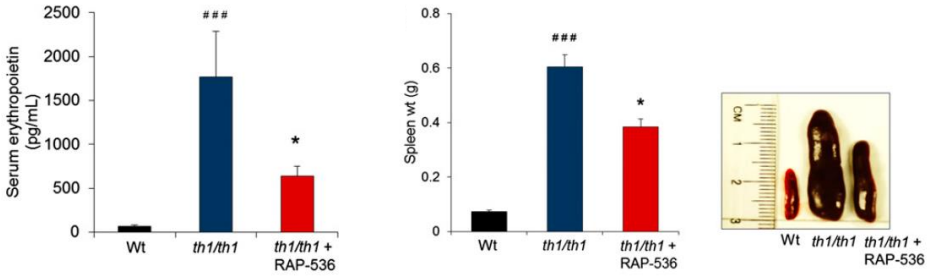


Figure 6. *RAP-536 promotes erythroid differentiation in Hbb$^{th1/th1}$ mice,* Left: Serum EPO concentrations (n=6-11 mice per group). Right: Spleen weight (n=13-17 mice per group) and Spleen size shown in representative images obtained in a study separate from that shown at left but using the same dosing schedule in 10- to 12-month-old mice.

RAP-536 treatment in β-thalassemic mice reduces membrane aggregates of a-globin and intracellular levels of ROS during erythroid differentiation, leading to peripheral red cells with reduced levels of basal oxidative stress, improved resistance to stress, improved morphology, reduced hemolysis, and increased cell survival. Finally, RAP-536 corrected bone pathology and reduced abnormal iron homeostasis, two major complications with serious consequences in β-thalassemia (Suragani et al., 2014).

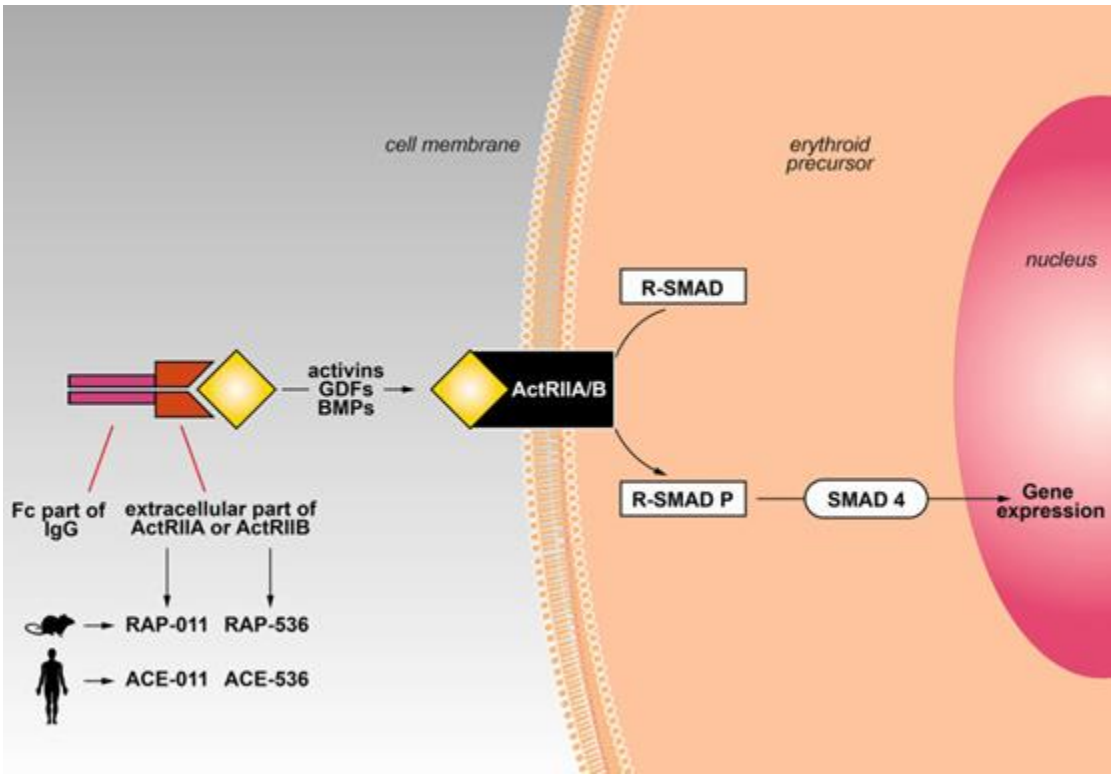


Figure 7. *TGF-b signaling pathway and its ligand traps* (Makis et al., 2017). TGF-b ligands, activins, GDFs and BMPs bind to a type II receptor (ActRIIA, ActRIIB), which phosphorylates receptor-regulated Sma and Mad related proteins (R-SMAD). R-SMAD/coSMAD complexes gather in the nucleus where enable transcription and participate in the regulation of target gene expression. Sotatercept and Luspatercept are modified receptors of activin (activin receptor-II trap ligands) that have been developed from the fusion of the extracellular part of activing receptor (ActRIIA or ActRIIB) and the Fc part of IgG immunoglobulin. They function as selective trap ligand that inhibits the activation of the SMAD pathway.

Hence, Luspatercept provides a potential treatment option for anemia associated with haematological disorders, characterized by ineffective erythropoiesis regardless high EPO levels, accumulation of immature erythroid precursors and decreased maturation of these cells, such as MDS and β-thalassemia.

Intriguingly, development of Luspatercept begun, aiming to evaluate its potential benefits and risks, with phase 1, 2 and 3 clinical trials in β-thalassemia and MDS.

Phase 1 trials in healthy volunteers (Attie at al., 2014)

The first-in-human, phase 1, dose finding clinical trial was randomized, double-blind, placebo-controlled and was conducted between September 2011 and September 2012 at a single clinical research center to assess safety, tolerability, pharmacokinetics, and pharmacodynamic of ascending Luspatercept doses in healthy volunteers (ClinicalTrials.gov NCT01432717). Thirty-two healthy post-menopausal women were randomized in sequential cohorts of eight subjects (six active and two placebo control subjects per cohort) each to receive up to two doses of either ACE-536 (0.0625, 0.125, and 0.25 mg/kg) or placebo (3:1 randomization) given subcutaneously every 2 weeks. Postmenopausal women were chosen due to the potential risk of ACE-536 binding to activin(s) and inhibiting their activity with subsequent potential effects on fertility.

Among the inclusion criteria were age between 45–75 years, body mass index (BMI) of 20–32 kg/m^2, platelet count ≥100,000/μL and hemoglobin levels ≥11.0 g/dL and ≤14.5 g/dL.

Subjects who donated or lost ≥500 mL of whole blood within 2 months prior to day 1 were excluded. Moreover, no prior erythropoietin-stimulating agent (ESA) treatment, systemic glucocorticoid therapy, or anticoagulant therapy within 6 months prior to day 1 was aloud.

Safety and pharmacodynamics (PD) analyses were assessed at baseline and regularly throughout the study, until day 57, with follow-up visits at 2 and 10 week follow-up period.

Results

Thirty-two postmenopausal women were enrolled, including 24 subjects randomized to active treatment and eight randomized to placebo.

Mean baseline age was 59.4 years, and hemoglobin was 13.2 g/dL.

Safety and tolerability

Luspatercept was well tolerated at dose levels up to 0.25 mg/kg over the 1-month treatment period and no serious or severe adverse events were reported.

The incidence of AEs was comparable across treatment groups, including placebo. The majority of AEs (59 of 71 events, 83%) were mild in severity and resolved without sequelae.

The most frequently reported AE was headache (four active-treated patients, three placebo-treated, all considered not related).

The majority of AEs were considered unrelated to the study treatment. Among the 24 subjects treated with ACE-536, seven (29%) experienced at least one AE considered at least possibly related to the study drug. Those included injection-site hemorrhage (three subjects), injection-site macule (two subjects), and dry skin, macule, hyperesthesia, muscle spasms, myalgia, generalized pruritus, and papular rash (one subject each).

Pharmacokinetics

Mean concentrations (Area Under the Curve ($AUC_{0–14d}$) and Maximum Concentration (C_{max})) of Luspatercept increased proportionally after the first dose; mean T_{max} (time to reach the maximum concentration) was 7-10 days and mean $t_{1/2}$ (half-life: time taken for T_{max} to drop in half) was 15-16 days, and they were not dose proportional (Figure 8).

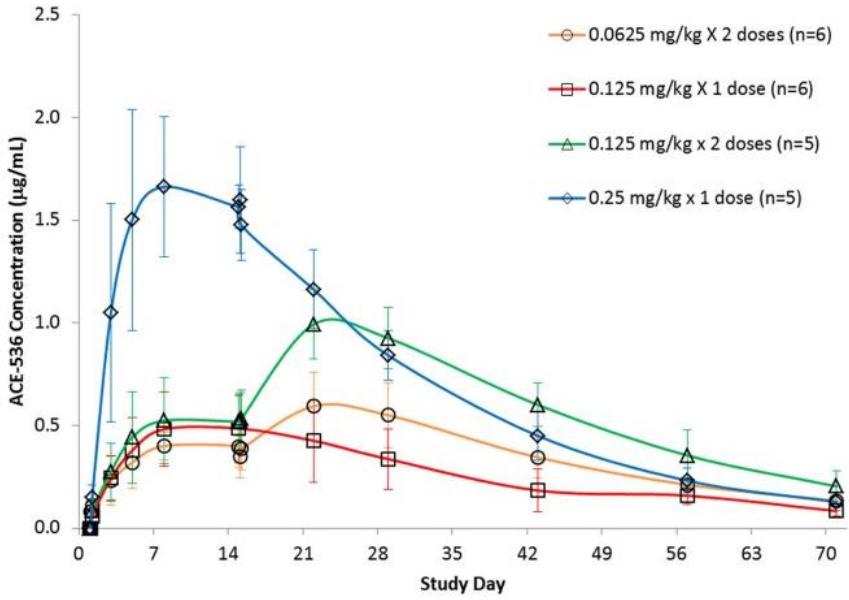


Figure 8*. Pharmacokinetic profile of ACE-536 concentrations* following one or two subcutaneous doses in healthy postmenopausal women treated with ACE-536 (mean ± SD). PK: pharmacokinetic, SC: subcutaneous.

The mean change of hemoglobin concentration increased from baseline 7 days after initiation of treatment, was dose-dependent and maintained for several weeks following treatment (Figure 9).

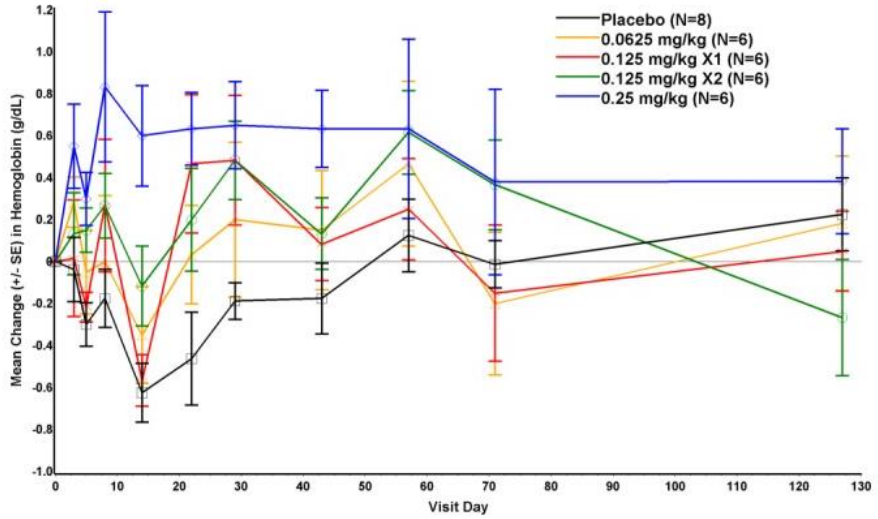


Figure 9. *Change from baseline in hemoglobin concentrations* (g/dL, mean ± SE), baseline hemoglobin was last non-missing value prior to dosing. Hb: hemoglobin, SE: standard error

The proportion of subjects with hemoglobin increase from baseline ≥1.0 g/dL increased in a dose-dependent manner to 83.3% of subjects in the highest dose group, 0.25mg/kg (Figure 10).

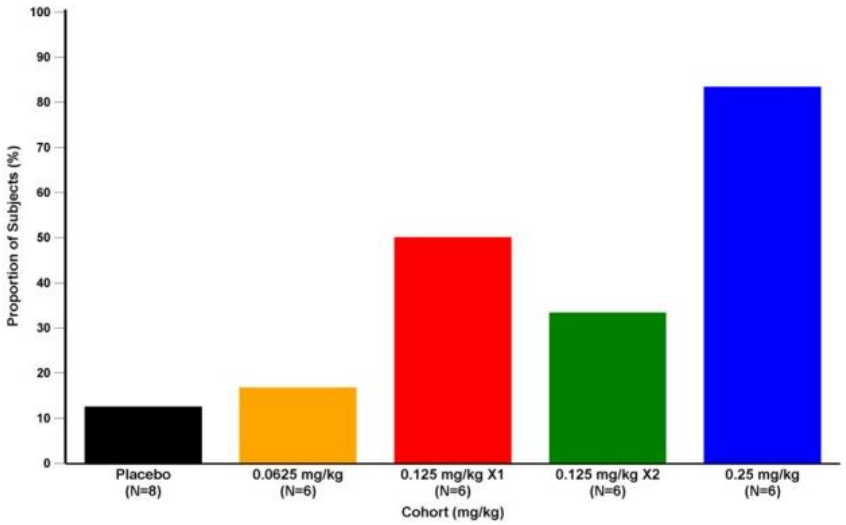


Figure 10. *Proportion of subjects (%) with hemoglobin increase ≥1.0 g/dL* following dosing with ACE-536 or placebo.

