30 April 2020
EMA/270924/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Reblozyl

International non-proprietary name: luspatercept

Procedure No. EMEA/H/C/004444/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Administrative information

Name of the medicinal product:	Reblozyl
Applicant:	Celgene Europe BV Winthonlaan 6N 3526 KV Utrecht NETHERLANDS
Active substance:	luspatercept
International Non-proprietary Name/Common Name:	luspatercept
Pharmaco-therapeutic group (ATC Code):	other antianemic preparations, (B03XA06)
Therapeutic indication(s):	treatment of: - adult patients with transfusion-dependent anaemia due to very low, low and intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts, who had an unsatisfactory response to or are ineligible for erythropoietin-based therapy - adult patients with transfusion-dependent anaemia associated with Beta-thalassaemia
Pharmaceutical form(s):	Powder for solution for injection
Strength(s):	25 mg and 75 mg
Route(s) of administration:	Subcutaneous use
Packaging:	vial (glass)
Package size(s):	1 vial

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List of abbreviations

ACE-036	tbd by the applicant (OC)
ACE-536	Luspatercept
ActRIIB	Activin receptor type IIB
ADA	Antidrug antibodies
AE	Adverse event
ALT	Alanine transaminase
ANCOVA	analysis of covariance
AST	Aspartate transaminase
AUC	Area under the concentration-time curve
AUC0-14d	Area under the concentration-time curve over a 14-day dosing interval
AUC0-21d	Area under the concentration-time curve over a 21-day dosing interval
AUC0-∞	AUC from time zero extrapolated to time infinity
AUCave	average AUC
AUCavg	Average AUC prior to the first event
AUCavg15	Average AUC from Week 1 to Week 15
AUCavg24	Average AUC from Week 1 to Week 24
AUCavg48	Average AUC from Week 1 to Week 48
AUCss	Area under the concentration-time curve at steady state
AUROC	Area under the receiver operating characteristic curves
Baso E	basophilic erythroblast
BFU-E	burst forming units-erythroid (early stage)
BLQ	Below the limit of quantification
BM	bone marrow
BMD	bone mineral density
BMI	body mass index
BMP	bone morphogenetic protein
BRE	bone morphogenetic protein responsive element
BSC	best supportive care
BW	Body weight
CBC	complete blood count
CFU-E	colony forming units-erythroid (later stage)
CI	confidence interval
CL/F	apparent clearance
Cmax	maximum plasma concentration of drug
Cmax,ss	Maximum concentration at steady state
CSR	clinical study report

CV	Coefficient of variation
DART	Developmental and reproductive toxicity
DMC	Data Monitoring Committee
DS	Day of study
dw	dry weight
DXA	dual-energy x-ray absorptiometry
ECD	extracellular domain
ECOG	Eastern Cooperative Oncology Group
EFD	Embryo-fœtal development
eGFR	estimated glomerular filtration rate
EOS	end of study
EOT	end of treatment
EPO	erythropoietin
E-R	exposure-response
ESA	erythropoiesis-stimulating agent
ETA	Random inter-subject effect (η)
FACIT-F	Functional Assessment of Chronic Illness Therapy-Fatigue
FACT-An	Functional Assessment of Cancer Therapy-Anemia Scale
FDA	Food and Drug Administration
FSH	Follicle stimulating hormone
GD	Gestational day
GDF	growth differentiation factor
GLP	Good laboratory practice
hActRIIB-hIgG1	human activin type IIB receptor fused to the Fc portion of human IgG1
hActRIIB-mIgG2a	human activin type IIB receptor ECD fused to murine Fc
Hb	haemoglobin
Hbb	haemoglobin beta
Hbbth1/th1	mouse β-thalassemia model
Hct	haematocrit
Hgb	Hemoglobin
HgbE	hemoglobin E
HR	Hazard ratio
HRQoL	health-related QoL
IC50	half maximal inhibitory concentration
ICF	informed consent form
ICT	iron chelation therapy
IIV	Interindividual variability
ITT	intent-to-treat
IV	intravenous

Ka	Absorption rate constant
KLH	Keyhole limpet hemocyanin
LD	Lactation day
LH	Luteinising hormone
LIC	liver iron concentration
LS	least squares
MAA	marketing authorisation application
MDS	Myelodysplastic syndromes
moA	mechanism of action
MRI	magnetic resonance imaging
N	Number of subjects
NCI-ODWG	National Cancer Institute-Organ Dysfunction Working Group
NOAEL	No observed adverse effect level
NTD	nontransfusion-dependent
NUP98-HOXD13	MDS mouse model
OR	Odds ratio
Ortho E	orthochromatic erythroblast;
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PND	Postnatal day
Poly E	polychromatic erythroblast
Pro E	pro-erythroblast
Q3W	Once every three weeks
QoL	quality of life
QTc	Corrected QT interval
QTcF	Corrected QT interval using Fridericia's equation
RAP-536	same modified human ActRIIB as in ACE-536, but with murine Fc
RBC	red blood cell
RBC-T	red blood cell transfusion
RBC-TI	red blood cell transfusion independence
Retic	reticulocyte
RV	Residual variability
s.c.	subcutaneous
SAP	statistical analysis plan
SC	Subcutaneous
SCD	sickle cell disease
SCE	Summary of Clinical Efficacy
SD	standard deviation
SF-36	36-item Short Form Health Survey

Sig	Statistically significant
SPR	surface plasmon resonance
t1/2	Terminal half-life (noncompartmental analysis) or elimination half-life (compartmental analysis)
TD	transfusion-dependent
TDAR	T-cell dependent antibody response
TEADA	Treatment-emergent anti-drug antibodies
TEAE	Treatment-emergent adverse event
TGFß	transforming growth factor beta
Tmax	Time to reach Cmax
TranQoL	transfusion-dependent QoL questionnaire
ULN	Upper limit of normal
V1/F	Apparent volume of distribution of the central compartment
WOCBP	Women of child bearing potential
ΔQTcF	Baseline-adjusted QTcF
ΔΔQTcF	Placebo-corrected baseline-adjusted QTcF

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Celgene Europe BV submitted on 26 April 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Reblozyl, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

Reblozyl was designated as an orphan medicinal product EU/3/14/1300 on 29.07.2014 in the following condition: treatment of beta (β)-thalassaemia intermedia and major.

Reblozyl was designated as an orphan medicinal product EU/3/14/1331 on 22.08.2014 in the following condition: treatment of myelodysplastic syndromes (MDS).

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Reblozyl as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/en/medicines/human/EPAR/reblozyl

The applicant applied for the following indication: Reblozyl is indicated for the treatment of adult patients with very low, low and intermediate risk ring sideroblast positive myelodysplastic syndromes (MDS) associated anaemia who require red blood cell transfusions and have received or are not eligible for erythropoietin-stimulating agent therapy.

Reblozyl is indicated for the treatment of adult patients with beta-thalassaemia (β-thalassaemia) associated anaemia who require red blood cell transfusions.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0130/2019 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMEA-001521-PIP01-13-M02 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan mediciIntnal products.

Applicant's request(s) for consideration

New active Substance status

The applicant requested the active substance luspatercept contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Milena Stain Co-Rapporteur: Ewa Balkowiec Iskra

The application was received by the EMA on	26 April 2019
The procedure started on	23 May 2019
The Rapporteur's first Assessment Report was circulated to all CHMP members on	12 August 2019
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	12 August 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	27 August 2019
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	5 September 2019
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	19 September 2019
The applicant submitted the responses to the CHMP consolidated List of Questions on	20 December 2019
The following GCP inspections were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GCP inspection at 3 sites (Thailand, US and Canada) between August and October 2019. The outcome of the inspection carried out was issued on.	2 December 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	03 February 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 February 2020
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	27 February 2020

The applicant submitted the responses to the CHMP List of Outstanding Issues on	31 March 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	15 April 2020
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	N/A
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Reblozyl on	30 April 2020
The CHMP adopted a report on similarity of Reblozyl with Revlimid and Zynteglo on (Appendix 1)	30 April 2020

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Myelodysplastic syndromes (MDS)

MDS are a group of clonal bone marrow (BM) neoplasms, which represent the most common class of acquired BM failure syndromes in adults (Ades, 2014; Bejar, 2014). Myelodysplastic syndromes are characterised by ineffective haematopoiesis, in which haematopoietic progenitor cells have reduced ability to differentiate and increased likelihood of apoptosis (Ades, 2014; Foran, 2012). This manifests in abnormal 'dysplastic' cell morphology in one or more haematopoietic cell lines and development of peripheral cytopenias (Ades, 2014; Bejar, 2014; Foran, 2012).

In approximately 30% of patients with MDS, abnormal cell morphology/biology results in the potential for clonal evolution and development of acute myeloid leukemia (AML) (Bejar, 2014; da Silva-Coelho, 2017).

In the MDS Multicentre Registry study, the median time of survival from diagnosis was 75 months (range, 1.7 to 350 months). The 2- and 5-year survival probabilities were 86% and 61%, respectively. Transfusion-dependent (TD) patients had a median survival of 44 months compared to 97 months for transfusion-independent patients (Germing, 2012).

β-Thalassaemia

The β-thalassaemias are a group of inherited disorders characterised by absent or reduced production of the β-globin chains of haemoglobin (Hgb), the oxygen-carrying molecule in human RBCs. There are two beta globin genes (one on each chromosome). The mutations can result in reduced expression (beta+) or complete absence of expression (beta0) of the β-globin chains. The severity of disease correlates with the imbalance between alpha and beta globin chains. Because beta globin expression begins during infancy (gamma globin is the beta-like chain used in foetal and early infant haemoglobins), the thalassaemia phenotype generally begins to manifest during the first year of life (typical age of presentation, four to six months).

2.1.2. Epidemiology

MDS

An analysis of 64 cancer registries from European countries indicates that the incidence of MDS was 1.5 per 100,000 individuals per year in 1995–2002 (Visser, 2012). Data from the Dusseldorf registry in Germany suggest that the crude incidence of MDS in 2002–2005 was approximately 4 per 100,000 person-years (3.40 for MDS as defined by World Health Organization [WHO] subtypes versus 4.15 using French-American-British [FAB] classification) (Neukirchen, 2011). The annual incidence of MDS reported by the Haematological Malignancy Research Network (HMRN) in the UK between 2010 and 2016 is 3.5 per 100,000 (HMRN, 2019). Incidence of MDS extracted from population-based registries such as SEER in the US and similar databases worldwide may not accurately capture the true number of MDS cases due to underdiagnoses and underreporting (Cogle, 2011).