Luspatercept was well tolerated and resulted in sustained increases in hemoglobin levels in healthy postmenopausal women.

Nevertheless, Luspatercept given subcutaneously every 2 weeks showed long-acting pattern of exposure and although C_{max} & $AUC_{0–14d}$ were generally dose-proportional, T_{max} & $t_{1/2}$ were not.

These results therefore suggest that a less frequent SC dosing, for example, every 3 weeks may be appropriate for future studies. With this in mind, phase 2 clinical trials were designed accordingly.

Clinical trials of Luspatercept in β-thalassemia

Phase 2 clinical trials (Piga et al., 2018)

The aim of this step was to determine a tolerable and active dose level and schedule of Luspatercept in adult patients with transfusion-dependent or non-transfusion-dependent β-thalassemia. Phase 2 single-arm, multicenter, dose-finding base and open-label extension studies (ClinicalTrials.gov NCT01749540 & NCT02268409), were conducted to assess preliminary efficacy and safety of Luspatercept in 64 adult patients with transfusion-dependent (TD) or non-transfusion dependent (NTD) β-thalassemia.

The primary endpoint was erythroid response defined as a hemoglobin increase from baseline of ≥1.5 g/dL for ≥2 weeks (in the absence of RBC transfusions) for non-transfusion-dependent patients, and as a reduction in RBC transfusion burden over a 12-week interval of ≥20% as compared with pre-treatment for transfusion-dependent patients. Secondary endpoints included erythroid response, time to and duration of, as well as safety, health-related quality of life and reduction in liver iron concentration (LIC) in both TD and NTD patients.

Methods

Among inclusion criteria was age ≥ 18 years and in the dose-escalation stage prior splenectomy or spleen size <18 cm in the longest diameter, were permitted. Patients with folate deficiency, symptomatic splenomegaly, and history of thromboembolic events were excluded. Patients with any clinically significant pulmonary, cardiovascular, endocrine, neurologic, hepatic,

gastrointestinal, infectious, immunologic, or genitourinary disease were also excluded unless adequately controlled.

Eligible patients were classified as either TD patients, defined as ≥4 RBC units transfused in the 8 weeks prior to first dose confirmed over 6 months, or NTD patients, defined as <4 RBC units transfused in the 8 weeks prior to first dose with baseline Hb <10.0 g/dL.

Study design.

In the 3-month dose finding study, patients were treated with 6 escalating dose levels from 0.2 to 1.25 mg/kg of Luspatercept SC every 3 weeks (n=35). In the expansion cohort doses from 0.8 to 1.25 mg/kg were administered (n=29). The base study is completed and patients who completed the base study were enrolled into an open-label, ongoing, 24-month extension study for up to 5 years to determine long-term efficacy and gather long-term safety data.

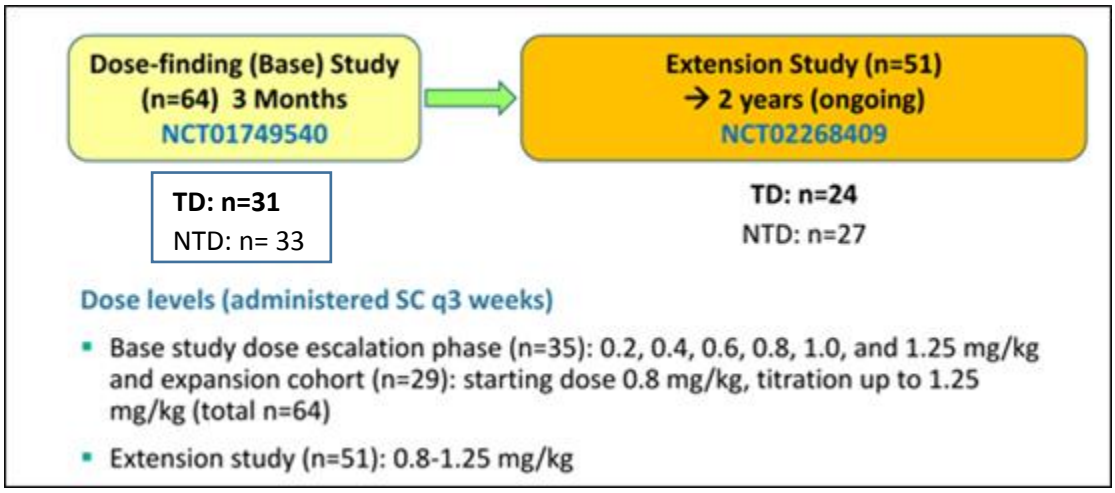


Figure 11. *Study design describing the initial dose finding and the extension stages*

Results

The study enrolled patients between 11 February 2013 and 6 July 2015 at 8 sites in Italy and Greece. Baseline characteristics for the 63 patients treated with higher dose levels of Luspatercept (≥0.6 mg/kg) were median age 38.5 years (range 20-62) and 52% male (n= 33), while 67% (n= 43) had prior splenectomy. At baseline, median transfusion burden was 8 RBC units/12 weeks (range 4–18) for the TD patients and mean liver iron concentration (LIC) was 5.0 ± 5.3 mg/g dry wt. NTD patients had median Hemoglobin 8.5 g/dL (range 6.5–9.8) and mean (SD) liver iron concentration 5.4 ± 3.8 mg/g dry weight (3.6).

Primary endpoint was achieved as protocol-defined erythroid response occurred in 71% of NTD patients and 81% of TD patients receiving higher doses of Luspatercept.

Although the study was single-group, open-label design without a control group for comparison, the lower-dose groups were considered subtherapeutic and pharmacokinetic analysis confirmed that 1.0 mg/kg Luspatercept is an appropriate starting dose for further studies in patients with β-thalassemia, with titration up to 1.25 mg/kg.

Efficacy in Non-Transfusion-Dependent Patients

Primary endpoint

A total of 33 NTD pts enrolled in the base study. Of 31 of them, who were treated with Luspatercept dose levels ≥0.6 mg/kg, 18 (58%) achieved a mean hemoglobin increase from baseline of ≥1.5 g/dL over 14 consecutive days (95% CI, 39.1-75.5).

22 (71%) and 14 (45%) of 31 NTD patients treated at dose levels of 0.6-1.25 mg/kg, achieved increases in mean Haemoglobin level ≥1.0 g/dL and ≥1.5 g/dL respectively over a 12-week period on treatment versus baseline.

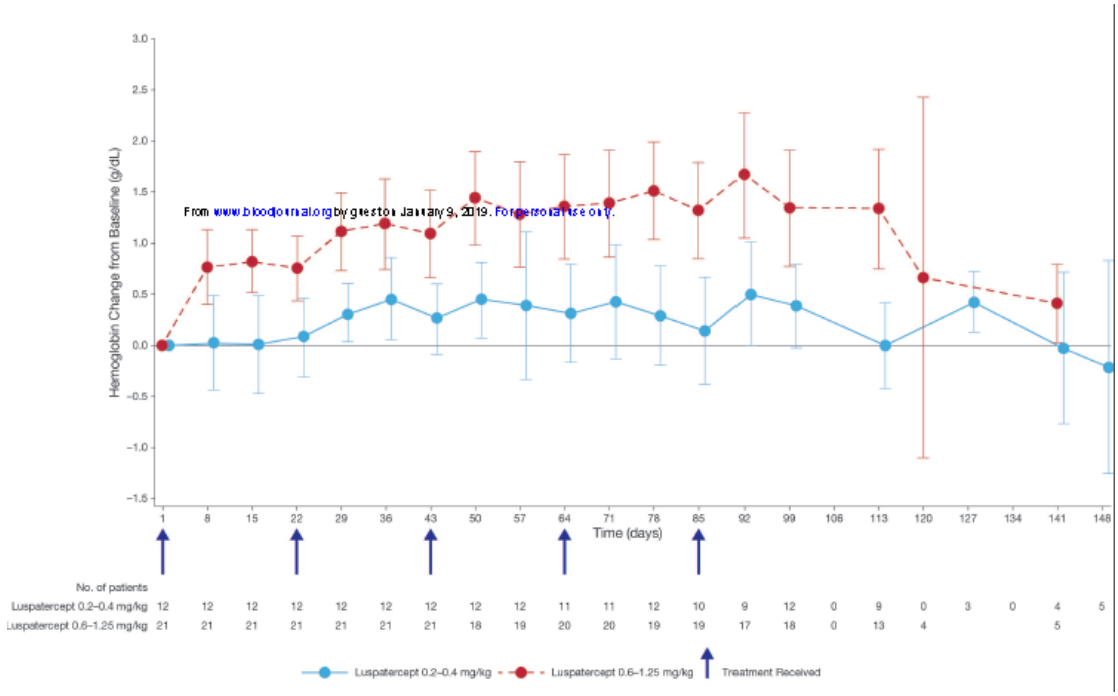


Figure 12. *Mean hemoglobin changes from baseline* for those treated with Luspatercept at dose levels of 0.2-0.4 mg/kg and 0.6-1.25 mg/kg in the initial stage of the study

Median time to hemoglobin response among NTD patients in the initial stage of the study was 8 days (range, 7-30).The increase in Hemoglobin levels was sustained throughout the treatment with Luspatercept.

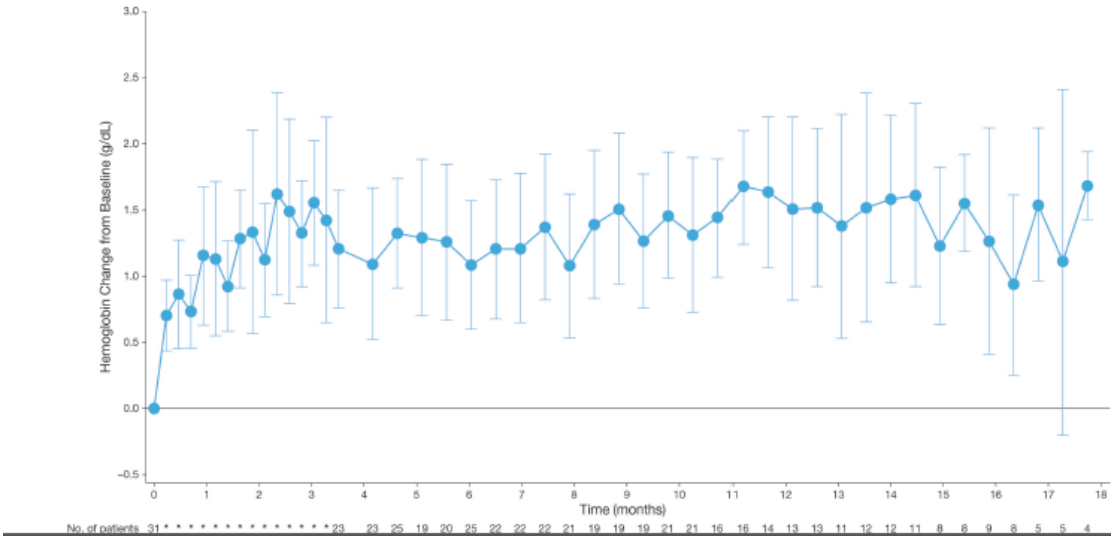


Figure 13. *Mean change in hemoglobin over the study duration*, including the extension stage up to 18 months total duration, for all 31 NTD patients treated with dose levels 0.6-1.25 mg/kg

Efficacy in Transfusion-Dependent (TD) Patients

Primary endpoint

A total of 32 TD pts were treated with Luspatercept at ≥0.6 mg/kg during the base and extension studies. 26 out of 32 (81%) TD pts achieved a ≥ 20% reduction in RBC units transfused over any 12-week period pre-treatment (rolling) compared with the 12 weeks prior to baseline (95% CI, 63.6-92.8).

23 (72%) patients achieved RBC transfusion burden reduction of ≥33% (95% CI, 53.3-86.3) and 20 (63%) patients of ≥ 50% (95% CI, 43.7-78.9).