Data consistently demonstrate that MDS is most commonly diagnosed in patients with a median age at diagnosis in the seventh decade (Ma, 2012; Neukirchen, 2011) and is reported more frequently in men versus women (Ma, 2007; Neukirchen, 2011; Visser, 2012).

β-Thalassaemia

Large-scale epidemiologic studies of patients with β-thalassemia are lacking. Most available epidemiologic data have been estimated using carrier frequency rates determined in screening programs, or stem from expert opinion of researchers conducting large-scale observational studies. About 80 to 90 million people (~1.5% of the global population) are carriers of β-thalassaemia with approximately 60,000 symptomatic individuals born annually (Modell, 2007). The annual incidence of symptomatic individuals is estimated at 1 in 100,000 worldwide and 1 in 10,000 in the European Union (EU) (Galanello, 2010). Incidence is highest in the Mediterranean region, the Middle East, North America and South East Asia (particularly India, China, Thailand and Indonesia; this region accounts for approximately 50% of affected births).

The highest prevalence of the structural variant HgbE is observed in the Indian subcontinent and East and Southeast Asia, where carrier frequencies may reach as high as 80% (Olivieri, 2010; Weatherall, 2005; Weatherall, 2010). Haemoglobin E/β-thalassaemia currently affects around 1,000,000 people worldwide (Olivieri, 2011) and more than 19,000 affected children are born each year, half of them having TD β-thalassaemia (Modell, 2008; Weatherall, 2010). It should also be noted that, in view of continued migration, β-thalassaemia is now also becoming increasingly common in large multi-ethnic cities in Europe and North America, as evident from newborn screening programs (Harteveld, 2010; Lorey, 2000; Lorey, 2001; National Sickle Cell and Thalassaemia Screening Programme, 2018; Piel, 2016). Thalassaemia today continues to pose a public health concern owing to its chronic disease burden and need for long-term management (Weatherall, 2010).

2.1.3. Biologic features, aetiology and pathogenesis

MDS

The cause of MDS is known only in 15% of cases (Ades, 2014; Fenaux, 2014). In approximately 30% of pediatric patients with MDS, the disease is due to an inherited predisposition, such as Down's syndrome, Fanconi anaemia, and neurofibromatosis (Ades, 2014; Fenaux, 2014). In adult patients without inherited predisposition, MDS may be attributed to a number of factors, including older age, prior treatment with chemotherapy agents or radiotherapy, and exposure to environmental irritants (Ades, 2014; Fenaux, 2014; Foran, 2012). Advanced age is the single greatest risk factor (Sekeres, 2010).

An abnormal karyotype is observed in approximately 40% to 60% of *de novo* MDS cases and 50% to 80% of cases in which prior therapy resulted in MDS (Ades, 2014; Bejar, 2014; Haase, 2007; Visconte, 2014a). Unlike AML, MDS is characterised by partial or complete loss or gain of chromosomes, the most frequent being: deleted 5q; −7 or deleted 7q; +8; deleted 20q; and deleted 17p (Ades, 2014). The chromosomal abnormality del(5q) leads to haplo-insufficiency of a variable number of genes according to the extent of the deleted region (Ades, 2014) and is one of the best understood pathogenic mechanisms in MDS.

Almost 80% of patients with MDS carry at least one mutation in one gene (Papaemmanuil, 2013). While founder mutations drive asymptomatic local expression, further mutations acquired within a clone will subsequently impair normal haematopoiesis, alter blood counts, and ultimately result in overt MDS (Kennedy, 2017; Papaemmanuil, 2013). Indeed, genomic instability (genetic defects, mutations) increases the propensity to develop AML (Papaemmanuil, 2013; Visconte, 2014a).

β-Thalassaemia

The β-thalassaemias are a group of inherited disorders characterised by absent or reduced production of the β-globin chains of haemoglobin (Hgb). Mutations in the β-globin gene can be passed on from each of the 2 carrier parents to affected offspring in a recessive Mendelian manner.

2.1.4. Clinical presentation, diagnosis

MDS

Many patients with MDS are asymptomatic; the lack of specific symptoms among patients with lower-risk MDS is a major diagnostic challenge (Foran, 2012). Diagnosis is often made during assessment of comorbidities, when peripheral blood or bone marrow (BM) features associated with MDS are revealed. Differential diagnosis is informed by the patient's medication history; exclusion of diseases, such as autoimmune disorders, renal failure, malignancies, chronic infections or inflammations, aplastic anaemia, and paroxysmal nocturnal haemoglobinuria, is also important (Fenaux, 2014).

The clinical presentation of MDS is heterogeneous and varies depending on the subtype and severity of the cytopenia (Foran, 2012). Anaemia is the most common peripheral erythroid maturation defect, occurring in 80% to 85% of cases (Steensma, 2006). Many patients with MDS who develop anaemia, particularly those with Revised International Prognostic Scoring System (IPSS-R) lower-risk MDS (approximately 40%), become transfusion dependent (Zeidan, 2013; Ades, 2014). Thrombocytopenia is observed in 30% to 45% of patients with MDS and neutropenia in 40% of patients (Steensma, 2006). Symptoms are usually nonspecific but may be suggestive of the cytopenia involved. Fatigue and a decline in activities of daily living or quality of life (QoL) are indicative of anaemia. Recurrent infections may be related to neutropenia, and frequent and unexplained bruising or bleeding is suggestive of thrombocytopenia (Ades, 2014; Foran, 2012; Toma, 2012).

Patients with higher-risk MDS present with more severe symptoms (Foran, 2012). Analysis of the BM and blood samples is central to the diagnosis (Ades, 2014; Fenaux, 2014) and facilitates the exclusion of non-MDS causes of cytopenias (Ades, 2014; Bejar, 2014; Zini, 2017). Analysis of somatic mutations and flow cytometry analysis of BM cells can be useful when a diagnosis of MDS is uncertain (Fenaux, 2014).

β-Thalassaemia

As the adult human Hgb molecule is made up of two α- and 2 β-globin chains, the reduced synthesis of β-globin in patients with β-thalassaemia leads to an imbalance in the α/β-globin chain ratio. Accumulation of excess unpaired α-globin chains leads to formation of hemichromes and inclusion bodies, and premature death of RBCs and their precursors in the bone marrow and peripheral circulation. This process, termed ineffective erythropoiesis, results in a state of chronic anaemia and subsequent pathophysiologic mechanisms involving iron accumulation in vital organs, resulting in significant clinical co-morbidities in affected patients (Taher, 2018).

Patients with TD β-thalassaemia (which includes conventional β-thalassaemia major and severe forms of HgbE/β-thalassaemia) commonly come to clinical attention in early childhood (before 2 years of age) with severe anaemia (< 7 g/dL). These patients require life-long, regular blood transfusion therapy. Patients with NTD β-thalassemia (which includes conventional β-thalassemia intermedia and mild to moderate forms of HgbE/β-thalassemia) present later in childhood or adolescence with mild to moderate anaemia and require no or only occasional transfusions in instances of blood loss or worsening anaemia due to periods of physiological stress (e.g., during infections, surgery, or pregnancy).

Patients with thalassaemia intermedia invariably develop increasing clinical complications from β-thalassaemia, such as thrombotic events or pulmonary hypertension and can become transfusion dependent during their lifetime, regardless of whether they required transfusions earlier in life (Guidelines Management of Non Transfusion Dependent Thalassaemia, 2013).

2.1.5. Management

MDS

The main clinical guidance for the management of MDS in Europe is the European Society for Medical Oncology (ESMO) (Fenaux, 2014). Management of patients with MDS differs depending on the ultimate goal of treatment for each individual (Zeidan, 2013; Zeidan, 2017). Treatment of MDS is complicated by the advanced age of the affected population and, thus, high incidence of comorbid conditions and relative inability of patients to tolerate intensive treatment approaches (Greenberg, 2017). In addition, there is a growing role for consideration of genetic and epigenetic lesions in therapeutic decision-making.

- Patients with lower-risk MDS have a lower likelihood of AML progression and a longer survival expectancy (Fenaux, 2014); management of this population is focused on treating cytopenias (predominantly anaemia) and optimising QoL (Fenaux, 2014).

- Patients with higher-risk MDS are at greater risk of progression to AML and have shorter survival expectancy (Fenaux, 2014). Consequently, the aim of treatment in this population is to modify the natural disease course (Fenaux, 2014).

All patients with MDS receive supportive care (NCCN, 2018), which may include blood transfusions, clinical monitoring, management of iron overload, QoL assessment, and psychosocial support (Fenaux, 2014; NCCN, 2018).

Treatment of IPSS-R Lower-risk Patients with MDS

The main focus of management in patients with IPSS-R lower-risk MDS is the treatment of cytopenia(s) and the improvement of QoL (Fenaux, 2014). The specific treatment approach will differ according to the type of cytopenia(s) observed, and there are benefits and challenges associated with currently available first-line treatment options. The majority (80% to 85%) of patients with MDS develop anaemia (Steensma, 2006). Erythropoiesis-stimulating agents (ESAs) are the first-line treatment option for anaemia in lower-risk MDS patients without del(5q) (Fenaux, 2014; NCCN, 2018). Despite an initial response to ESA treatment, approximately 70% of patients will become unresponsive to ESAs (Park, 2017). Careful monitoring for disease progression and consideration of the patient's preferences remain important factors in the tailoring of treatment in this population (NCCN, 2018).