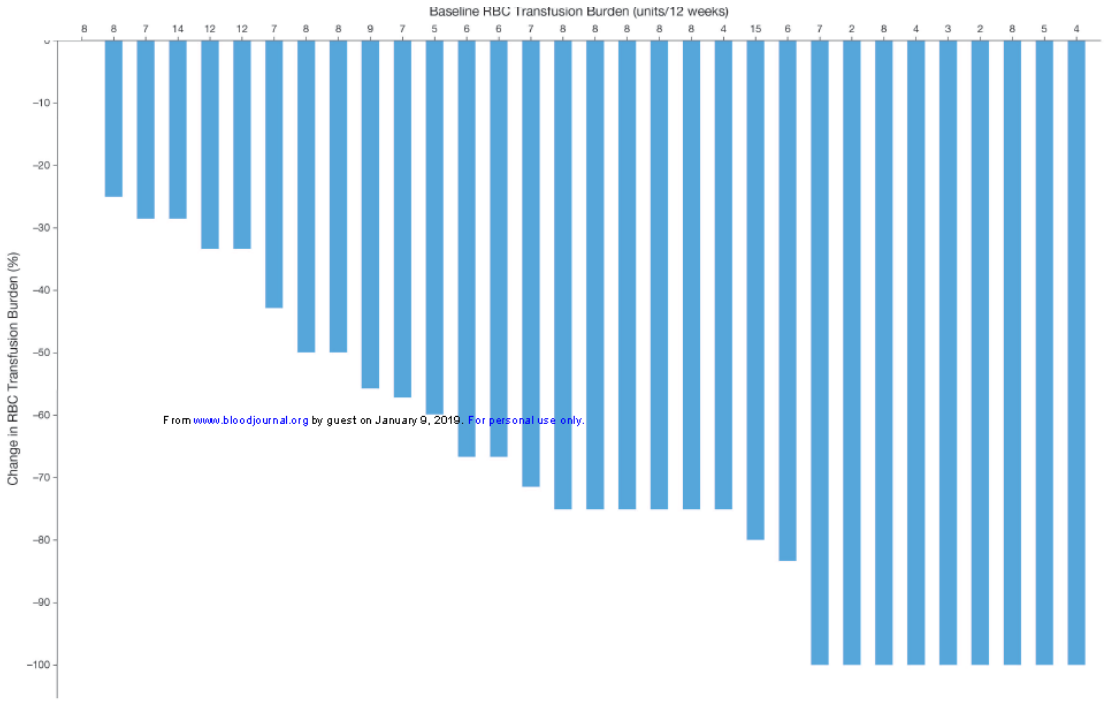


Figure 14. *Reduction in transfusion burden of ≥2 RBC units* versus baseline for all TD patients who received 0.6-1.25 mg/kg Luspatercept

Moreover, the mean transfusion burden over the best recorded 12-week responses on treatment was 3.0 RBC units (SD ± 3.76), compared with 7.6 units (SD ± 3.56) at baseline.

Liver iron concentration

Luspatercept treatment was associated with clinically significant decreases in LIC, particularly in patients who had baseline LIC ≥3 mg/g dry weight and who were receiving concomitant iron chelation therapy, although it is unclear whether decreases in LIC may be due in part to the decrease of transfusion burden and/or increase in iron utilization related to Luspatercept treatment.

Of 15 patients with NTD beta-thalassemia with baseline LIC ≥3 mg/g dry weight who were treated for ≥4 months, 5 (33%) achieved a decrease in LIC ≥2 mg/g dry weight (95% CI, 11.8-61.6). The mean LIC for NTD patients at the end of the initial stage of treatment was −0.36 mg/g dry weight (SD ± 1.59) compared with 5.38 mg/g (SD ± 3.79) at baseline.

Of 9 patients with TD with baseline LIC ≥3 mg/g dry weight who were treated for ≥4 months, 5 (56%) achieved a decrease in LIC ≥2 mg/g dry weight (95% CI, 21.2-86.3). The mean LIC for TD patients at the end of initial stage of treatment was −0.27 mg/g dry weight (SD ± 1.64) compared with 5.03 mg/g (SD ± 5.32) at baseline.

Exploratory endpoints

Leg ulcers

Six patients suffered from 1 to 6 leg ulcers at baseline. Of the 6 patients with leg ulcers, which were often long-standing and resistant to other therapies, all had complete healing of 1 or more leg ulcers, with a total of 9 ulcers fully healed, 2 partially healed, and 3 not improved. The median time to complete healing was 10 weeks (range, 6-33).

Bone mineral density

Patients receiving Luspatercept were assessed for changes in bone marrow density (BMD), as skeletal deformities and decreased BMD can be observed in β-thalassemia due to ineffective erythropoiesis.

However, many patients had normal baseline BMD and in addition with the small numbers of patients, analysis and interpretation of the effects of Luspatercept are rather limited. After 24 weeks of treatment at dose levels ≥0.6 mg/kg, there were increases of the mean percentage change in BMD (+5.3 ± 15.8% for total hip (P = 0.23; n = 14) and +3.1 ± 6.6% for lumbar spine (P = 0.07; n = 17)) but they were not found to be statistically significant.

Quality-of-life

Quality-of-life changes were measured using the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) which is a validated 13-question patient-reported outcome (PRO) questionnaire used to assess anemia-related symptoms such as fatigue and weakness. Improvements in FACIT-F score in

NTD patients was significantly correlated with the mean 12-week change in hemoglobin (r = 0.64; P = 0.002; n = 21). Of the NTD patients with a baseline FACIT-F deficit (<44 points) compared with normative data, 7 of 9 (78%) improved by ≥3 points at 24 weeks. The majority of these patients (86%) also had an improvement in mean hemoglobin of ≥1.0 g/dL over a 12-week period. No significant changes were observed in TD patients.

Safety Summary

Luspatercept was generally safe and well tolerated in all 64 patients treated at all dose levels with the majority of AEs being grade 1 or 2, mainly during the first 8 weeks, with a clear trend to decrease. The most frequent related AEs (≥10%) were bone pain, headache, myalgia, arthralgia, musculoskeletal pain, back pain, and injection site pain. Bone pain, was more frequently observed in TD patients and may be due in part to the withdrawal of transfusions in response to treatment.

Grade 3 AEs considered treatment-related were uncommon: 6 reported in 5 (8%) patients: bone pain (n=3), asthenia (n=2), and headache (n=1).

No treatment- related grade 4 AEs or serious AEs or treatment-related deaths were reported. Out of 64, only 4 (6%) patients discontinued treatment due to AEs. AEs were more frequent during the first 8 weeks, with a trend to decrease over time.

Pharmacokinetics (PK) of Luspatercept

In the meantime, a secondary analysis of first data collected from these two phase 2 studies (base and extension) aimed to characterize the pharmacokinetics (PK) of Luspatercept, and to explore the exposure-response relationship for efficacy and safety in pts with β-thalassemia, thereby informing selection of the starting dose for phase 3 studies of Luspatercept in β-thalassemia (Chen et al., 2016).

The main exposure endpoint was Area Under the Luspatercept serum concentration-time Curve (AUC). Clinical endpoints included Hb increase, transfusion reduction, and drug-related adverse events (AEs) in weeks 1-15. Responders were defined as NTD pts with a mean Hb increase ≥ 1.0 g/dL/15 weeks, and TD pts with a transfusion reduction ≥ 33%/15 weeks.

Results

As of July 20, 2016, preliminary data were available for 89 pts, including 49 NTD pts (baseline Hb 6.5-9.8 g/dL) and 40 TD pts (baseline transfusion burden 4-18 units/12 weeks). Median age was 37 years (range 20-62), and 47% were female.

Luspatercept PK was adequately described by a 1-compartment PK model with linear absorption and elimination. The half-life of Luspatercept in serum was~10 days across doses. Body weight positively correlated with Luspatercept clearance and its volume of distribution. TD pts had~23% lower volume of distribution than NTD pts.

In NTD pts, higher Luspatercept serum AUC correlated with a greater increase in Hb levels ($P < 0.01$). The median AUC was similar for NTD responders and TD responders (~100d·μg/mL vs ~120d·μg/mL). As AUC increased, the frequency of responders increased for NTD pts, TD pts, and the 2 groups combined. Population PK simulation predicted that a starting dose of 1.0 mg/kg Luspatercept would result in > 90% of NTD pts and ~50% of TD pts achieving the median AUC concentration observed in responders. By contrast, < 50% pts (NTD or TD) were predicted to achieve the target AUC at the 0.8 mg/kg dose.

Grade 2-3 drug-related AEs (all types) were more frequent with higher AUC ($P < 0.05$). In both NTD and TD pts, bone and muscle pain were the most common AEs; however, these AEs did not have a significant relationship with Luspatercept serum exposure.

Conclusions: Higher Luspatercept serum exposure correlated with greater erythroid hematopoietic response as well as more frequent grade 2-3 related AEs. Exposure-response modeling and PK simulation support a phase 3 starting dose of 1.0 mg/kg and intra-patient dose escalation up to 1.25 mg/kg according to patients' response.

Ongoing Phase 2 clinical trials

BEYOND trial is a Phase 2, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of Luspatercept (ACE-536) versus placebo in adults with non-transfusion dependent β-thalassemia. The study is divided into the Screening Period, Double-blind Treatment Period (DBTP) and Post-Treatment Follow-up Period (PTFP) (ClinicalTrials.gov NCT03342404).

It is planned to randomize approximately 150 subjects at a 2:1 ratio of Luspatercept versus placebo.

The primary objective is to evaluate the effect of Luspatercept versus placebo on anemia, as measured by mean hemoglobin concentration in the absence of transfusions over a continuous 12-week interval, from Week 13 to Week 24, compared to baseline.

Greece is participating in BEYOND with 3 sites: Children's Hospital ‘Agia Sophia’, Laiko General Hospital of Athens & Alexandra General Hospital.

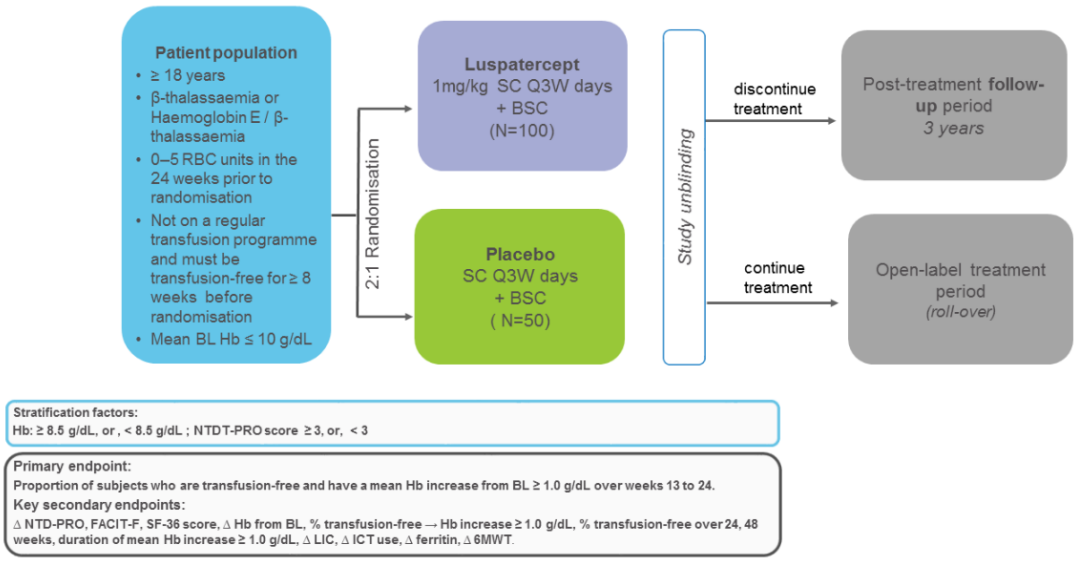


Figure 15. *Study design of the Phase 2 BEYOND trial (*ClinicalTrials.gov NCT03342404*).* 6MWT*: six-minute-walk test,* BL*: baseline,* BSC*: best* supportive care*, discon., discontinue, FACIT-F: Functional Assessment of Chronic Illness Therapy-Fatigue; ICT: iron chelation therapy, LIC: liver iron concentration, PRO: patient-reported outcome, SF 36: 36 item short form survey.*

Phase 3 clinical trials

Based on the Phase 2 data, a Phase 3 trial, named BELIEVE, was designed (ClinicalTrials.gov NCT02604433).

BELIEVE is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of Luspatercept (ACE-536) plus Best Supportive Care (BSC) versus placebo plus BSC in adults who require regular red blood cell transfusion due to β-thalassemia.

Greece is participating in the study with 5 sites (Children's Hospital ‘Agia Sophia’, Laiko General Hospital of Athens, General Hospital of Athens "G. Gennimatas", Ippokrateio General Hospital of Thessaloniki & General University Hospital of Patras).

Results of the BELIEVE trial at the unblinding status after 48 weeks of treatment, were orally presented in 60th American Society of Hematology (ASH) annual meeting by M. D. Cappellini (Cappellini et al., ASH 2018).

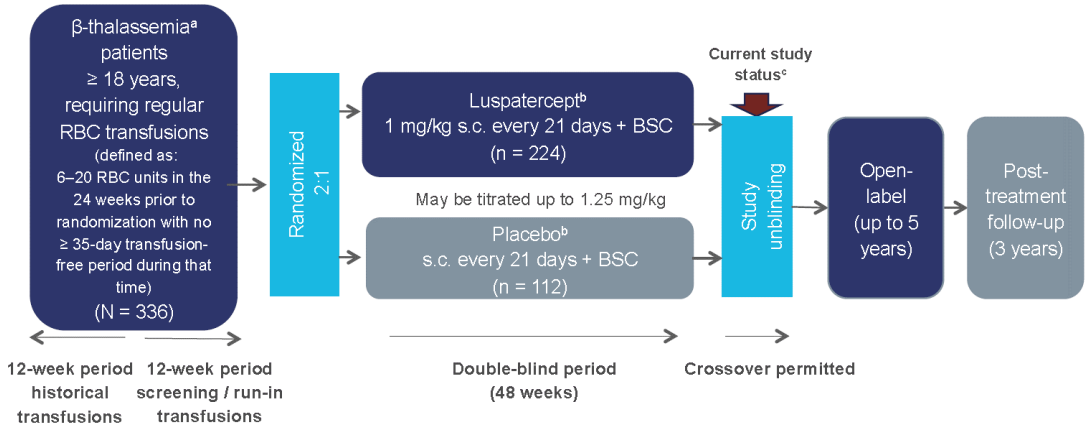


Figure 16. *Study design of the Phase 3 BELIEVE trial*; [a] β-thalassemia or hemoglobin E / β-thalassemia (β-thalassemia with mutation and / or multiplication of α-globin was allowed. [b] RBC transfusions and iron chelation therapy to maintain each patient's baseline hemoglobin level. [c] The trial is fully enrolled and patients continue to receive treatment or follow-up. BSC: best supportive care, RBC: red blood cell, s.c.: subcutaneously.

Methods

Inclusion criteria included transfusion dependent adult patients with β-thalassemia or hemoglobin E/β-thalassemia; transfusion dependency was defined as requirements of 6–20 RBC units in the 24 weeks prior to randomization and additionally, no transfusion-free period ≥ 35 days during that time.

Patients were randomized 2:1 to receive either Luspatercept, at a starting dose level of 1.0 mg/kg with titration up to 1.25 mg/kg, or placebo, subcutaneously every 3 weeks for ≥ 48 weeks. Patients in both treatment arms were under Best

Supportive Care and continued to receive RBC transfusions and iron chelation therapy to maintain the same baseline Hb level.

The primary endpoint was a ≥ 33% reduction in RBC transfusion burden of ≥ 2 units during weeks 13 to 24.

Key secondary endpoints included ≥ 33% reduction in transfusion burden in later time period, at weeks 37 to 48, as well as ≥ 50% reduction in transfusion burden at weeks 13 to 24, and at later time period, at weeks 37 to 48, and mean change in RBC transfusion burden at weeks 13 to 24.

An additional end point of maintaining of ≥ 33% reduction in RBC transfusion burden over any consecutive 12 weeks on study was also evaluated.

Results

†Between July 2016 and June 2017, 336 patients were randomized at 65 sites in 15 countries and 332 were treated. Median age was 30 years (range 18–66) and 58% of patients were female. β^0/β^0 genotype (classification according to the HbVar database, where both β-chain genes are completely deleted or inactive) was observed in 68 of 224 (30.4%) and 35 of 112 (31.3%) patients in the Luspatercept and placebo arms, respectively, identifying a rather difficult to treat population. Median hemoglobin, defined as the mean of all documented pre-transfusion hemoglobin values during the 24 weeks prior to first dose for each patient, was above 9 g/dL and patients received a median of 6 RBC units in the 12 weeks prior to treatment, whereas 58% of patients in each arm had prior splenectomy. LIC was 6.14 mg/g d/w (range 0.8-125.0) for patients in the Luspatercept arm and 5.05 mg/g (range 0.2-53.2) in the placebo arm, while

more patients in the Luspatercept arm had median LIC >7mg/g d/w (46.0% versus 40.2%).

Characteristic	Luspatercept (n = 224)	Placebo (n = 112)
Age, median (range), years	30 (18–66)	30 (18–59)
Female, n (%)	132 (58.9)	63 (56.3)
β^0/β^0 n (%)	68 (30.4)	35 (31.3)
Hemoglobin (24 week),[a] median (range), g/dL	9.31 (4.5–11.4)	9.15 (5.8–11.7)
RBC transfusion burden, median (range), units/12 weeks	6.12 (3–14)	6.27 (3–12)
RBC transfusion burden, median (range), units/24 weeks	14 (6–24)	15 (6–26)
Splenectomy, n (%)	129 (57.6)	65 (58.0)
Serum ferritin, median (range), µg/L	1,447 (88–6,400)	1,304 (136–6,400)
LIC, median (range), mg/g dry weight	6.14 (0.8–125.0)	5.05 (0.2–53.2)
> 7 mg/g dry weight, n (%)	103 (46.0)	45 (40.2)
Myocardial iron by T2*, median (Q1–Q3), ms	34.7 (27.4–40.3)	36.3 (29.0–42.0)

[a] Defined as the mean of all documented pre-transfusion hemoglobin values during the 24 weeks prior to first dose for each patient

Figure 17. *Demographics and Baseline Disease Characteristics*

The primary endpoint was met by a significantly greater proportion of patients in the Luspatercept arm: 48 of 224 (21.4%) versus 5 of 112 (4.5%) patients receiving placebo (odds ratio 5.79, $P < 0.0001$) achieved a ≥ 33% reduction in RBC transfusion burden of ≥ 2 units during weeks 13 to 24.

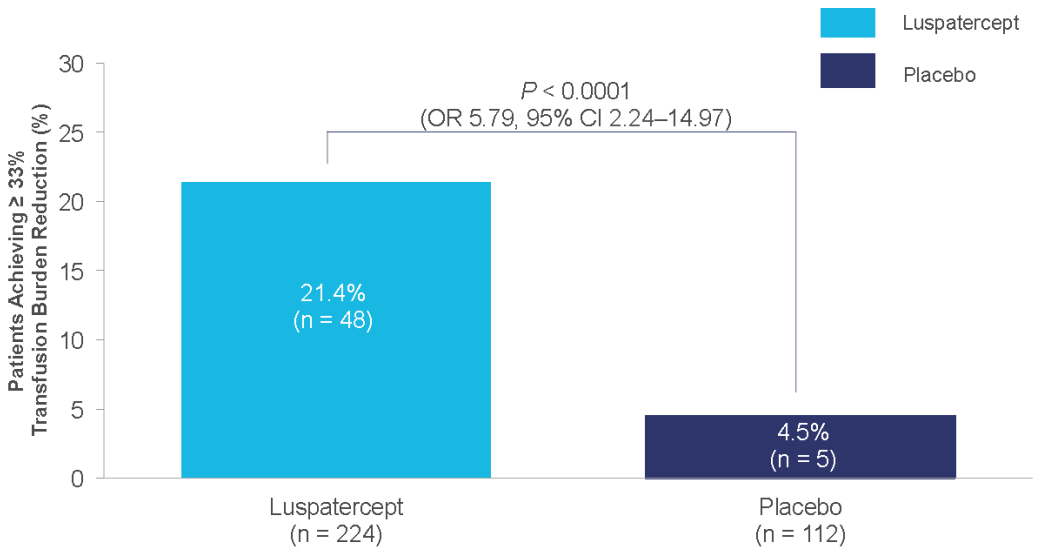


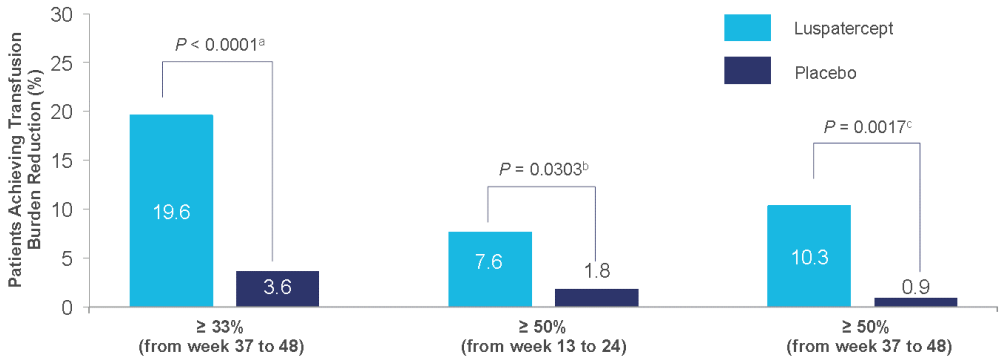
Figure 18. *Proportion of patients achieving ≥ 33% reduction in RBC transfusion burden*

Forest plot analysis for the primary end point demonstrated that all subgroups consistently benefit from Luspatercept treatment.

Sub-groups	Luspatercept n/N (%)	Placebo n/N (%)	OR (95% CI)	*P* value
Overall	48/224 (21.4)	5/112 (4.5)	5.79 (2.24, 14.97)	< 0.0001
Region: North America & Europe	23/100 (23.0)	1/51(2.0)	14.94 (1.95, 114.12)	0.0009
Region: Middle East & North Africa	11/52 (21.2)	2/26 (7.7)	3.22 (0.66, 15.77)	0.1351
Region: Asia–Pacific	14/72 (19.4)	2/35 (5.7)	3.98 (0.85, 18.62)	0.0629
Age: ≤ 32 years	22/129 (17.1)	4/63 (6.3)	3.00 (0.98, 9.20)	0.0476
Age: > 32 years	26/95 (27.4)	1/49 (2.0)	17.50 (2.27, 134.98)	0.0004
Splenectomy: Yes	31/129 (24.0)	2/65 (3.1)	9.72 (2.22, 42.53)	0.0003
Splenectomy: No	17/95 (17.9)	3/47 (6.4)	2.94 (0.81, 10.69)	0.0918
Sex: Female	35/132 (26.5)	4/63 (6.3)	5.33 (1.80, 15.80)	0.0011
Sex: Male	13/92 (14.1)	1/49 (2.0)	8.05 (1.01, 64.16)	0.0218
β-thalassemia Gene: β^0/β^0	9/68 (13.2)	2/35 (5.7)	2.54 (0.48, 13.51)	0.2708
β-thalassemia Gene: Non-β^0/β^0	39/155 (25.2)	3/77 (3.9)	8.35 (2.47, 28.23)	< 0.0001
Baseline Transfusion Burden: ≤ 6 units/12 weeks	27/112 (24.1)	3/56 (5.4)	5.61 (1.60, 19.65)	0.0033
Baseline Transfusion Burden: > 6 units/12 weeks	21/112 (18.8)	2/56 (3.6)	6.16 (1.38, 27.44)	0.0082
Baseline Hemoglobin: < 9 g/dL	22/87 (25.3)	4/51 (7.8)	3.78 (1.25, 11.42)	0.0128
Baseline Hemoglobin: ≥ 9 g/dL	26/137 (19.0)	1/61 (1.6)	14.17 (1.85, 108.79)	0.0012
Baseline Liver Iron: ≤ 3 mg/g dry weight	12/70 (17.1)	1/37 (2.7)	7.18 (0.88, 58.63)	0.0335
Baseline Liver Iron: > 3 to ≤ 7 mg/g dry weight	13/51 (25.5)	0/30 (0)	Infinity	0.0053
Baseline Liver Iron: > 7 to ≤ 15 mg/g dry weight	10/38 (26.3)	1/19 (5.3)	5.41 (0.67, 43.34)	0.0741
Baseline Liver Iron: > 15 mg/g dry weight	13/65 (20.0)	3/26 (11.5)	1.79 (0.47, 6.78)	0.3831

Figure 19. *Forest plot subgroups analysis for the primary endpoint*

A ≥ 33% reduction in RBC transfusion burden at weeks 37–48 was achieved by 44 of 224 (19.6%) patients receiving Luspatercept compared with 4 of 112 (3.6%) patients receiving placebo (P < 0.0001). The more demanding end point of a ≥ 50% reduction in RBC transfusion burden at weeks 13–24 and 37–48 was achieved by 17 (7.6%) and 23 (10.3%) of the 224 patients receiving Luspatercept, respectively, compared with 2 (1.8%) and 1 (0.9%) of 112 patients receiving placebo and that was statistically significant (P = 0.0303 and P = 0.0017, respectively). The difference of mean change in transfusion burden from baseline at weeks 13 to 24 was 1.35 units/12 weeks (P < 0.0001).



- The least squares mean change in transfusion burden from baseline to weeks 13–24 (luspatercept versus placebo) was −1.35 RBC units/12 weeks (95% CI −1.77 to −0.93; *P* < 0.0001)

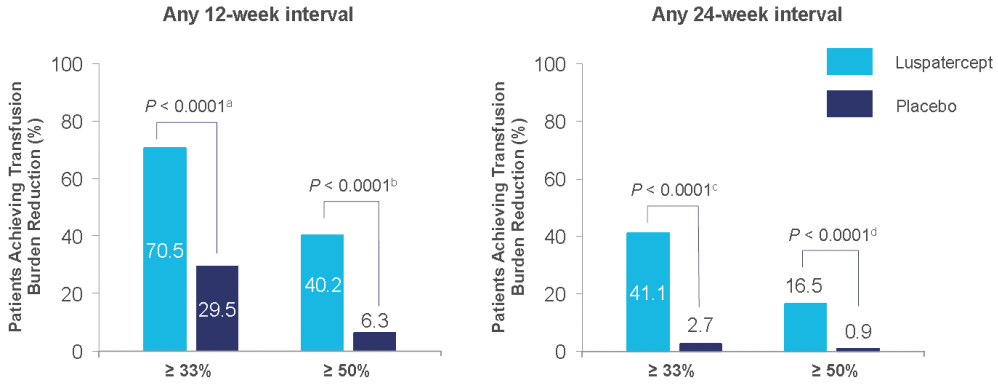
[a] OR 6.44, 95% CI 2.27–18.26. [b] OR 4.55, 95% CI 1.03–20.11. [c] OR 11.92, 95% CI 1.65–86.29.

Figure 20. *Secondary endpoints of ≥ 33% reduction in RBC transfusion burden* from week 37 to 48, and of ≥ 50% reduction in RBC transfusion burden from weeks 13 to 24 or 37 to 48.

Regarding the additional end point of achieving a ≥ 33% reduction in RBC transfusion burden over any consecutive 12 weeks on study, 158 of 224 (70.5%) patients receiving Luspatercept managed to reach this clinically

meaningful threshold, compared with 33 of 112 (29.5%) patients receiving placebo (P < 0.0001).