Erythropoiesis-stimulating Agents

As anaemia is the most common cytopenia among patients with lower-risk MDS (Steensma, 2006), it tends to be the focus of treatment in this population (Fenaux, 2013; Fenaux, 2014). Erythropoiesis-stimulating agents, with or without granulocyte-colony stimulating factor (G-CSF), are used in patients with lower-risk MDS without del(5q) (Fenaux, 2014; NCCN, 2018). The major favourable prognostic factors for response to ESAs are low (< 2 units/month) or no RBC transfusion requirement and baseline serum EPO level < 500 U/L (Fenaux, 2013). The European ESA Scoring System uses a serum EPO level of ≤ 200 U/L as a prognostic factor for ESA responsiveness (Santini, 2013). Despite an initial response to ESA treatment, approximately 70% of patients become unresponsive to ESAs (Park, 2017).

Erythroblast differentiation and maturation during late-stage erythropoiesis is independent of the effect of erythropoietin (Eshghi, 2007; Hattangadi, 2011). Mutations in SF3B1 can cause impaired late-stage RBC differentiation (Obeng, 2016).

There are limited treatment options for lower-risk MDS patients who fail treatment with ESAs or have poor prognostic factors of response to ESAs (Santini, 2016). The second-line treatment options are restricted to anti-lymphocyte globulin and anti-thymocyte globulin (immunosuppressive therapies) in the < 60 to 65-year age category, and azacitidine and lenalidomide, both of which are myelosuppressants. Outcomes remain suboptimal despite the use of these second-line treatment options, and many patients will ultimately require long-term RBC transfusions (Fenaux, 2014; NCCN, 2018).

Lenalidomide

Lenalidomide (Revlimid) is recommended treatment of lower-risk patients with MDS with del(5q) (Fenaux, 2014; NCCN, 2018). In Europe, lenalidomide is indicated for use in adult patients with transfusion-dependent anaemia due to low- or intermediate-1-risk MDS associated with an isolated deletion 5q cytogenetic abnormality when other therapeutic options are insufficient or inadequate (Revlimid SmPC, 2019). Lenalidomide in MDS patients with del(5q) yields RBC-transfusion independence (RBC-TI) in two-thirds of this population for a median duration of 2 to 3 years, and cytogenetic responses in 50% to 70% of patients (Ades, 2014). The most common Grade 3 or 4 adverse events included myelosuppression (neutropenia, 55%; thrombocytopenia, 44%) that often requires treatment interruption or dose reduction (NCCN, 2018).

Red Blood Cell Transfusions

RBC transfusion forms the mainstay of treatment in patients with lower-risk MDS and anaemia. Many patients become transfusion dependent, which is associated with increased morbidity, a reduced QoL, and high social burden (Fenaux, 2013; Hellström-Lindberg, 2013; Platzbecker, 2012).

There is no internationally recognised haemoglobin level threshold below which transfusion should be given, and the use of transfusion should be tailored to the individual patient based on comorbidities (e.g., lung and cardiac function, the individual's level of physical activity) (Hellstrom-Lindberg, 2013). Guidelines based on expert opinion, such as those provided by ESMO, recommend administering transfusions at haemoglobin levels starting in the 8 to 10 g/dL range, since patients with comorbidities, poor functional tolerance, or poor QoL may experience significant clinical benefit from transfusions (Fenaux, 2014).

Red blood cell transfusions are time consuming, can have a deleterious effect on a patient's social functioning, increase the patient's dependence on the medical system, affecting the use of hospital resources and the supply of RBC concentrates (Fenaux, 2013; Hellström-Lindberg, 2013; Platzbecker, 2012). Chronic transfusions lead to secondary iron overload. The relatively long survival of low- and intermediate-risk MDS groups (Greenberg, 2006; Valent, 2007) places them at an increased risk of damage by iron overload from prolonged red blood cell transfusions compared to high-risk patients with a markedly reduced survival (Steensma, 2006). The MDS population consists mainly of elderly patients with comorbid conditions and a propensity to develop cardiac failure, infection, haemorrhage, and hepatic cirrhosis; iron overload may exacerbate these pre-existing conditions (Malcovati, 2005).

Iron Chelation Therapy

Iron chelation may be required in patients receiving frequent transfusions in order to avoid iron-related cardiac, hepatic, and endocrine toxicities (Fenaux, 2014; Platzbecker, 2012; Steensma, 2013). As such, monitoring of the number of RBC transfusions, cardiac function with magnetic resonance imaging, and serum ferritin levels is recommended (NCCN, 2018).

There is a lack of consensus on the use of iron chelation in patients with lower-risk MDS. Initiation of iron chelation is generally recommended in management guidelines although specific treatment thresholds and prescribing practice vary (Fenaux, 2014; NCCN, 2018). Guidelines generally advocate starting iron chelation in patients with a relatively favourable prognosis (e.g., IPSS low- or intermediate-1-risk MDS), who have received 20 to 60 RBC concentrates, or if serum ferritin rises above 1000 U/L (Bennett, 2008; Fenaux, 2014; Malcovati, 2013).

Unmet Medical Need

The majority (80% to 85%) of patients with MDS develop anaemia (Steensma, 2006). Erythropoiesis-stimulating agents are the first-line treatment option for anaemia in lower-risk patients with MDS without del(5q); lenalidomide is the recommended treatment for patients with del(5q) (Fenaux, 2014; NCCN, 2018). Despite an initial response to ESA treatment, approximately 70% of patients will become unresponsive to ESAs (Park, 2017). In addition, ESAs are less effective in patients with either endogenous EPO level $\geq$ 200 U/L or those requiring RBC transfusion of $\geq$ 2 units/month.

Treatment options remain suboptimal in the lower-risk MDS patients who are not eligible or no longer respond to ESAs, and many patients will ultimately require long-term RBC transfusions (Fenaux, 2014; NCCN, 2018). Red blood cell-transfusion dependence and lower haemoglobin levels have been associated with a deleterious impact on outcomes and increased mortality in patients with MDS (Platzbecker, 2012; Fenaux, 2013; Hellström-Lindberg, 2013).

<u>β-Thalassaemia</u>

In patients with TD β-thalassemia, transfusion and iron chelation therapy (ICT) have been the mainstay of therapy from childhood to adulthood. The objective of disease management in TD and NTD β-thalassemia is to prevent the development or progression of serious and irreversible morbidities as patients transition to adulthood, through interventions targeting the key pathophysiologic mechanisms of ineffective erythropoiesis, anaemia, and iron overload. The current wider scope of management also includes monitoring for and treatment of specific clinical complications as well as lifestyle modifications, and psychosocial therapy. As such, management of patients requires close monitoring and regular follow up from early childhood throughout adulthood through multidisciplinary teams from various medical specialties (Cappellini, 2014a; Taher, 2013a; Taher, 2018).

Transfusion Therapy

Transfusion works by supplying normal erythrocytes and thus decreasing the demand for erythropoiesis, ultimately suppressing ineffective erythropoiesis and its associated pathophysiology. Management guidelines recommend initiation of regular transfusion therapy in patients with TD β-thalassemia with a total Hgb level of < 7 g/dL (in the absence of infection) or > 7 g/dL, but with poor growth and development, aiming at a pre-transfusion Hgb target of 9 to 10.5 g/dL (11 to 12 g/dL in those with heart disease) (Cappellini, 2014a; Musallam, 2013a).

The main challenge with regular transfusion therapy is secondary iron overload. Transfusional iron overload causes significant morbidity and mortality. In patients with TD β-thalassemia, iron accumulation in vital organs such as the liver and heart is evident in children aged as young as 2 and 6 years, respectively (Berdoukas, 2013; Borgna-Pignatti, 2014a; Wood, 2008; Yang, 2014), with cumulative iron overload subsequently leading to organ dysfunction in the heart, liver, and endocrine glands (Cappellini, 2014a). Iron-related cardiomyopathy has been the leading cause of death in patients with TD β-thalassemia (Borgna-Pignatti, 2004; Cappellini, 2014a), and mortality from liver disease is also increasing (Voskaridou, 2012).

Iron Chelation Therapy

In view of the detrimental effects of iron overload in patients with TD β-thalassemia (secondary, via transfusions) and NTD β-thalassemia (primary, via intestinal absorption), prompt diagnosis and management are essential in these patient populations.

Three iron chelators are currently approved for the treatment of iron overload in patients with TD β-thalassemia: Deferoxamine in SC or intravenous injection, oral deferiprone in tablet or solution form, and oral deferasirox in dispersible tablet and, more recently, film-coated tablet forms (Cappellini, 2014a; Musallam, 2013a; Taher, 2018). Although progress in ICT was associated with clear improvement in survival in long-term follow-up studies evaluating different birth cohorts of patients with TD β-thalassemia (Borgna-Pignatti, 2004; Modell, 2000), the situation is not yet ideal. Recent data from a large global sample of patients with TD β-thalassemia (N = ~1000) showed that over half of patients continue to show high-risk iron overload levels in the heart and liver, reflecting that optimal control of iron overload in the global population of TD β-thalassemia has not yet been achieved (Aydinok, 2015).

All available chelators are also associated with several adverse events (AEs) (e.g. ocular, auditory, bone growth retardation, local reactions, allergy and gastrointestinal, arthralgia, agranulocytosis/ neutropenia, increased creatinine, increased hepatic enzymes). Although these AEs are usually non-serious and manageable, they require close clinical and laboratory monitoring (e.g., neutrophil count, creatinine, and hepatic enzymes), which remains cumbersome in a chronic disease with lifelong therapy (Cappellini, 2014a; Musallam, 2013a; Taher, 2018). Additionally, ensuring adherence to ICT is essential as it correlates with both effective management and patient survival (Delea, 2007a; Gabutti, 1996).

The limitations in efficacy, safety, and adherence are also coupled with poor access to, or affordability of, oral ICT, especially in resource-constrained countries where the disease is most prevalent.

Splenectomy

Although not supported by data from clinical trials, splenectomy has been performed traditionally as an alternate or adjunct to transfusion therapy in certain patients for improvement of Hgb concentration, growth, and quality of life (Premawardhena, 2005). Splenectomy has become almost obsolete in patients with TD β-thalassemia (Piga, 2011), but the procedure is still used sporadically in patients with NTD β-thalassemia to "boost" Hgb levels (Musallam, 2012b; Olivieri, 2012; Olivieri, 2008; Taher, 2011; Vichinsky, 2012). Recent guideline recommendations, however, have restricted the use of splenectomy to patients who are unable to receive transfusion and ICT, as well as those with clinically symptomatic splenomegaly or hypersplenism (Cappellini, 2014a; Taher, 2013a).