Moreover, 40.2% patients receiving Luspatercept achieved a ≥ 50% reduction in RBC transfusion burden over any consecutive 12 weeks on study compared to 6.3% patients on placebo (P < 0.0001). Furthermore, 41,1% versus 2,7 and 16,5% versus 0.9% of patients receiving Luspatercept and placebo respectively, manage to achieve a ≥ 33% & a ≥ 50% reduction in RBC transfusion burden over any consecutive 24 weeks on study (both P < 0.0001).



[a] OR 5.69, 95% CI 3.46–9.35. [b] OR 9.95, 95% CI 4.44–22.33. [c] OR 25.02, 95% CI 7.76–80.71. [d] OR 20.37, 95% CI 2.86–144.94.

Figure 21. *'Rolling' endpoint of ≥ 50% reduction in RBC transfusion burden over any consecutive 12 weeks*

As far as iron parameters were concern to the limited duration of 48 weeks on study, those were also reported, as iron accumulation is a major complication of transfusions in β-thalassemia, despite the iron chelation therapy. The least square mean difference in serum ferritin and in myocardial iron were statistically significant between treatment arms, although this is rather not clinically

relevant. LIC changes from the baseline were not statistically significant between treatment arms and this is probably due to the chronic nature of the disease and should be evaluated in a longer follow up.

	Luspatercept (n = 224)	Placebo (n = 112)	LS Mean of Difference	95% CI	*P* Value
Mean change from baseline at week 48					
Serum ferritin, µg/L (SD)	−245 (780)	105 (521)	−340	−502, −177	< 0.0001
LIC, mg/g dry weight (SD)	0.10 (5.760)	0.08 (5.229)	0.11	−1.16, 1.38	0.8685
Myocardial iron by T2*, ms (SD)	−1.83 (15.084)	0.02 (6.843)	−2.39	−4.67, −0.12	0.0391

SD, standard deviation.

Figure 22. *Iron parameters*

Safety summary

Treatment-emergent adverse events (TEAEs) observed in the study were generally consistent with data already reported from phase 2 data. Additionally, treatment-emergent AEs leading to dose delay or dose reduction were similar between treatment arms and no deaths were reported on the Luspatercept arm.

TEAEs of all grades with ≥ 10% frequency were similar between treatment arms, apart from bone pain, arthralgia and dizziness that were more frequent in Luspatercept arm, probably due to the activity in the bone marrow.

TEAEs BY FREQUENCY ≥ 10% IN EITHER ARM (ALL GRADES)

n (%)	Luspatercept (n = 223[a])	Placebo (n = 109[a])
Back pain	61 (27.4)	32 (29.4)
Upper respiratory tract infection	59 (26.5)	36 (33.0)
Headache	58 (26.0)	26 (23.9)
Bone pain	**44 (19.7)**	**9 (8.3)**
Arthralgia	**43 (19.3)**	**13 (11.9)**
Pyrexia	36 (16.1)	23 (21.1)
Cough	32 (14.3)	12 (11.0)
Fatigue	30 (13.5)	14 (12.8)
Oropharyngeal pain	28 (12.6)	12 (11.0)
Diarrhea	27 (12.1)	11 (10.1)
Dizziness	**25 (11.2)**	**5 (4.6)**
Asthenia	22 (9.9)	11 (10.1)
Myalgia	22 (9.9)	11 (10.1)
Pharyngitis	20 (9.0)	13 (11.9)

[a] Safety population.

Figure 23. *Table of Treatment-emergent adverse events (TEAEs) of all grades with ≥ 10% frequency*

Conclusions

Luspatercept managed to demonstrate significant reductions in RBC transfusion burden in adults with transfusion-dependent β-thalassemia and potentially offer a new treatment option for these patients who require regular RBC transfusions.

† As of May 11, 2018, cutoff date.

Phase 3 study, BELIEVE, is ongoing and Luspatercept is expected to result in more clinical meaningful differences in the longer follow up, with a manageable safety profile.

Clinical trials of Luspatercept in Lower Risk MDS

Similarly with β-thalassemia, as previously shown, Luspatercept managed to increase hemoglobin in a myelodysplastic syndromes mouse model and a phase 1 study of healthy postmenopausal women. In contrast with ESAs, Luspatercept promotes late-stage erythroid differentiation and a phase 2 study was designed to assess the safety and efficacy of Luspatercept in lower-risk myelodysplastic syndromes patients, refractory to or ineligible to receive ESAs.

Phase 2 clinical trials: PACE-MDS (Platzbecker et al., 2017)

Methods: Study design and participants

PACE-MDS is a Phase 2 multicentre, open-label, dose-finding study, with long-term extension, for adult patients with lower-risk MDS or chronic myelomonocytic leukemia (CMML), having anemia with or without need for red blood cell transfusion support.

Enrolling patients were classified as low transfusion burden (LTB), defined as requiring ≤ 4 red blood cell (RBC) units in the 8 weeks before treatment and baseline haemoglobin <10 g/dL, or high transfusion burden (HTB), defined as requiring ≥ 4 RBC units in the 8 weeks before treatment.

Specifically, the study was composed by the base study, an initial dose-finding study (ClinicalTrials.gov NCT01749514 consisting of the dose-finding cohorts

and expansion cohorts), and the extension study (ClinicalTrials.gov NCT02268383). Luspatercept was administered SC every 21 days at dose concentrations ranging from 0.125 mg/kg to 1.75 mg/kg.

In the base study, all patients were to receive Luspatercept at one of seven pre-specified dose concentrations ranging from 0.25 mg/kg to 1.75 mg/kg for up to five doses and for a maximum of 12 weeks. Patients in the expansion cohort were to receive 1.0 mg/kg Luspatercept with dose titration up to 1.75 mg/kg for a maximum of 5 years. Following assessment for response and safety from the safety review team at the end of 12 weeks, patients from the base study could be enrolled into the extension study, and continue with 1.0 mg/kg Luspatercept SC every 21 days.

Outcomes

Due to the short duration of treatment, a modified endpoint was introduced as a primary endpoint in the 12-week base study: the proportion of patients achieving modified Haematological Improvement-Erythroid (mHI-E). To a greater extent, that was defined for LTB patients as Hb concentration increase of ≥1.5 g/dL from baseline for ≥14 days, and for HTB patients as a reduction in RBC transfusion of ≥4 RBC units, or a 50% or higher reduction in RBC units, over 8 weeks versus baseline transfusion burden. Nevertheless, as International Working Group (IWG)-defined haematological improvement–erythroid (HI-E) endpoints observed to be similar with mHI-E, the more stringent IWG HI-E is to be reported throughout the results. IWG HI-E was defined as a ≥ 1.5 g/dL increase in haemoglobin concentration over 8 weeks for LTB patients

and a reduction in RBC transfusion requirements of ≥ 4 units over 8 weeks for HTB patients (Cheson et al., 2006).

Secondary endpoints included evaluation of safety and tolerability of Luspatercept, percentage of responders with erythroid, neutrophil, and platelet haematological improvements (as defined by IWG 2006 criteria), time to, and duration of mHI-E and IWG defined HI-E, frequency of RBC transfusions and percentage of patients achieving RBC-TI for ≥8 weeks for TD patients, as well as PK profile and other PD effects.

Results

Between 21st January 2013 and 12th February 2015, 58 MDS patients were enrolled in the 12-week base study [27 patients in the dose-escalation cohorts (0.125- 1.75 mg/kg) and 31 patients in the expansion cohort (1.0-1.75 mg/kg)] and received subcutaneous Luspatercept Q3W.

Dose concentrations of 0.125- 0.5 mg/kg were deemed sub-therapeutic following review of efficacy data and only patients treated at higher dose concentrations (N=51/58) were included in the efficacy evaluable population.

Of the 51 patients who received 0.75-1.75 mg/kg Luspatercept across both base and extension studies, 17 had a LTB and 34 had a HTB, whereas 42 patients were evaluable for RBC-TI. Among LTB patients, median Hb concentration was 8.7 g/dL (range: 6.4–10.1) at baseline and for HTB patients, median transfusion burden was six RBC units per 8 weeks (range: 4.0–18.0).

Follow up period was 3 months and median total duration of treatment for all patients across both the base and extension studies was 6.8 months (range: 2.0–19.8; interquartile range: 3.5–15.2), with 20 patients ongoing at the data cut-off of 4th March 2016.

IWG Haematological Improvement- Erythroid

Of the 51 patients treated with higher Luspatercept dose concentrations (0.75–1.75 mg/kg), across both base and extension studies, 32 patients (63% [95% CI: 48–76]) achieved IWG HI-E.

Of the 17 LTB patients, 11(65%) achieved HI-E, 13 showed sustained increases in mean hemoglobin from baseline for at least 15 months and 11 (85%) of those 13 patients, manage to maintain the hemoglobin change for a median duration of 8.3 months.

Among HTB patients, 21 (62%) of 34 patients treated at higher dose concentrations (0.75–1.75 mg/kg) achieved HI-E, while 15 (79%) out of 19, achieved IWG HI-E with a median duration of response of 11.6 months (95% CI 2.3 –NE). Mean time to response was 0.5 months (SD 0.9; 95% CI 0.3–0.8).

Achievement of HI-E seemed to be similar, regardless of prior use of ESAs (62% of patients with prior ESA usage and 65% of ESA- naïve patients) or prior use of Lenalidomide (63% of patients with prior Lenalidomide use and 63% of Lenalidomide- naïve). Moreover, 76% of patients with baseline serum erythropoietin <200 IU/L, 58% of patients with ≥200 and ≤500 IU/L, and 43% of patients with baseline serum erythropoietin >500 IU/L achieved HI-E, thus

Luspatercept seemed to be effective, even in patients with higher erythropoietin concentrations, where ESAs have poor response.

Luspatercept treatment had more frequent and more robust responses in patients with positive ring sideroblasts ≥ 15% (69% achieved IWG HI-E) or SF3B1 mutations (77%).

RBC Transfusion Independence

Regarding RBC transfusion independence, 16 (38%) out of evaluable 42 patients (including 8 patients with LTB and 34 patients with HTB) achieved RBC-TI across both studies, when most patients had previously failed ESA treatment.

For these 42 previously transfused patients, the max percentage change from baseline in RBC transfusion burden over 8 weeks, is shown in figure 24.

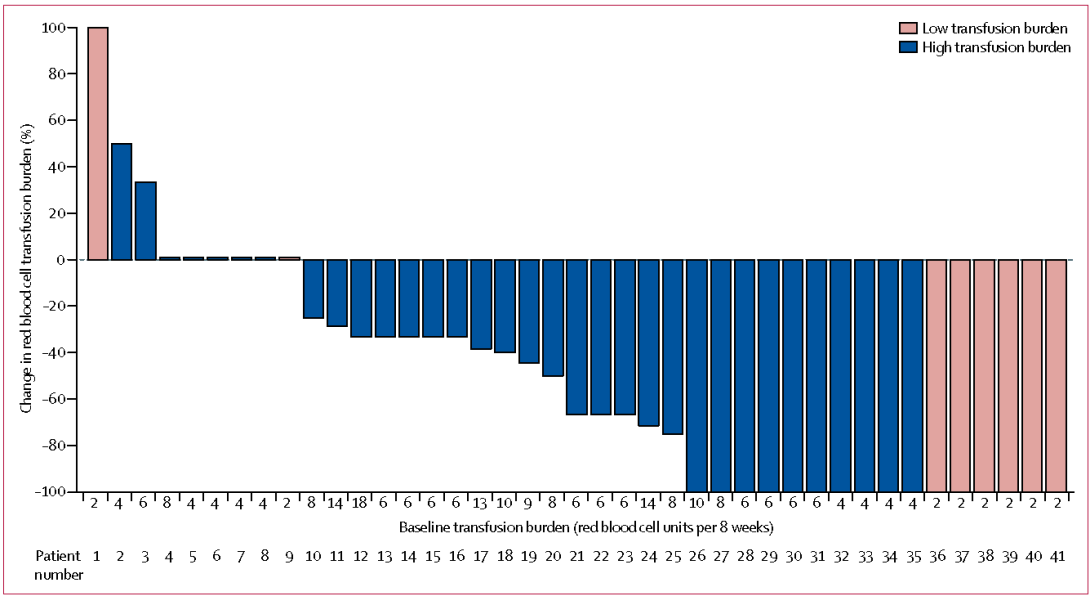


Figure 3: Maximum percentage change in red blood cell transfusion burden in previously transfused patients
One RBC-TI evaluable patient is not shown due to discontinuation before completing 8 weeks of treatment. RBC-TI=red blood cell transfusion independence.

Figure 24. *Change of RBC transfusion burden per patient*

Out of the 22 previously transfused patients who continued receiving Luspatercept into the extension study, 11 (50%) remained transfusion-free for ≥ 8 weeks with median duration of RBC-TI 15.3 months (95% CI 3.6- NE [study ongoing]).

In the multivariable logistic regression analysis performed to assess the association between baseline factors and RBC-TI, serum erythropoietin concentration (<500 IU/L vs ≥500 IU/L; p=0.02) and iron chelation therapy use (yes vs no; p=0.01) were significant. Erythropoietin concentration (<200 IU/L vs ≥200 IU/L) did not have a significant effect in the multivariable analysis (p=0.81).

Safety data

Safety data were analyzed for all 58 patients who received at least one dose of study drug. The most common grade 1–2 adverse events determined to be at least possibly related to study drug were fatigue in four (7%) patients, bone pain in three (5%), and diarrhea in three (5%). Grade 3 adverse events considered related to treatment were reported in only three (5%) patients.

Conclusions

To conclude, these findings suggest that Luspatercept 1.0 mg/kg with titration up to 1.75 mg/kg is the appropriate starting dose for further studies in patients with myelodysplastic syndromes.

Moreover, Luspatercept was effective in patients with high endogenous EPO regardless of prior ESAs use, and response was more frequent and robust in positive ring sideroblasts patients with ≥15% RS, or SF3B1 mutations.

Phase 3 clinical trials

The MEDALIST study

Based on previous indications from phase 2 studies, a phase 3, double-blind, randomized, placebo-controlled, multicenter study was designed, to evaluate the efficacy and safety of Luspatercept (ACE-536) versus placebo in subjects with anemia due to IPSS-R very low, low, or intermediate MDS with ring sideroblasts who require RBC transfusions, the MEDALIST study (ClinicalTrials.gov NCT02631070) (Fenaux et al., ASH 2018).

Methods: Study design and participants

Eligible patients were adults, with IPSS-R-defined Very low-, Low-, or Intermediate-risk MDS with +RS according to the WHO 2016 criteria; were refractory, intolerant, or ineligible to receive erythropoiesis-stimulating agents (ESAs); and required RBC transfusions. Patients were randomized 2:1 to receive either Luspatercept, at a starting dose level of 1.0 mg/kg with dose escalation up to 1.75 mg/kg, if required to achieve optimal exposure, or placebo, subcutaneously every 3 weeks for ≥ 24 weeks.

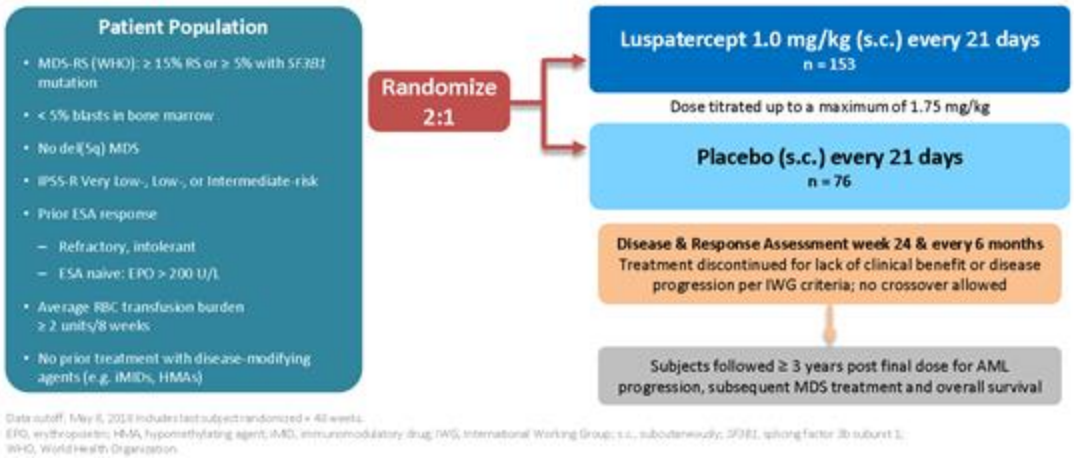


Figure 25. *Study design of the Phase 3 MEDALIST trial* (ClinicalTrials.gov NCT02631070). AML: acute myeloid leukemia, BSC: best supportive care, ESA: erythropoiesis stimulating agent, IPSS-R: Revised International Prognostic Scoring System, MDS: myelodysplastic syndromes, RBC: red blood cell, RS: ring sideroblasts, SC: subcutaneous, WHO: world health organization

The primary endpoint of the MEDALIST study is the proportion of patients who are RBC transfusion free over any consecutive 56-day period (TI ≥8 weeks) within week 1 through week 24.

Secondary endpoints include:

- proportion of patients who are RBC transfusion free over any consecutive 84-day period (TI ≥ 12 weeks) within week 1 through 24 and week 1 through 48,
- proportion of patients who are RBC transfusion free over any consecutive 56-day period (TI ≥ 8 weeks) within week 1 through 48,

- mean change in RBC units transfused over a fixed 16-week period within week 9 through 24 and week 33 through 48
- proportion of patients achieving HI-E (IWG modified 2006 criteria; Cheson et al. Blood. 2006) over any consecutive 56-day period during treatment,
- proportion of patients achieving a mean Hb increase ≥1.5 g/dL from baseline over any consecutive 56-day period in absence of RBC transfusions,
- as well as OS, adverse events, and/or PK parameters.

Results

Patients were randomized between March 2016 and June 2017 at 65 sites in Belgium, Canada, France, Germany, Italy, the Netherlands, Spain, Sweden, Turkey, UK, and USA. The enrolment target has been met earlier than expected, and initial data were orally presented in 60th American Society of Hematology (ASH) annual meeting by Alan F. List.

As of May 8, 2018 cutoff date, a total of 229 patients were randomized and received Luspatercept (N=153) and placebo (N=76). Median age was 71 years (range 26–95), median time from diagnosis was 41.8 months (range 3–421), and 62.9% were male.

Overall, both treatment groups were balanced with similar patient baseline characteristics.

According to WHO classification, 145 (94.8%) and 74 (97.4%) of the patients in the Luspatercept and placebo respectively, had refractory cytopenia with multilineage dysplasia and ring sideroblasts.

Patients had 7.6g/dL, rather low median pre-transfusion hemoglobin and received a median of 5 RBC units/8 weeks (range 1–20) transfusion burden during the 16 weeks prior to treatment (43.2% of patients had ≥ 6 RBC units/8 weeks, 27.9% had ≥ 4 to < 6 RBC units/8 weeks, and 28.8% had < 4 RBC units/8 weeks).

At baseline, 138 (60.3%), 58 (25.3%), and 32 (14.0%) patients had serum erythropoietin levels < 200 IU/L, 200–500 IU/L, and > 500 IU/L, respectively.

A total of 218 (95.2%) patients had previously received ESAs and 206 (90.0%) patients had an SF3B1 mutation.

Characteristic	Luspatercept (n = 153)	Placebo (n = 76)
Age, median (range), years	71 (40–95)	72 (26–91)
Male, n (%)	94 (61.4)	50 (65.8)
Time since original MDS diagnosis, median (range), months	44.0 (3–421)	36.1 (4–193)
WHO classification		
RCMD-RS, n (%)	145 (94.8)	74 (97.4)
RBC transfusion burden, median (range), units/8 weeks[a]	5 (1–15)	5 (2–20)
≥ 6 units/8 weeks, n (%)	66 (43.1)	33 (43.4)
< 6 units/8 weeks, n (%)	87 (56.9)	43 (56.6)
Pre-transfusion Hb, median (range), g/dL	7.6 (6–10)	7.6 (5–9)
IPSS-R risk category[b]		
Very Low, Low, n (%)	127 (83.0)	63 (82.9)
Intermediate, n (%)	25 (16.3)	13 (17.1)
SF3B1 mutation, n (%)	141 (92.2)	65 (85.5)[c]
Serum EPO		
< 200 U/L, n (%)	88 (57.5)[c]	50 (65.8)
≥ 200 U/L, n (%)	64 (41.8)[c]	26 (34.2)

[a]In the 16 weeks prior to randomization. [b]1 (0.7%) patient in the luspatercept arm was classified as IPSS-R High-risk. [c]Data were missing for 1 patient.
RCMD-RS, refractory cytopenia with multilineage dysplasia with RS.

Figure 26. *Demographics and Baseline Disease Characteristics*

Regarding treatment exposure, 83.7% and 89.5% of the patients completed ≥ 24 weeks of Luspatercept and placebo treatment respectively, but 51.0% and 15.8% completed the 2nd response assessment period of ≥ 48 weeks. 45.8% of patients treated with Luspatercept remained on treatment compared to 7.9% on placebo. 54.2% compared to 92.1% had to discontinue from Luspatercept and placebo respectively, mainly due to lack of benefit (33.3% vs 65.8%). Almost 23% of patients had 1.0 mg/kg of Luspatercept and 18% and 59% received dose escalation (1.33 and 1.75 mg/kg).

Parameter	Luspatercept (n = 153)	Placebo (n = 76)
Treatment duration, median (range), weeks	**49 (6–114)**	**24 (7–89)**
Completed ≥ 24 weeks of treatment (primary phase), n (%)	128 (83.7)	68 (89.5)
Completed ≥ 48 weeks of treatment, n (%)	78 (51.0)	12 (15.8)
Number of doses received, median (range)	16 (2–37)	8 (3–30)
Maximum dose escalation, n (%)*		
1.0 mg/kg	35 (22.9)	5 (6.6)
1.33 mg/kg	28 (18.3)	8 (10.5)
1.75 mg/kg	90 (58.8)	63 (82.9)
Patients remaining on treatment, n (%)	**70 (45.8)**	**6 (7.9)**
Patients discontinued from treatment, n (%)	**83 (54.2)**	**70 (92.1)**
Lack of benefit	51 (33.3)	50 (65.8)
Patient withdrawal	14 (9.2)	10 (13.2)
AE	10 (6.5)	4 (5.3)
Disease progression	3 (2.0)	2 (2.6)
Other	5 (3.3)	4 (5.3)

*Dose may be titrated up to a maximum of 1.75 mg/kg.
AE, adverse event.

Figure 27. *Parameters of treatment exposure*

Primary endpoint was met and 58 out of 153 (37.9%) patients receiving Luspatercept, achieved the primary endpoint of RBC transfusion independence for ≥ 8 weeks compared with 10 of 76 (13.2%) patients receiving placebo and this difference was statistically significant (odds ratio [OR] 5.1, P < 0.0001).

RBC-TI ≥ 8 weeks	Luspatercept (n = 153)	Placebo (n = 76)
Weeks 1–24, n (%)	**58 (37.9)**	**10 (13.2)**
95% CI	30.2–46.1	6.5–22.9
P value[a]	< 0.0001	

[a] Cochran–Mantel–Haenszel test stratified for average baseline RBC transfusion requirement (≥ 6 units vs < 6 units of RBCs/8 weeks) and baseline IPSS-R score (Very Low or Low vs Intermediate).
CI, confidence interval.

Figure 28. *Primary Endpoint of Red Blood Cell Transfusion Independence for ≥ 8 Weeks*

Forest plot analysis for the primary end point demonstrated that all subgroups consistently benefit from Luspatercept treatment. Intriguingly, patients with RBC transfusion burden < 6 units/8 weeks during the 16 weeks prior to treatment and patients with higher baseline platelet count benefited the most.

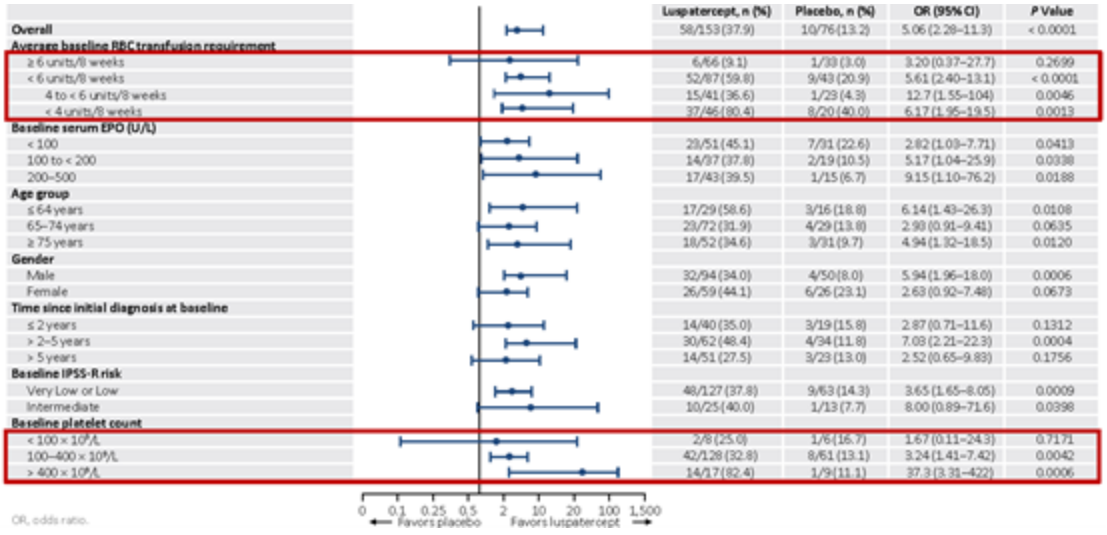
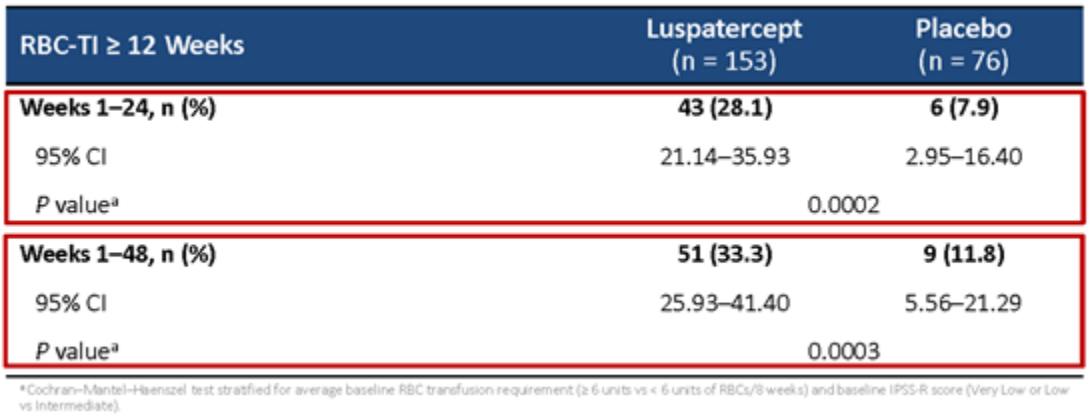


Figure 29. *Forest plot subgroup analysis for the primary endpoint*

The key secondary endpoints of RBC-TI for ≥ 12 weeks (in weeks 1–24 period) was achieved by 43 of 153 (28.1%) patients in Luspatercept arm compared with 6 of 76 (7.9%) in the placebo arm (P = 0.0002) and RBC-TI for ≥ 12 weeks (in weeks 1- 48 period) was achieved by 51 of 153 (33.3%) patients treated with Luspatercept compared with 9 of 76 (11.8%) patients receiving placebo (P = 0.0003).