Hematopoietic Stem Cell Transplantation

Haematopoietic stem cell transplantation (HSCT) is the only available curative therapy for patients with β-thalassaemia. Almost 90% of patients with TD β-thalassaemia who undergo HSCT at experienced centres in Europe now survive, with 2-year disease-free survival rates of > 80% (Baronciani, 2016). Availability of matched donors remains a key limitation (available in only ~20% to 25% of cases) (Angelucci, 2014; Cappellini, 2014a). Moreover, several factors continue to limit the acceptability of HSCT: an overall mortality risk of 12% within 2 years of transplantation, acute and chronic graft versus host disease and graft failure, and the need for complete myeloablation that can result in infertility and other toxicities (Baronciani, 2016; Cappellini, 2014a; Taher, 2013a). The best clinical outcomes of HSCT are reported in patients with TD β-thalassemia aged < 14 years at transplantation (Baronciani, 2016); this is likely due to older patients having existing morbidity related to iron overload and other complications (Higgs, 2012). The use of unrelated donors and umbilical cord blood are not uncommon, but still mostly experimental.

Zynteglo

In May 2019, Zynteglo has been approved in the EU, for the treatment of patients 12 years and older with transfusion dependent β-thalassaemia who do not have a β0/β0 genotype and for whom HSCT is appropriate, but an HLA matched related HSC donor is not available.

Unmet Medical Need

There is no available therapy approved or widely used to address the underlying ineffective erythropoiesis and anaemia of β-thalassemia, the source of clinical morbidity and diminished quality of life. In patients with TD β-thalassemia, transfusion and ICT have shown a clear survival benefit. However, administration of regular and chronic transfusion therapy continues to pose challenges for patients' quality of life, also presenting a public health burden. Challenges with access and affordability of transfusion exist especially in resource-constrained countries. Moreover, transfusional iron overload necessitates iron overload monitoring and ICT. The efficacy and safety of current iron chelators are not ideal, with persisting challenges in adherence, access, and affordability.

Hematopoietic stem cell transplantation is an established option in patients with TD β-thalassemia, but its use is limited to small groups of younger patients with available matched donors who can tolerate the procedure. Splenectomy is primarily restricted to patients with splenomegaly or hypersplenism because of the high risk of infections and vascular disease following the procedure.

Clearly, there is an unmet need for the development of novel therapies that can ameliorate the ineffective erythropoiesis and anaemia of β-thalassemia, and subsequently prevent the development of morbidity and poor quality of life.

About the product

Luspatercept (ACE-536) is a recombinant fusion protein that binds selectively to transforming growth factor-beta (TGF-β) superfamily ligands. By binding to specific endogenous ligands (e.g., GDF-11, activin B), luspatercept inhibits Smad2/3 signalling, resulting in erythroid maturation through differentiation of late-stage erythroid precursors (normoblasts) in the bone marrow. Smad2/3 signalling is abnormally high in disease models characterized by ineffective erythropoiesis, i.e., myelodysplastic syndromes (MDS) and β-thalassemia, and in the bone marrow of MDS patients.

<u>MDS-associated anaemia</u>

The initially claimed indication was "Reblozyl is indicated for the treatment of adult patients with anaemia requiring transfusions due to very low- to intermediate-risk myelodysplastic syndromes (MDS), who have ring sideroblasts and loss or lack of response to, are ineligible for, or intolerant to erythropoiesis-stimulating agent (ESA) therapy".

The indication was later slightly amended to "Reblozyl is indicated for the treatment of adult patients with transfusion-dependent anaemia due to very low, low and intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts, who had an unsatisfactory response to or are ineligible for erythropoietin-based therapy (see section 5.1).

<u>Beta-thalassaemia associated anaemia</u>

The initially claimed indication was 'Reblozyl is indicated for treatment of adult patients with anaemia requiring transfusions due to beta thalassaemia (β thalassaemia).'

This was changed during assessment to "Reblozyl is indicated for the treatment of adult patients with transfusion-dependent anaemia associated with beta-thalassaemia (see section 5.1)" to more adequately reflect the target population.

Type of Application and aspects on development

The global clinical development program for luspatercept consists of a total of 9 clinical studies across 3 different disease states: myelodysplastic syndromes (MDS), β-thalassaemia, and myelofibrosis.

Of these studies, 3 are included in the global clinical development program for MDS, all of which are ongoing and provide efficacy and safety data for this submission. These studies include a pivotal Phase 3, randomized, double-blind, placebo-controlled study (ACE-536-MDS-001; MEDALIST) and 2 supportive Phase 2, open-label, single-arm studies (multiple ascending-dose base Study A536-03 and extension Study A536-05). Efficacy and safety data through the clinical data cut-off date for each study are included in this submission, including at least 48 weeks of double-blind treatment data in Study ACE-536-MDS-001.

4 studies are included in the global clinical development program for β-thalassaemia, 3 of which have completed enrolment and provide efficacy and safety data for this submission. These studies include a pivotal Phase 3, randomized, double-blind study (ACE-536-B-THAL-001; BELIEVE) and 2 supportive, sequential Phase 2, open-label, single-arm studies (multiple ascending-dose base Study A536-04 and extension Study A536-06). Study A536-04 is complete; the long-term extension Phase 2 Study A536-06 is ongoing for a total treatment duration of up to 5 years; and the long-term extension Phase 3 Study ACE-536-B-THAL-001 is ongoing until all subjects initially assigned to luspatercept in the double-blind treatment phase complete a total treatment duration of 5 years from first dose. Efficacy and safety data through the clinical data cut-off data for each study are included in this submission, including at least 48 weeks of double-blind treatment data in Study ACE-536-B-THAL-001.

MDS

In the development of the study design, Appendix 4 to the Guideline on the Evaluation of Anticancer Medicinal Products in Man (EMA/CHMP/703715/2012, dated 13 Dec 2012) was taken into account. Celgene also received scientific advice on the Phase 3 MDS study design from the Medicines Evaluation Board (MEB; Netherlands) on 28 Jul 2015 and from the Medical Products Agency (MPA; Sweden) on 26 Aug 2015. The final study design of Study ACE-536-MDS-001 was amended to incorporate the advice received from both agencies on the proposed patient population, stratification factors, randomization, the placebo comparator arm, primary and key secondary endpoints, the statistical analysis plan, and the proposed long-term follow up period.

β-Thalassaemia

Scientific advice from the CHMP was given in June 2015 (EMEA/H/SAH/044/1/2015/PA/III). Key feedback was received on the antidrug antibody (ADA) testing plan and the β-thalassemia clinical development plan, specifically the design of the TD Phase 3 study (Study ACE-536-B-THAL-001) that provides pivotal clinical data to support the use of luspatercept as a treatment for β-thalassemia in this Marketing Authorization Application (MAA). The key points raised by the CHMP on the immunogenicity testing plan for luspatercept were incorporated into the testing plan for the studies and the immunogenicity risk management strategy.

In addition, the applicant received EMA Scientific advice concerning the CMC development (EMA/CHMP/SAWP/716191/2017) in September 2017. The questions addressed the introduction of a new working cell bank (WCB2), the data package for the change from Fiolax to TopLyo vials as primary container closure system for drug product, the robustness studies for drug product lyophilisation progress, the proposals for drug substance and drug product manufacturing process validation, the approach to establish the control strategy, and finally the control strategy for N-glycans. In general, the recommendations given in this advice have been taken into account; appropriate information to address the concerns raised in the scientific advice has been included into the dossier.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as powder for solution for injection containing 25 mg or 75 mg of luspatercept as active substance. After reconstitution, each mL of solution contains 50 mg luspatercept.

Other ingredients are: citric acid monohydrate (E330), sodium citrate (E331), polysorbate 80, sucrose, hydrochloric acid (for pH adjustment), sodium hydroxide (for pH adjustment) as described in SmPC section 6.1.

The product is available in 3 mL Type I glass vial with a hydrophobic inner coating closed with a bromobutyl rubber stopper and aluminium seal with yellow or orange (for the 25 mg and the 75 mg strength respectively) polypropylene flip-off cap, as described in the SmPC section 6.5.

2.2.2. Active Substance

General information

Luspatercept is a recombinant fusion protein consisting of two identical chains, each consisting of the modified form of the extracellular domain (ECD) of human activin receptor type IIB (ActRIIB) linked to the human IgG1 Fc domain (including the hinge, CH2 and CH3 domains) through a linker.

The average molecular mass calculated using the most abundant glycoform observed on each N-glycosylation site and the most abundant form of O-glycan occupancy is 94kDa. A list of physicochemical and other relevant properties of the active substance, including the mode-of-action is given.

Luspatercept binds selectively to TGF-β superfamily ligands. By binding to specific endogenous ligands (e.g., GDF-11, activin B) luspatercept inhibits Smad2/3 signalling, resulting in erythroid maturation through differentiation of late-stage erythroid precursors (normoblasts) in the bone marrow. Smad2/3 signalling is abnormally high in disease models characterised by ineffective erythropoiesis, i.e. myelodysplastic syndromes (MDS) and β-thalassemia, and in the bone marrow of MDS patients.

Manufacture, characterisation and process controls

The manufacturing of the active substance takes place at Lonza Biologics Tuas Pte Ltd located in Singapore.

The process is typical for a monoclonal antibody/recombinant fusion protein with the Fc domain of an IgG1: The commercial manufacturing process begins with the thawing of a single vial from the Working Cell Bank. The cell culture is expanded in a series of shake flasks followed by further expansion in cell bags. The contents of the cell culture bags are used to inoculate a production bioreactor. The production bioreactor conditions and nutrient feeds are monitored and controlled. The culture is clarified using depth filtration into a bioprocess container, where it is held prior to further downstream processing. Information on the culture duration and agitation speed were provided.

The downstream process includes three chromatography steps, low pH viral inactivation, viral filtration, ultrafiltration/diafiltration (UF/DF) and final filtration/bulk fill/freezing steps. Luspatercept is stored at ≤ -65°C. The pooling strategy for the chromatography fractions in the downstream process was

sufficiently explained. The batch numbering system has been explained and the batch and scale definition is given.