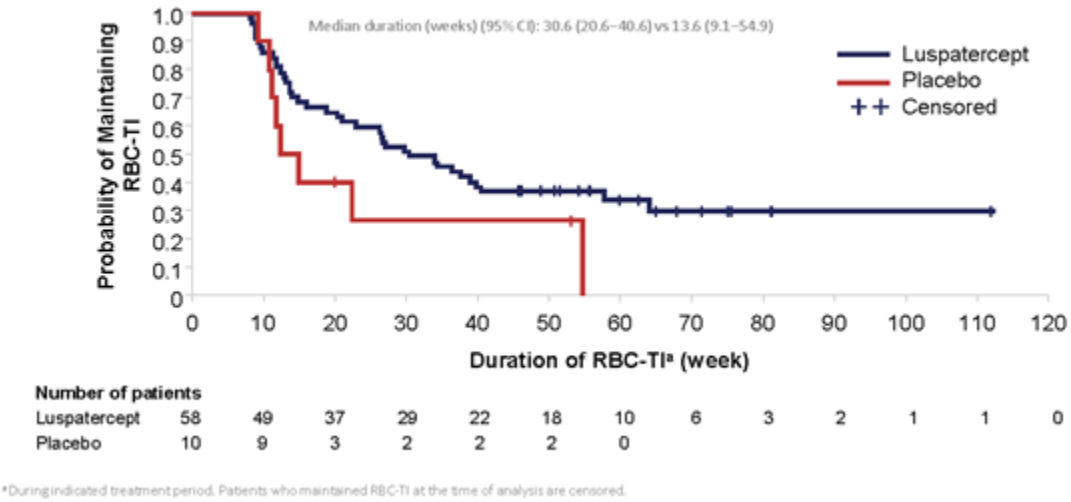


RBC-TI ≥ 12 Weeks	Luspatercept (n = 153)	Placebo (n = 76)
Weeks 1–24, n (%)	**43 (28.1)**	**6 (7.9)**
95% CI	21.14–35.93	2.95–16.40
P value[a]	0.0002	
Weeks 1–48, n (%)	**51 (33.3)**	**9 (11.8)**
95% CI	25.93–41.40	5.56–21.29
P value[a]	0.0003	

[a]Cochran–Mantel–Haenszel test stratified for average baseline RBC transfusion requirement (≥ 6 units vs < 6 units of RBCs/8 weeks) and baseline IPSS-R score (Very Low or Low vs Intermediate).

Figure 30. *Key Secondary Endpoint of Red Blood Cell Transfusion Independence ≥ 12 Weeks*

Median duration (in weeks) of RBC-transfusion independence in primary endpoint responders was 30.6 (range 20.6-40.6) for Luspatercept compared to 13.6 (range 9.1-54.9) for placebo.



*During indicated treatment period. Patients who maintained RBC-TI at the time of analysis are censored.

Figure 31. *Duration of RBC-TI Response in Primary Endpoint Responders*

Secondary Endpoint: Erythroid Response (HI-E): proportion of patients achieving HI-E (IWG modified 2006 criteria, Cheson et al. Blood. 2006) over any consecutive 56-day period during treatment.

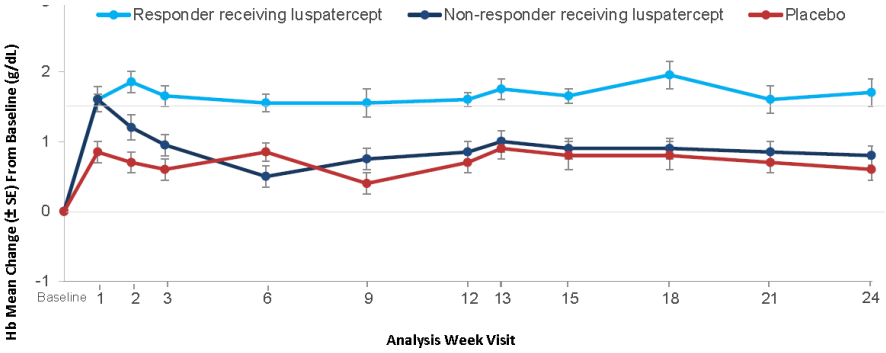
	Luspatercept (n = 153)	Placebo (n = 76)
Achieved HI-E[a] (weeks 1–24), n (%)	**81 (52.9)**	**9 (11.8)**
Reduction of ≥ 4 RBC units/8 weeks (baseline transfusion burden ≥ 4 units/8 weeks)	52/107 (48.6)	8/56 (14.3)
Hb increase of ≥ 1.5 g/dL (baseline transfusion burden < 4 units/8 weeks)	29/46 (63.0)	1/20 (5.0)
95% CI	44.72–61.05	5.56–21.29
P value[b]	< 0.0001	
Achieved HI-E[a] (weeks 1–48), n (%)	**90 (58.8)**	**13 (17.1)**
Reduction of ≥ 4 RBC units/8 weeks (baseline RBC transfusion burden ≥ 4 units/8 weeks)	58/107 (54.2)	12/56 (21.4)
Hb increase of ≥ 1.5 g/dL (baseline RBC transfusion burden < 4 units/8 weeks)	32/46 (69.6)	1/20 (5.0)
95% CI	50.59–66.71	9.43–27.47
P value[b]	< 0.0001	

[a]Defined as the proportion of patients meeting the HI-E criteria per IWG 2006 criteria (Cheson et al. 2006) sustained over a consecutive 56-day period during the indicated treatment period.
[b]Luspatercept compared with placebo, Cochran–Mantel–Haenszel test.

Figure 32. *Secondary Endpoint: Erythroid Response (HI-E)*

Patients receiving Luspatercept were more likely to achieve an mHI-E response, defined as a reduction in transfusion of ≥ 4 RBC units/8 weeks or a mean hemoglobin increase of ≥ 1.5 g/dL/8 weeks in the absence of transfusions, compared with patients receiving placebo (52.9% vs 11.8% during weeks 1–24; P < 0.0001 and 58.8% vs 17.1% during weeks 1-48; P< 0.0001).

Patients that responded to Luspatercept, improved their hemoglobin levels and stabilized it overtime, with median peak hemoglobin increase 2.55 (1-4.1) g/dL.



- Median peak hemoglobin increase in luspatercept responders: 2.55 g/dL (1–4.1 g/dL)

Number of patients

Responder[a]	153	24	36	55	53	52	50	42	47	50	42	45
Non-responder		33	51	61	52	60	53	34	45	56	48	35
Placebo	76	32	36	41	47	44	52	29	44	47	44	32

[a]LS mean difference (95% CI) for luspatercept responders versus placebo: 1.08 (0.84, 1.31), *P* < 0.0001.
Only patients with RBC-TI ≥ 8 weeks during weeks 1–24 are included. Hb measurement was excluded within 14 days after a RBC transfusion unless within 3 days prior to another RBC transfusion. Mean and SE were not calculated if the number of patients was < 8 in the luspatercept non-responder group or < 4 in the placebo group. SE, standard error.

Figure 33. *Change in Hemoglobin Concentration*

Safety Summary

The safety profile of Luspatercept was consistent with that reported in the phase 2 PACE-MDS study (Platzbecker et al., 2017).

Treatment-emergent adverse events, TEAE, were balanced between the arms. The most common grade 3 or 4 TEAEs reported in Luspatercept-treated

patients were anemia (6.5% of patients), fall (4.6%), and fatigue (4.6%). TEAEs leading to death In Luspatercept arm were sepsis (n = 2), multiple organ dysfunction syndrome, renal failure, and hemorrhagic shock; in placebo arm were sepsis, urosepsis, general physical health deterioration, and respiratory failure. Progression to AML occurred in 4 patients (3/153 [2.0%] in the Luspatercept arm; 1/76 [1.3%] in the placebo arm).

Conclusions

In, as IPSS-R-defined, Very low-, Low-, or Intermediate-risk MDS patients with positive RS, who require RBC transfusions, treatment with Luspatercept resulted in a significantly higher percentage of patients who achieved RBC-TI, major RBC transfusion burden reduction, or hemoglobin increase, compared with placebo.

Erythroid responses were durable, with approximately 40% of patients achieving RBC-TI sustained at 12 months of treatment and Luspatercept was generally safe and well tolerated in this patient population.

Luspatercept is a potential new therapy for the treatment of patients with lower-risk, RS-positive MDS with RBC transfusion-dependent anemia

The COMMANDS study

A phase 3, Open-label, Randomized Study is subsequently designed to compare the efficacy and safety of Luspatercept (ACE-536) versus Epoetin Alpha for the treatment of anemia due to IPSS-R very low, low or intermediate risk due to Myelodysplastic Syndrome (MDS) in ESA naive subjects who require Red Blood Cell transfusions, the COMMANDS study (ClinicalTrials.gov Identifier: NCT03682536).

Primary Outcome of the study will be Red Blood Cell-transfusion independence (RBC-TI) over the first 24 weeks. Among secondary endpoints, are Hematologic improvement - erythroid response (HI-E) per International Working Group (IWG) within 24 weeks, time to and duration of HI-E, duration of RBC-TI ≥ 24 weeks and ≥ 84 days, evaluation of changes in QoL & Anemia through EORTC QLQ-C30 & FACT-An questionnaires, pharmacokinetic, as well as time to AML and OS.

Patients eligible for the study must have documented diagnosis of MDS according to WHO 2016 classification that meets IPSS-R classification of very low, low, or intermediate risk disease, with < 5% blasts in bone marrow and an endogenous serum erythropoietin (sEPO) level of < 500 U/L. Presence of ring sideroblasts is not an eligibility criterion.

This trial is recruiting patients and aims to investigate the potential benefit of Luspatercept as a late-stage erythroid maturation agent compared with the activity of Epoetin-a in the early stages of hematopoiesis.

Development of Luspatercept in Primary Myelofibrosis

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) that is characterized by a clonal myeloproliferation of the stem-cells, leading to bone marrow stromal reaction including reticulin fibrosis, abnormal cytokine expression, anemia, hepatosplenomegaly, extramedullary hematopoiesis (EMH), constitutional symptoms, cachexia, leukemic progression, and shortened survival. Diagnosis of PMF is based on bone marrow morphology and is supplemented by the presence of JAK2 mutation in ~ 90% of the patients (Tefferi A., 2018).

Currently, the only treatment capable of prolonging survival or potential cure, is allogeneic stem cell transplant (ASCT) but it is associated with almost 50% transplant-related deaths or severe morbidity (eg, graft vs. host disease). Alternative treatment options are mainly palliative with no modification of the natural history of the disease. Hydroxyurea is the drug of choice for MF-associated splenomegaly and is effective in reducing spleen size by half in approximately 40% of patients (Martinez et al., 2010).

JAK inhibitors have a nonspecific ability to suppress inflammatory cytokines but do not show anti-tumor activity or ability to reverse bone marrow fibrosis and induce cytogenetic or molecular remissions (Tefferi A., 2018). Ruxolitinib (Jakavi®) is a JAK 1 / 2 inhibitor (JAK1 and JAK2) indicated for the treatment of disease-related splenomegaly or symptoms in adult patients with primary myelofibrosis (EMA, Jakavi®, SPC 2018). Ruxolitinib was evaluated compared to placebo or best supportive care in two randomized studies, COMFORT-1 &

COMFORT-2. In the COMFORT-1 trial, ruxolitinib demonstrated spleen response rate approximately 42% compared to <1% for placebo. Intriguingly, the benefit of the drug was antagonized by ruxolitinib-associated anemia (31% vs. 13.9%) (Verstovsek et al., 2012). Similarly, in the COMFORT-2 trial, spleen response was 28.5% with ruxolitinib compared with 0% for best available therapy (n = 219), but unfortunately, patients receiving ruxolitinib presented with increased thrombocytopenia (44.5% vs. 9.6%) and anemia (40.4% vs. 12.3%). (Harrison et al., 2012).

Potential therapeutic pathways in Primary Myelofibrosis

TGF-β is expressed at high levels in the bone marrow, while inhibition of TGF-β signaling can prevent fibrosis development and reactivate normal hematopoiesis, particularly erythropoiesis, in several models (Gastine et al., 2007, Ceglia et al., 2016). In particular, TGF-β interaction with activins induces intracellular signaling via phosphorylation of Smad2/3 transcription factors. Activation of Smad2/3 regulates to the expression of several profibrotic genes. As a consequence, activation of activin/TGF-β-mediated SMAD-2/3 signaling promotes fibrosis and thus, new therapeutic strategies targeting this signaling, may potential contribute to the pathogenesis of myelofibrosis.