Control of materials

Raw materials used in the production process have been listed and their quality grade is provided. Compendial materials are tested to the referenced compendia whereas for non-compendial raw materials in-house specifications are in place. The composition of buffers, solutions and cell culture media is given.

Concerning the source, history, and generation of the cell substrate sufficient information on the origin, culture and storage conditions of the host cell line has been included in the dossier. A master cell bank (MCB) was manufactured from a research cell bank, consistent with current Good Manufacturing Practices (cGMPs) and the relevant ICH guidelines. From this master cell bank, two working cell banks have been generated and were used for the production of early clinical batches and for process validation and post validation batches.

Both working cell banks as well as the master cell bank have been extensively characterised and tested for safety. A protocol for the preparation of a replacement of working cell banks has been provided.

Control of critical steps and intermediates

Process parameters and critical process parameters as well as in-process controls (IPCs) and critical in-process controls together with their acceptable ranges or action limits have been provided in a tabulated overview. No IPCs have been established with respect to purity during the purification, this strategy was appropriately justified. The Applicant indicated that in case of exceeding acceptance limits or action limits investigations on possible root causes and the impact of the deviation on the affected batch will be initiated. It was further clarified how excursions of critical in-process controls will be managed. Excursions that lead to batch rejection have been listed. The applicant was asked to perform an evaluation of proline supplementation to improve process robustness as part of lifecycle management. This evaluation will be provided once available; this strategy is supported.

Process validation

Process validation was performed on consecutive process performance qualification (PPQ) batches. The process parameters and in-process control ranges used in the validation were based on protocols with pre-established ranges. Process validation was performed and confirmed that the active substance manufacturing process can perform effectively and reproducibly to produce an active substance meeting its predetermined specifications and quality attributes. This conclusion is further supported by batch data from other post-PPQ batches which met all release criteria. A few deviations which occurred during validation activities have been sufficiently explained and the root cause has been identified, corrective actions have been implemented and the potential impact on product quality and process consistency could be excluded. Thus, the observed deviations do not jeopardize the validity of the conducted process performance qualification. Following the execution of process validation, process parameters and in-process controls were re-evaluated and certain changes were made leading to the commercial process parameters and in-process controls. These changes are considered to enhance the commercial control strategy and, since the PPQ batches meet the acceptance criteria for the post-PPQ changes, do not impact the validated status of the process.

In addition to the process validation, supportive studies including lifetime studies of the ultrafiltration membranes and the chromatography resins, impurity clearance, stability/hold time studies of process intermediates and shipping qualification studies have been conducted. Regarding the chromatography

resin lifetime studies the maximal numbers of cycles have been defined and process parameters set based on appropriate studies; overall the information provided in this respect is deemed satisfactory.

Finally, the validation of reprocessing for a unit operation will be considered complete when a minimum of one reprocessed run has been executed successfully at the commercial scale. This approach is in line with the relevant EMA guideline on process validation.

Manufacturing process development

Product critical quality attributes (CQAs) have been identified via a risk assessment including both impact and uncertainty of the knowledge affecting efficacy and safety. Process characterisation started with an initial risk assessment for each unit operation to identify process parameters for evaluation in process characterisation studies. These parameters were assessed based on their potential impact on CQAs. For each unit operation, a subset of parameters was selected for univariate, multivariate, and excursion studies. The impact of the process parameters on product quality attributes based on risk assessment, results from process characterisation and validation studies is summarised in a graphical layout for each single process manufacturing step. The approach for process characterisation is endorsed.

The two production scale bioreactors used at the manufacturing site for the production of the active substance were qualified for commercial production of luspatercept during validation with successful PPQ batches. Equivalency of the two bioreactors was assessed and confirmed.

The process development starting from Process I, the changes leading to Process II and later to an early Process III, and finally to the intended commercial version of Process III together with the rationale for the process changes has been sufficiently described. After each development step a separate comparability study has been conducted. Data from these comparability studies indicate a comparable quality profile of luspatercept produced via the different process versions. In addition, the change fromone WCB to another has been investigated by comparison of process performance parameters which indicate a comparable process performance.

Characterisation

Concerning the elucidation of structure and other characteristics a panel of standard and state-of-the-art methods have been used to characterise the physicochemical as well as biological quality attributes of the luspatercept molecule. Various orthogonal techniques were used to study the primary structure, carbohydrate structure, higher order structure, the mass, size and charge heterogeneity, and the biological activity. In addition, a number of structure-function relationship studies have been performed which provide further inside in degradation pathways and the criticality of structural changes. The characterisation work was done with the process performance qualification batches as well as with clinical batches derived from the intended commercial version of the active substance manufacturing process. Further information on the qualification status of the characterisation methods has been provided. In addition, possible misfolded forms/incorrect disulfide bonding structures, the Pro-Ala exchange, and the relationship between deamidation and the charged variant profile of heat-stressed samples have been discussed.

Regarding the impurities, a comprehensive discussion on potential process and product-specific impurities as well as on potential elemental impurities in luspatercept has been provided. For process-specific impurities clearance studies have been conducted in the course of process validation. Clearance of process-specific impurities has been shown on a number of luspatercept batches. These studies demonstrate a robust removal of process-specific impurities by the purification process. This conclusion is further supported by additional spiking studies which have been performed for a subset of process-related impurities. Taken together, sufficient evidence is provided that the manufacturing process is capable of consistently removing process-specific impurities to sufficient low levels.

Therefore, it is agreeable that no in-process or release testing of process-related impurities are needed. Product-related impurities have been extensively investigated in structure-function relationship studies in which luspatercept has been exposed to different stress factors. In addition, clearance of high and low molecular weight species has been studied during process validation activities. Furthermore, for high molecular weight species an additional spiking study has been conducted. As for the process-related impurities sufficient evidence is provided that the manufacturing process is capable of consistently removing product-specific impurities to sufficient low levels. This conclusion is also supported by the available batch release data, which confirm that the luspatercept manufacturing process consistently delivers material of high purity. Finally, potential elemental impurities have been addressed via a risk assessment according to ICH Q3D. The conclusion that the overall risk of elemental impurities in active substance is low and thus, no routine testing for these impurities is required is agreed.

Specification

The updated release specifications include tests for identity, protein concentration, purity and impurities, potency as well as tests for general quality attributes.

Concerning the acceptance limits, the provided justifications for each specification are considered acceptable and so are the justifications for parameters omitted such as glycosylation and osmolality. The release specifications for Luspatercept active substance are considered acceptable.

Analytical methods

Method descriptions for the non-compendial methods were provided. Of note, a tabulated overview of method changes during the clinical development has been also provided. Bridging studies to compare the old versus the new methods have been conducted and support these changes.

Non-compendial methods have been validated in accordance with ICH Q2(R1) whereas compendial methods have been verified. The analytical methods for commercial release and stability testing are considered suitable for their intended use.

Batch analysis

Batch release data from 35 batches of luspatercept produced with different process versions have been provided. The release data complied with the release specifications in place at the time of testing and indicate a batch-to-batch consistency.

Reference materials

No national or international reference standard is available for luspatercept. Thus, internal reference standards have been established; the standards were representative for material used in the respective development phase where the standards were being used. A link between the current standards and the standards in use during earlier development phases has been established. The working reference standards have been appropriately characterised using standard and state-of-the-art methods. Storage conditions and the stability protocols for the primary and the working reference standards were given. A protocol for qualification for new reference standards will be submitted via a future variation.

Container closure system

A description as well as drawings of the container closure system have been provided. The suitability of the container closure system was demonstrated by physicochemical, biocompatibility and container closure integrity testing (CCIT). Specifications for the container closure were presented. Summaries of

relevant extractables and leachables studies were provided, which indicate that the 2L PETG bottle and high-density polyethylene (HDPE) closure are suitable for the storage of luspatercept active substance.

Stability

An initial shelf-life of 48 months for active substance when stored at the recommended long-term storage condition of ≤-65°C is proposed.

This shelf-life claim is primarily based on 48 month stability data available from three "registrational" batches. These stability batches were manufactured using the same process and scale as intended for commercial manufacturing and the completed process performance qualification. The container closure system used for these batches was representative to the commercial closure system; same material but smaller volume. It can be agreed that the registrational stability batches are representative of the proposed commercial batches. These three stability batches were stored for 48 months at long term conditions (≤-65°C) and for 6 months at accelerated conditions -20°C, 2-8°C, 25±2°C / 60% RH, and 40±2°C / 75% RH.

Data from another supporting stability batch with data available for 24 months at ≤ -65 °C and for 6 months at -20°C, 2-8°C, 25±2°C / 60% RH, and 40±2°C / 75% RH was presented too.

Additional stability data at long-term storage conditions from active substance batches are available covering 12 months from the process performance qualification batches. Moreover, stability data from two earlier process version batches for up to 36 months at ≤-65 °C and for 6 months at -20°C, 2-8°C, 25±2°C / 60% RH, and 40±2°C / 75% RH were provided.

In general, the provided data support the stability claim at the recommended storage temperature; whereas the observed out-of-specification results for protein concentration could be explained by improper sample preparation before measurement.

Furthermore, stability has also been investigated at accelerated and stress conditions (6 month data for four different temperature conditions -20°C, 5°C, 25°C and 40°C). As expected for recombinant proteins certain degradation trends were observed for various quality attributes when stored at higher temperature conditions. The ongoing long-term stability studies will be continued up to the 60 month time-point.

In general, a shelf-life claim of 48 month when stored at ≤-65°C as proposed above is acceptable.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Luspatercept is formulated as a single-use, sterile, preservative-free, lyophilized powder intended for subcutaneous administration after reconstitution with sterile water for injection (WFI). The bulk solution is compounded at 50 mg/mL. The finished product (FP) is available in two presentations, 25 mg/vial and 75 mg/vial.

The 25 mg/vial presentation contains 0.75 mL fill volume (37.5 mg luspatercept) which includes a 0.25 mL overfill. When reconstituted with 0.68 ml sterile WFI, the resulting solution contains 0.75 ml of 50 mg/mL luspatercept. This reconstituted volume accommodates delivery of up to 0.5 mL of 50 mg/mL luspatercept.