Additionally, given the proven safety and efficacy of ruxolitinib, it is most likely that ruxolitinib-based combinations with drugs that improve anemia is self-evident.

For all these reasons, Luspatercept, could be a fair candidate for such a combination.

Currently, Sotatercept is being evaluated in an on-going, phase 2, trial in subjects with Myeloproliferative Neoplasm (MPN)-associated Myelofibrosis and anemia as monotherapy or in combination with ruxolitinib. Initial data presented at ASH 2017 Annual Meeting, were encouraging as Sotatercept was well tolerated whereas 6 out of 17 (35%) and 1 out of 8 (12.5%) patients treated with Sotatercept alone or in combination with ruxolitinib respectively, achieved erythroid response, with good tolerance (Prithviraj et al., ASH 2017).

Phase 2 clinical trials

Following the previous rational, a phase 2 study was designed to evaluate safety and efficacy of Luspatercept in subjects with Myeloproliferative Neoplasm-associated Myelofibrosis who have anemia with and without Red Blood Cell-transfusion dependence (ClinicalTrials.gov Identifier: NCT03194542).

Primary Outcome of the study will be the anemia response as it relates to hemoglobin increase or to increased RBC-transfusion independence.

Additional endpoints include time to anemia response, duration of response, frequency of RBC transfusions, reduction in fatigue symptoms, Health-related QoL, changes in Functional Assessment of Cancer Therapy - Anemia (FACT-An) and pharmacokinetics.

The study is sponsored by University of Texas MD Anderson Cancer Center and it is currently in recruitment status.

Discussion

Ongoing clinical trials, additional questions and future directions

Luspatercept, a fusion protein consisted of a modified activin receptor IIB (ActRIIB), a member of the TGF-β superfamily, and the Fc domain of human immunoglobulin G (IgG1), has so far been studied in the treatment of β-thalassemia, Lower Risk Myelodysplastic Syndromes and Myelofibrosis. Luspatercept has not yet received any approval by EMA or FDA and is currently being evaluated in clinical trials.

Study title, Identifier	**Phase; Patients enrollment; Status**	**Primary endpoint**	**Disease**	**Treatment; Intervention**
NCT03342404; A Study to Determine the Efficacy and Safety of Luspatercept in Adults With Non Transfusion Dependent Beta (β)-Thalassemia **(BEYOND)**	Phase 2; 150; **Recruiting**	To evaluate the effect of luspatercept in non-transfusion dependent β-thalassemia-patient vs placebo on anemia, as measured by mean hemoglobin concentration in the absence of transfusions over a continuous 12-week interval, from Week 13 to Week 24, compared to baseline.	β-thalassemia	2:1 ratio Luspatercept vs placebo; Screening Period, Double-blind Treatment Period (DBTP) and Post-Treatment Follow-up Period (PTFP).

NCT03682536; Efficacy and Safety Study of Luspatercept (ACE-536) Versus Epoetin Alfa for the Treatment of Anemia Due to IPSS-R Very Low, Low or Intermediate Risk Myelodysplastic Syndromes (MDS) in ESA Naïve Subjects Who Require Red Blood Cell Transfusions **(COMMANDS)**	Phase 3; 350; **Recruiting**	Red Blood Cell Transfusion Independence (RBCTI) for 24 weeks [Time Frame: Randomization through Week 24]	Myelodysplastic Syndromes	Experimental: Experimental Arm: luspatercept (ACE-536) 1.0 mg/kg subcutaneous (SC) every 3 weeks (Q3W) Active Comparator: Control Arm: epoetin alfa 450 IU/kg subcutaneous (SC) weekly
NCT03194542 ; A Safety and Efficacy Study to Evaluate Luspatercept in Subjects With Myeloproliferative Neoplasm-associated Myelofibrosis Who Have Anemia With and Without Red Blood Cell transfusion Dependence	Phase 2; 70; **Recruiting**	•Anemia response as it relates to hemoglobin (Hgb)increase •Anemia response as it relates to increased red blood cell (RBC)-transfusion independence	•Primary Myelofibrosis •Anemia	Luspatercept; Screening Period, a Treatment Period followed by a Posttreatment Follow-up Period.
NCT02604433; An Efficacy and Safety Study of Luspatercept (ACE-536) Versus Placebo in Adults Who Require Regular Red Blood Cell Transfusions Due to Beta (β) Thalassemia **(BELIEVE)**	Phase 3; 335; Active, not recruiting	Proportion of subjects with hematological improvement from Week 13 to Week 24 compared to 12-week prior to randomization	•Erythrocyte Transfusion •β-thalassemia	Experimental: Luspatercept (ACE-536) plus BSC Luspatercept, SC once every 21 days at starting dose 1 mg/kg. Placebo Comparator: Placebo plus BSC normal saline solution subcutaneous (SC) once every 21 days

NCT02631070; A Study of Luspatercept (ACE-536) to Treat Anemia Due to Very Low, Low, or Intermediate Risk Myelodysplastic Syndromes **(MEDALIST)**	Phase 3; 229; Active, not recruiting	Red Blood Cell Transfusion Independence (RBC-TI) ≥ 8 weeks [Time Frame: Week 1 through week 24]	Myelodysplastic Syndromes	Experimental: Luspatercept (ACE-536) Starting dose of 1.0 mg/kg SC every 3 weeks. Placebo Comparator: Placebo SC every 3 weeks
NCT02268383; ACE-536 Extension Study - Myelodysplastic Syndromes	Phase 2; 75; Active, not recruiting	To evaluate the long-term safety and tolerability of ACE-536 in patients with low or intermediate-1 risk MDS who were previously enrolled in study A536-03	Myelodysplastic Syndromes	Luspatercept (ACE-536) 1.0 mg/kg once every 3 weeks by subcutaneous injection
NCT02268409; ACE-536 Extension Study - Beta Thalassemia	Phase 2; 75; Active, not recruiting	Long-term safety and tolerability of ACE-536 in patients with β thalassemia who were previously enrolled in study A536-04	β-thalassemia	Luspatercept (ACE-536) 0.8 mg/kg once every 3 weeks by SC injection
NCT01749540; Study to Evaluate the Effects of ACE-536 in Patients With Beta-thalassemia	Phase 2; 64; Completed	Proportion of patients who have an erythroid response. [Time Frame: Assessed at approximately 24 weeks from patient screening.]	β-thalassemia	Luspatercept (ACE-536) 1 of 7 possible dose levels. Subjects receive ACE-536 administered SC every 3 weeks for up to 5 cycles.
NCT01749514; Study of ACE-536 for the Treatment of Anemia in Patients With Myelodysplastic Syndromes (MDS)	Phase 2; 116; Completed	Proportion of patients who have a modified erythroid response (mHI-E). [Time Frame: Assessed at approximately 28 weeks from patient screening.]	Myelodysplastic Syndromes	Experimental: ACE-536 Subjects assigned to 1 of 7 possible dosing group. Subjects receive ACE-536 administered SC every 3 weeks for up to 5 cycles.

NCT02626689; To Document the Burden of Illness on the Quality of Life and the Impact on Healthcare Utilization in (Beta) β-thalassemia Subjects Who Are Transfusion Dependent (TD) and Non-transfusion Dependent (NTD) Receiving Standard of Care	Observational; 100; Completed	Change in the Physical component score (PSC) over the study period versus the country specific population norms using the 36-item Short Form (SF-36) Quality of Life instrument [Time Frame: Up to 6 months]	β-thalassemia	*β-thalassemia TD subjects* Participants will complete 3 QoL instruments (i.e. FACT-AN, SF-36v2, and the TranQol) once every 3 weeks, in addition to a TranQol instrument on the day of RBC transfusion. *β-thalassemia NTD subjects* Participants will complete 2 QoL instruments (i.e. FACT-An, SF-36v2l) once every 3 weeks, in addition to completing the NTD Patient Recorded Outcome (PRO) tool on a daily basis.
NCT01432717; Study of ACE-536 in Healthy Postmenopausal Women	Phase 1; 40; Completed	Number of participants with Adverse Events as a measure of safety and tolerability. [Time Frame: 22 weeks]	Anemia	Experimental: ACE-536 Subjects assigned to 1 of 5 possible dosing groups. Subjects receive a total of 2 SC doses of ACE-536 on Day 1 and 15. Placebo Comparator: Placebo: Subjects receive a total of 2 SC doses of placebo on Day 1 and 15.

Figure 34. *Clinical development of Luspatercept* (clinicaltrials.gov)

Phase 3 data presented in the latest 60th American Society of Hematology (ASH) annual meeting, showed promising efficacy in β-thalassemia and Lower Risk MDS in the 3-4 years that those trials are ongoing, although a longer follow-up is needed to have a precise footprint of its value.

Patients with β-thalassemia are in continuous need of regular or random transfusions, facing the complications of iron overload even in the era of chelators, living with burdened Quality of Life. Luspatercept managed to decrease the actual number of RBC units in BELIEVE study and what seems really significant, is the newly introduced 'rolling' endpoint, revealing the importance of achieving a decrease in transfusion burden, whenever this is achieved. Depiction of the actual transfused RBC units per year, per patient, could also be reasonably connected with a passive reduction in iron intake and a more near-to-normal hemoglobin.

To date, the precise mode of action of Luspatercept is not completely described and a lot are yet to be learned. Gene-profiling is not a routine process for patients with β-thalassemia and no specific mutations or markers have yet been identified to predict response. Nevertheless, a sub-analysis of endpoints in the responders of Luspatercept is mandatory in order to capture the real benefit in these set of patients.

As already mentioned, a longer follow up of Luspatercept is necessary to understand whether the inhibition of GDF11-ActRIIB-Smad2/3 dependent signaling and the promoting of late-stage maturation of erythroid precursors is evolved in mutagenesis by a parallel mechanism. Still, Luspatercept is well tolerated and outweighs the risks of gene therapy as age and performance

status is not a barrier and could at any time ‘bridge’ gene therapy in case of no or partial response.

On the other hand, in Lower Risk MDS, treatment options are very limited when patients don’t respond or lose response to ESAs and increase their needs in support with RBC transfusions. The dosing scheme of Luspatercept enables compliance and at the same time enlarges the treatment free interval, as it is administered subcutaneously every 21 days in the hospital leading to a positive effect in QoL.

The random finding that Luspatercept is beneficial especially to patients with positive ring sideroblasts, suggests that perhaps there is some association with the pathophysiology of iron metabolism in MDS. Further investigation of the responders mutation profiling will eventually give some guidance for the right candidates.

Finally, the activity of Luspatercept in osteoporosis is not negligible, if we consider that β-thalassemia and MDS patients are nowadays an ‘aging’ population, living longer with increased medical monitoring needs.

List of abbreviations and definition of terms

In this study the following abbreviations are used:

Abbreviation	Explanation
ActRIIB/A	Activin Receptor IIB (or A)
AE	Adverse Events
AML	Acute Myeloid Leukemia
ASCT	Allogeneic Stem Cell Transplant
ASH	American Society of Hematology
AUC	Area Under the Curve
BFU-E	Erythroid Burst Forming Unit
BMD	Bone Marrow Density
BMPs	Bone Morphogenetic Proteins
CFU-E	Erythroid Colony Forming Unit
Cmax	Maximum Concentration
CMML	Chronic Myelomonocytic Leukemia
EMH	Extramedullary Hematopoiesis
EORTC	European Organization for Research and Treatment of Cancer
EORTC QLQ-C30	EORTC Quality of Life Questionnaire – C30
EPO	Erythropoietin
EPO mAb	Monoclonal Antibody against EPO

EPOR	Erythropoietin Receptor
ESAs	Erythropoietin Stimulating Agents
FACIT-F	Functional Assessment of Chronic Illness Therapy-Fatigue
FasL	Fas-Fas Ligand
GATA-1	GATA binding protein 1
GDFs	Growth Differentiation Factors
Hb	Haemoglobin
HR MDS	Higher Risk Myelodysplastic Syndromes
HSP70	Heat Shock Protein 70
HTB	High Transfusion Burden
ICT	Iron Chelation Therapy
IgG1	Immunoglobulin G 1
IPSS-R	International Prognostic Scoring System-Revised
IWG	International Working Group
JAK2	Janus Kinase 2
LIC	Liver Iron Concentration
LR MDS	Lower Risk Myelodysplastic Syndromes
LTB	Low Transfusion Burden
MDS	Myelodysplastic Syndromes
MDS-RS	MDS with Ringed Sideroblasts
MEL	Mouse Erythroleukemic cells
mHI-E	Haematological Improvement - Erythroid
MPN	Myeloproliferative Neoplasm
NTD	Non Transfusion Dependent

PD	Pharmacodynamics
PK	Pharmacokinetics
PRO	Patient-Reported Outcome
QoL	Quality-of-life
RBC-TD	Red Blood Cell Transfusion Dependence
RBC-TI	Red Blood Cell Transfusion Independence
ROS	Reactive Oxygen Species
R-SMAD	Receptor-Regulated Smad
SC	Subcutaneous
SE	Standard Error
sEPO	serum Erythropoietin
STAD	Signal Transducer and Activator of Transcription
t1/2	Time half-life: time taken for Tmax to drop in half
TD	Transfusion Dependent
TEAEs	Treatment-Emergent Adverse Events
TGF-β	Transforming Growth Factor-β
Tmax	Time to reach the maximum concentration
WHO	World Health Organization
Wt	Wild-Type mouse

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