The 75 mg/vial presentation contains 1.75 mL fill volume (87.5 mg luspatercept) which includes a 0.25 mL overfill. When reconstituted with 1.6 ml sterile WFI, the resulting solution contains 1.75 ml of 50 mg/mL luspatercept. This reconstituted volume accommodates delivery of up to 1.5 mL of 50 mg/mL luspatercept.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

Formulation Development

A Quality Target Product Profile (QTPP) for luspatercept powder for solution for injection was established. Three formulations of luspatercept powder for solution for injection have been used throughout clinical development and they have been described. The formulation development comprised a systematic evaluation of multiple buffers, pH ranges, and excipient combinations and their impact on product stability. Based on the results of these studies the final formulation was appropriately defined using a citrate buffer. An overfill study was performed in order to ensure that the required volume of the label claim can be withdrawn from the vials. Reconstitution volume studies confirmed that consistent target volume and target protein concentration is reached after reconstitution. Physicochemical properties of the reconstituted FP were defined.

Manufacturing Process Development

Manufacturing processes evolution and the manufacturing sites involved were discussed.

Critical quality attributes (CQAs) were identified, ranked and discussed in the active substance part. Based on the totality of process experience, release and stability data and structure function characterization the analytical control strategy was developed.

Process parameters and ranges for the respective process steps were determined based on manufacturing experience at commercial site and scale. For each process parameter a risk assessment was performed that was informed by prior experience, and potential impact on product quality and/or performance. Critical process steps were assessed in characterization studies. Relevant quality attributes, were tested in these characterization studies. Critical process parameters (CPPs) were defined. Updated stability data still show that assessed quality attributes stay within their acceptance limits with no apparent trends, which is indicative of a stable lyophilisate. Stability assessment of lyophilization robustness batches is still ongoing.

Analytical comparability was shown between all four processes.

Extractable and leachable studies were performed on all product touching parts of the container closure system. Photostability studies as well as moisture ingress and container closure integrity studies were performed as well. Low endotoxin recovery was assessed and showed no masking of endotoxins. Rubber coring studies confirmed a low risk of stopper coring during routine reconstitution and withdrawal procedure. As these vials are also used for commercial supply, results of the crimping study are directly applicable for development of the commercial process.

For the in-use syringe study of an "aged" batch, the most advanced commercial process batch that was available was used. The in-use test was performed at a time-point, which is about half the time of the proposed shelf-life of 36 months, and thus in principle is not in accordance to expectations of the guideline on in-use stability (CPMP/QWP/2934/99). However, considering the overall FP stability profile, and the fact that in-use results from vials after 2 and 17 months of storage are comparable the results can be considered indicative for the results to be expected at the end of the shelf-life. It was

shown that there is no trend regarding the potency results during in-use syringe stability testing and no absorption to the syringes takes place. It was also confirmed that no degradation of the protein occurred.

Overall, the process development was adequately presented.

Manufacture of the product and process controls

Manufacturers

The FP manufacture is performed at Vetter Pharma-Fertigung GmbH & Co. KG. in Langenargen, Germany. Labeling and secondary packaging is performed at Sharp Packaging Solutions, Hamont-Achel, Belgium. Several laboratories are involved in quality control testing. Importation of active substance and batch release of FP is performed at Celgene Distribution B.V., Utrecht, The Netherlands.

Manufacturing process and controls

Luspatercept FP manufacturing process consists of active substance thawing, compounding, sterile filtration and filling, lyophilization, and vial crimping. The manufacturing process for the two dosage strengths is identical with the exception of the pre-lyophilization fill volume in the vials.

The general batch formula and exemplary formula which is the lower limit of the intended commercial batch size range as well as the respective approximate number of resulting vials were presented.

The results from shipment validation studies showed that there is no impact from transportation on luspatercept FP product quality or container closure integrity.

Critical process parameters (CPPs), process parameters (PPs), in-process controls (IPCs), and critical in-process controls (CIPCs) are appropriately defined and limits were established. The specified processing times and hold times are supported by physicochemical and microbiological data from small-scale studies, process validation or stability studies.

Manufacturing process validation

The commercial control strategy was established during the process design stage based on existing knowledge from process development, risk-based process characterisation studies, and commercial scale runs.

For PPQ (stage 2), three consecutive runs for each presentation was performed. The lower and upper batch size was validated for both dosage strengths. Hold times for each process step were appropriately assessed in the PPQ, including a cumulative hold time study with exceeding time limits for one of each strength to confirm the maximum ranges for the hold times. Suitable additional testing was performed at the process steps. Overall the PPQ strategy covers relevant aspects of the manufacturing process and is acceptable. All relevant process parameters were assessed and set points and ranges were justified by pharmaceutical development.

During execution of the PPQ runs, one Out of Specification (OOS) result for one parameter occurred for one batch which was then excluded from the PPQ and replaced by an additional manufacturing run. The OOS result was investigated and sufficiently discussed. The conclusion was that it was reflective of an aberrant run. Some further deviations concerning this batch were appropriately addressed and measures implemented. All further results of this excluded run were presented together with the PPQ batches and met the acceptance criteria. Overall, it is agreed that the OOS and the deviations had no overall impact on the outcome of the process validation.

Further validation activities appropriately addressed the aseptic processing, the qualification of sterilization equipment and filter validation. For EU commercial supply, a protocol for transport validation was described. The planned continued process verification was briefly described and is acceptable.

Product specification

The release and end of shelf-life specifications for luspatercept powder for solution for injection include appropriate test and limits for general characteristics of the lyophilized product cake, identity, purity, PS 80, endotoxin, and sterility as well as a bioassay for potency and an assay for protein content. The proposed specification parameters are accepteable and those omitted from the specification have been sufficiently justified. Thus, the panel of analytical methods established for batch release of the FP as well as the list of assays for stability assessment is considered adequate. However the finished product potency specification limits should be re-evaluated and the summary of the currently ongoing investigation of the sources of the potency method variability should be reported post approval after a specified number of batches.

Analytical methods

The test methods are mostly identical to those used for control of as the active substance. The non-compendial analytical methods specific for testing of the FP are sufficiently described. Method validations are in line with requirements according to ICH Q2 (R1) and thus acceptable.

For compendial methods a reference to the respective Ph. Eur./USP Monograph is provided and the methods were sufficiently verified. Stability indicating analytical methods were identified.

Overall, the established specification limits were appropriately justified and are considered acceptable.

Batch analysis

In total, batch analysis data of 33 batches throughout development were presented; The results complied with the specification at the time of analysisThe data demonstrate that the manufacturing process reliably delivers consistent and uniform product.

Impurities are the same as those described for the active substance and no new impurities are introduced during FP manufacturing.

Reference materials

The reference standard used for luspatercept FP is the same as that used for luspatercept active substance.

Container closure system

The finished product is packaged in a 3 mL Type I glass vial with a hydrophobic inner coating closed with a bromobutyl rubber stopper and aluminium seal with yellow or orange (for the 25 mg and the 75 mg presentation respectively) polypropylene flip-off cap. Vials are washed and depyrogenated prior to use. Stoppers and seals are received ready to sterilize. The suitability of the container closure system was tested by stability studies.

Acceptable supplier specifications for the container closure system and technical drawings for the components are presented. Extractables and leachables have been appropriately addressed. The glass type of the vial and the rubber material of the stopper comply with compendial requirements.

Stability of the product

A shelf-life of 36 months for the FP when stored at the recommended storage conditions of 2 - 8°C was proposed. This shelf-life claim is primarily based on stability data available from six "registrational" FP stability batches in the commercial vials and four "supporting" FP stability batches packaged in different vials.

The six registrational batches were stored for 24 months at 2 - 8°C, for 3 months at -20 ± 5°C, and for 6 months at 25±2°C / 60±5% RH and 40±2°C / 75±5% RH.

The supporting stability batches were stored for 48 months at 2 - 8°C, for 6 months at -20 ± 5°C, for up to 36 months at 25±2°C / 60±5% RH, for up to 24 months at 30±2°C / 75±5% RH and for 6 months at 40±2°C / 75±5% RH.

Three of the four supporting batches have reached and surpassed the initial proposed shelf life of 36 months. Since the supporting batches are endorsed to be representative for the registrational and PPQ batches using the commercial vials, the minimum requirement for three batches supporting the proposed shelf-life of 36 months based on real time, real-condition stability data is met.

In addition to the above mentioned registrational and supportive batches, another three batches in the proposed vial and three further batches from earlier process development in different vials were included in the stability program. Stability data of these batches reached up to 12 months at 2-8°C conditions, with no trends detected.

The shelf-life of 8 hours at room temperature (≤25°C) and up to 24 hours at 2 - 8°C claimed in the SmPC for the reconstituted FP is acceptable based on results presented on physicochemical stability and on a microbial challenge study.

A photostability study was performed in accordance with Option 2 of the ICH Harmonized Tripartite Guidelines Photostability Testing of New Drug Substances and Products (Q1B). There were no marked differences in the results obtained for the light-exposed and dark control samples. The samples were tested without secondary packaging confirming that such packaging will provide additional beneficial protection to the finished product vials.

The stability program and testing frequency conforms to requirements as laid down in ICH Q5C. No deviations from acceptance criteria are reported so far. It has been clarified that variability is seen for subvisible particles without an obvious trend, and this was justified by data and by the test methodology.

Overall the presented data are within their defined limits and do not show significant trends. Thus, the proposed shelf-life of 36 months and storage conditions "Store in a refrigerator (2 - 8°C)", "Do not freeze", and "Store in the original carton in order to protect from light" as stated in the SmPC are accepted. Stability studies also indicate the suitability of the container closure system. The post approval stability protocol and stability commitment presented is acceptable.

Adventitious agents

A complete list of raw materials used for the luspatercept manufacturing process were provided. Raw materials used in the upstream and downstream processes are plant based or chemically synthesized or produced by recombinant DNA technology. Specifications are implemented for all raw materials for release by the raw material supplier. Where appropriate, this includes testing for non-viral adventitious agents at the upstream and downstream raw material supplier site.

Raw materials used in the luspatercept manufacturing process do not contain materials of animal origin. All the cell banks were extensively tested for non-viral adventitious agents. All testing specification were met and no bacterial, fungi, and mycoplasma contamination was detected.

Appropriate characterisation and safety testing of the cell banks is in place; data derived thereof do not raise a concern. Unprocessed bulk from the cell culture is tested for adventitious agents, mycoplasma and mouse minute virus (MMV). The manufacturing process includes two dedicated virus inactivation/reduction steps, but also the three chromatography steps were included into the virus clearance studies. Virus clearance studies have been conducted with four model viruses in small scale models. The scale-down models have been appropriately qualified. The data demonstrate that the luspatercept purification process provides substantial clearance of viruses with a wide range of physicochemical properties through a combination of inactivation and removal.

In summary, the provided information for both non-viral and viral adventitious agents is satisfactory, and no issues arise.

GMO

Not applicable.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product have been presented in a satisfactory manner. No major deficiencies have been found.

The active substance manufacturing process is well described. Raw materials and cell banks used in the manufacture of the active substance are of appropriate quality. An extensive process characterisation work has been conducted to gain process knowledge. The development of the process is well described, and comparability of material derived from the different process versions has been demonstrated. The quality control strategy is acceptable. The batch data and the performed validation of the manufacturing process are satisfactory and indicate that the process is capable to consistently deliver material meeting its predetermined specifications and quality attributes.

The finished product formulation development process was presented in detail. Appropriate process development studies were performed based on the risk of process parameters to CQAs or process performance. Comparability between the different process versions was shown. The established control strategy was successfully confirmed in the process validation studies. The finished product manufacturing process has been sufficiently validated and the process was shown to be capable of delivering consistent quality. Stability results so far show satisfactory stability even at accelerated and stressed temperature.

Three recommendations are proposed concerning the re-evaluation of the finished product potency specification limits, the outcome of the investigation of the sources of the potency method variability, and an evaluation of proline supplementation.

Overall the results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends points for investigation.

Three recommendations are proposed concerning the re-evaluation of the finished product potency specification limits, the outcome of the investigation of the sources of the potency method variability, and an evaluation of proline supplementation.

2.3. Non-clinical aspects

2.3.1. Introduction

Luspatercept, an erythroid maturation agent, is a recombinant fusion protein that binds selected transforming growth factor-β (TGF β) superfamily ligands. By binding to specific endogenous ligands (e.g. GDF 11, activin B) luspatercept inhibits Smad2/3 signalling, resulting in erythroid maturation through differentiation of late-stage erythroid precursors (normoblasts) in the bone marrow. Smad2/3 signalling is abnormally high in disease models characterised by ineffective erythropoiesis, i.e. MDS and β thalassaemia, and in the bone marrow of MDS patients.

2.3.2. Pharmacology

The Applicant submitted non-clinical pharmacology studies designed to characterise the biological activity of luspatercept with respect to mechanism of action (MoA) and pharmacodynamic effects. Pharmacodynamic studies were performed in diverse *in vitro* systems, as well as in healthy laboratory animals and in animal models of anaemia.

Table 1

Test Article: Luspatercept or RAP-536				
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**
Primary Pharmacodynamics				
Ligand Binding and Specificity of Luspatercept	Biacore (luspatercept captured by immobilized goat anti-human Fc antibody)	In vitro	Acceleron Pharma, Cambridge, MA	PPR089
In Silico Sequence Alignment Across Multiple Species; and Surface Plasmon Resonance Analysis of Luspatercept and RAP-536 for transforming growth factor beta (TGF-β) Ligands	In silico; and Biacore (luspatercept captured by immobilized goat anti-human Fc antibody)	In silico, in vitro	Acceleron Pharma, Cambridge, MA	PPR125
Inhibition of Ligand-induced Reporter Gene Expression by Luspatercept	pGL luciferase reporter gene under the control of different promotor motifs transfected into different cell lines (A204, HepG2, T98G), depending on the transforming growth factor beta (TGF-β) superfamily ligands to be assessed. Negative control renilla reporter plasmid (pRL-CMV)	In vitro	Acceleron Pharma, Cambridge, MA	PPR122

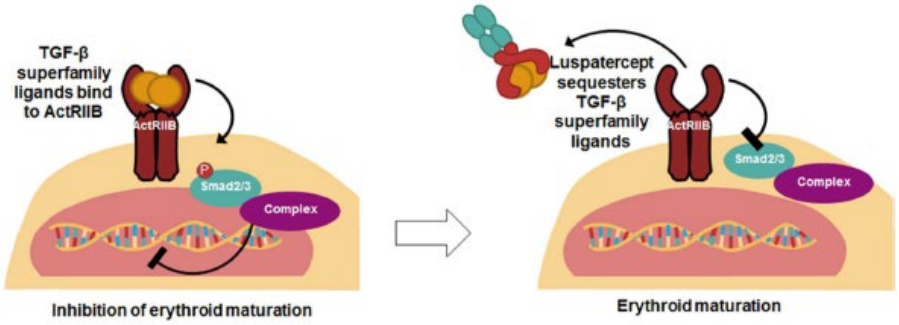
Primary pharmacodynamic studies

Mechanism of Action

Erythropoiesis is a multi-step process of proliferation and differentiation leading to development of mature RBCs. Transforming growth factor-β superfamily ligand trap ACE-536/Luspatercept aims to correct anaemia by promoting late-state erythropoiesis (Suragani, 2014).

The Applicant demonstrated that luspatercept binds to GDF11 and other TGF-β superfamily ligands (like GDF8, and activin B), and inhibits signalling downstream of activin receptors by phospho-Smad2/3. Inhibition of this signalling pathway promotes differentiation of late-stage erythroid precursors or normoblasts in the bone marrow, and consequently leads to an increase in production of mature erythrocytes.

The MoA of luspatercept is thus represented by enhancement of maturation of later stage erythroid precursors. This is distinct from the MoA of erythroid stimulating agents (ESAs), which promote proliferation and survival of erythroid progenitors.



ActRIIB = activin receptor type IIB; TGF-β = transforming growth factor-beta

Figure 1

In vitro data

Binding specificity of luspatercept was determined by *in vitro* ligand binding experiments with ligands of the TGF-β superfamily in both, cell-free and cell-based assay systems.

Binding of various TGF-ß superfamily ligands to ACE-536 (modified ActRIIB-human Fc) (study PPR089) and to both, ACE-536 and RAP-536 (same modified human ActRIIB as in ACE-536, but with murine Fc) has been assessed by SPR analysis. Results showed that both molecules bound the tested ligands with similar affinities. The highest affinity ligands for both ACE-536 and RAP-536 were GDF11, GDF8, activin B, and BMP6. BMP-2, -6, and -9 have the biggest osteoinductive potential. In this regard and especially in view of the significant increase in sternum malformations/ variations in rats and the hyoid bone in rabbits in the EFD studies, binding to the major osteoinductive ligands (BMP-2, -6, and -9), and possible safety related effects from binding to these ligands. Luspatercept treatment results in skeletal effects during foetal development.

A second-tier assessment by cell-based assays showed that the investigational medicinal product inhibited signalling by GDF8 and GDF11, but not Activin A, Activin B, BMP6, BMP9, or BMP10.

Members of the TGF-β superfamily, including ligands, receptors and accessory molecules, are highly conserved across mammalian species. *In silico*, amino acid sequence alignment of the human ActRIIB receptor protein with the bovine, rodent (rat & mouse), rabbit and *Cynomolgus* monkey sequence indicated a > 99% sequence homology (study PPR125) of the human sequence with that of each of these species.

In vivo studies

Homology between the human and *Cynomolgus* monkey sequences is 100%. Given the high degree of homology between the species, luspatercept is predicted to bind human, *Cynomolgus* monkey, rabbit, and rodent ligands with similar affinity, indicating the relevance of the laboratory animals used.

In *in vivo* studies in physiologically normal mice, rats and *Cynomolgus* monkeys, luspatercept/RAP-536 increased RBC, HGB, haematocrit (HCT) and reticulocytes. In general, all haematology values returned toward control or baseline values after cessation of dosing.

The pharmacological effect of luspatercept was not only explored in healthy mice, rats, and *Cynomolgus* monkeys, but also in different rodent models in which anaemia contributes significantly to morbidity, including models of anaemia associated with nephrectomy, acute blood loss, sickle cell anaemia and chemotherapy. Defined pharmacology studies focused specifically on the efficacy of luspatercept in a mouse model of MDS (NUP98-HOXD13) and a model of β-thalassemia (haemoglobin beta (Hbb) major-/-, Hbbth1/th1 mice), representing relevant models for the proposed initial target indications of the drug.

The NUP98-HOXD13 transgenic mouse mirrors several features of MDS in humans, including peripheral blood cytopenias, bone marrow dysplasia, anaemia, and increased apoptosis in the presence of a hypercellular or normocellular bone marrow. Likewise, the mouse β-thalassemia model (Hbbth1/th1) resembles human disease, as it presents with increased reticulocyte counts, splenomegaly due to ineffective erythropoiesis, and severe anaemia.

Some of the rodent pharmacology studies used a murine orthologue of luspatercept, referred to as RAP-536. The RAP-536 molecule contains the identical extracellular domain (ECD) found in luspatercept, but with an Fc portion derived from murine IgG2a, instead of human IgG1. Such murine orthologues are generally less immunogenic in mice compared to the humanised molecules (i.e., luspatercept), facilitating the conduct of longer-term pharmacology studies (generally greater than 3 weeks) by minimising the confounding influence of immunogenicity. No differences in ligand binding specificity or affinity between luspatercept and RAP-536 were observed, attributable to the identical ECD between the two molecules. Studies conducted with either luspatercept or the murine orthologue of luspatercept, RAP-536 had similar effects on maturation of erythroid precursors and increases in RBC, HCT, and HGB *in vivo*.

Results:

When luspatercept was administered twice on Day 0 and Day 4 to healthy mice, a rapid increase in RBC was measurable as soon as 12 hours following administration and sustained for up to 34 days (Report PPR084). The increase in RBC was associated with a concomitant decline in EPO expression. The decline in EPO expression is likely a negative feedback mechanism associated with an increase in circulating rRBC HCT and Hgb. After luspatercept treatment, an increase in serum EPO levels was seen after 14 days, but there was no increase 30 days after the final dose, suggesting that the increase in EPO levels induced by luspatercept is transient and completely reversible after withdrawal of treatment.

To investigate the effects of Luspatercept on long term erythropoietin expression, mice received ACE-536 at 10 mg/kg for 3, 7 and 28 days, or 1mg/kg for 28 days (Report PPR083). The study demonstrated that ACE-536 was effective in increasing RBC parameters independent of EPO for the first three days. However, repeated ACE-536 (10mg/kg) treatment for 28 days resulted in both increased RBC and serum EPO levels, although the increase in EPO levels was not considered very high (statistically not relevant), as observed in patients suffering from anemia. At 1mg/kg while EPO levels

were not changed, RBC parameters were significantly increased demonstrating that at low dosage the physiological levels of EPO are enough to support the erythropoietic response induced by ACE-536.

As luspatercept and EPO target different cell populations in the erythropoiesis, it was hypothesised that treatment of mice with both luspatercept and EPO would result in larger increases in RBCs than either substance alone would. To prove this hypothesis, the combined effect of luspatercept and recombinant erythropoietin on red blood cell maturation was tested (study report PPR087). Three days after a single dose of luspatercept (10 mg/kg IP) and EPO (1800 U/kg) to mice, the combination of the two agents resulted in a greater increase in RBC than the single treatments alone.

The murine orthologue of luspatercept, RAP-536, improved haematology parameters (increased RBC, HGB, HCT, improved RBC morphology), increased bone mineral density, and decreased RBC inclusion bodies, bilirubin, and spleen size in the NUP98-HOXD13 transgenic mouse model of myelodysplastic syndromes (MDS), and/or in the β-thalassemia mouse model (haemoglobin beta (Hbb)th1/th1 mice). However, it should be noted that although RAP-536 treatment significantly increased red blood parameters (RBC, HGB, and HCT) compared to vehicle, dosing with RAP-536 simultaneously led to very modest decreases in mean cell parameters (MCV, MCH, and MCHC) compared to vehicle, probably caused by a relative lack of iron (iron deficiency anemia) in comparison to the increased RBC production.

As measured by whole body DEXA scans, treatment of Hbbth1/th1 mice with RAP-536 significantly increased bone mineral density (by 17%), trabecular bone volume (by 100%), trabecular number (by 14%), and trabecular thickness (by 14%), compared to vehicle treatment, completely restoring these parameters to wild type levels. Bone parameters were unchanged by RAP-536 treatment in wild type mice, on the other hand.

Effects of the *in vivo* administration of the test compound RAP-536 on the frequency of haematopoietic progenitors in mouse bone marrow and spleen was analysed in another study (ACL04). The RAP-536 administered mice showed an increase in WBC counts, albeit not statistically significant and considered consistent with indirect changes related to inflammatory processes, such as the glomerulonephritis documented in all of these studies. None of the WBC increases was accompanied by abnormal white blood cell morphology or distribution, as indicated by microscopic examination of hematopoietic or lymphoid tissues.

The Applicant did not carry out longer term experiments, nor has an analysis of bone mineral density in the recovery phase been conducted. Due to the lack of data on the stability of an increase in bone mineral density in Hbb major-/- mice treated with RAP-536, it is not possible to estimate the significance of this effect for therapeutic use of luspatercept. In the current situation, despite the extensive pharmacological and toxicological documentation, no non-clinical data are available to describe the stability of bone mineralisation observed after luspatercept administration.

RAP-536 was also tested in a murine model of sickle cell disease. The treatment improved SC disease phenotype indicated by reduced spleen weights, oxidative stress, reticulocytes, percentage of sickle cells and RBC haemolysis compared to vehicle treated mice. However, RAP-536 at 1mg/kg to SCD mice did not result in a statistically significant increase in the RBC parameters.

In another study (Report PPR063), the effects of luspatercept on red blood cell production were tested in the absence or presence of an EPO neutralizing monoclonal antibody (EPO mAb). The study consisted of two time points (days 4 and 7) in order to assess the effects of luspatercept and EPO mAb during the acute and the subacute phases of treatment. On days 4 and 7 of treatment, luspatercept administration significantly increased total RBCs, haemoglobin and haematocrit, while the EPO mAb significantly decreased the same parameters. When administered together, luspatercept was able to

compensate the decline in all RBC parameters associated with EPO mAb only at Day 4 but not at Day 7. This supports that the effect of luspatercept on RBCs is different from that of EPO.

In sham-operated and unilateral nephrectomised mice (15/group), luspatercept administration (10 mg/kg IP twice weekly for 4 weeks) significantly increased total RBC, HGB, and HCT, while unilateral nephrectomised mice dosed with TBS (IP twice weekly for 4 weeks) showed a significant decline in these same parameters over the 4-week experimental period (Report PPR0043).

Secondary pharmacodynamic studies

Studies of the secondary pharmacodynamics of luspatercept have not been conducted.

Safety pharmacology programme

Stand-alone safety pharmacology studies with luspatercept were not conducted, in agreement with International Conference on Harmonisation (ICH) S6 guidance for the Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (ICH S6 (R1), 2011).

However, relevant safety pharmacology measurements (e.g., heart rate, blood pressure, respiratory rate, body temperature, neurological examinations, and electrocardiograms [ECGs]) were included as part of the 1- and 3-month repeat-dose general toxicology studies in *Cynomolgus* monkeys. Of note, in the juvenile toxicity study in rats a significantly lower mean body temperature (considered adverse) was measured in males dosed at 10 mg/kg during the dosing period.

The findings of 1- and 3-month toxicology studies in *Cynomolgus* monkeys do not reveal relevant concerns on safety pharmacology endpoints. However in the study No.PPR083 & study No.PPR084 luspatercept treatment results in a moderate increase in the circulating levels of EPO. In summary, luspatercept binds members of the TGF-β superfamily that participate in the later stages of erythropoiesis, stimulating differentiation of late stage cells already committed to the erythroid lineage. The non-clinical evidence for an increased production of mature erythrocytes was generated on the level of *in vitro* and *in vivo* studies (in healthy rats and *Cynomolgus* monkeys, in murine models of myelodysplastic syndromes (MDS) and beta-thalassaemia and acute and chronic anaemia models), in some of them by making use of a murine orthologue (RAP-536).

Pharmacodynamic drug interactions

Specific pharmacodynamic drug interaction studies have not been conducted.

2.3.3. Pharmacokinetics

The pharmacokinetic (PK) and toxicokinetic (TK) parameters of luspatercept were assessed in Sprague Dawley rats, NZW rabbits, and *Cynomolgus* monkeys, the same species used in the pivotal toxicology studies. Both, single-dose and repeat-dose studies were carried out by the Applicant to characterise the PK/TK of luspatercept in these animals. Luspatercept was administered as a solution in tris-buffered saline (TBS), the same formulation as used in clinical studies. The PK was characterised for the single-dose intravenous (IV) (monkey) and in subcutaneous (SC) routes of administration (rat, rabbit, and monkey), which is the intended route of administration in humans.

Bioanalytical methods were validated for the determination of luspatercept concentrations and for the detection of anti-drug antibodies (ADA) to luspatercept in toxicokinetic (TK) studies.

An ELISA-based sandwich immunoassay method was used for the measurement of luspatercept concentrations in rat milk and serum. The mean ratio of luspatercept concentrations in milk to concentrations in serum was 0.12, with an individual range of 0.06 to 0.17 across the time-points tested. The mean percent lacteal transfer (calculated as mean ratio*100) was 12% (Study number ACE-536-DMPK-2549).

In the embryo-foetal development studies, luspatercept was found in the embryo, indicating that luspatercept can cross the placental membrane in rats and rabbits. As part of the PPND study, the placental transfer of luspatercept was quantitatively determined after SC administration at 3, 10, and 30 mg/kg to Sprague Dawley rats to assess the exposure of the animals to luspatercept *in utero*. The ratio of luspatercept concentrations in pooled foetal serum to maternal serum was calculated for each dam, ranging from 4% to 33%. The mean ratios of luspatercept concentrations in pooled foetal serum compared to concentrations in maternal serum collected at the same time-point were 18% and 9% at 8 and 24 hours, respectively. The mean percent placental transfer (calculated as mean ratio*100) was 13.4%.

As luspatercept is a large protein that is not expected to be renally eliminated and whose catabolism is expected to take place through the normal proteolytic pathways, studies on metabolism and excretion of the drug in animals have not been conducted. Equally, due to luspatercept being a large biotherapeutic molecule, drug interactions have not been conducted.

2.3.4. Toxicology

The Applicant conducted a broad range of non-clinical studies to assess the toxicology of luspatercept. All pivotal and dose-range finding studies with the purpose of investigating the toxicological profile of luspatercept were conducted in accordance to GLP regulations. In all studies, luspatercept has been administered subcutaneously, the administration route which is also intended for its clinical use.

Single dose toxicity

No stand-alone studies on single dose toxicity have been conducted.

Repeat dose toxicity

Repeat-dose toxicity was studied in Sprague-Dawley rats in two studies of 1 month and 13 weeks, respectively, and in *Cynomolgus* monkeys in three studies of 4 weeks, 13 weeks and 6 months, respectively. A Q14D treatment regimen was applied in all of these studies.

In the 1-month study in rats (study 024488), the animals were dosed at 6, 20 and 60 mg/kg and terminated on Day 29; a subset of animals was maintained for a two months recovery period and terminated on Day 92. A NOAEL could not be established in this study. Apart from dose-related increases in RBC and related parameters, which are considered pharmacological effects of luspatercept, important adverse findings included increased platelet volume and decreased platelet count at high dose, increase in monocytes, WBC and lymphocytes at 20-60 mg/kg, increases in several serum parameters in males in all dose groups and in females at ≥20 mg/kg, decreased heart and lung weights at ≥6 mg/kg, membrano-proliferative glomerulonephritis in the kidney in all dose groups, hepatocellular coagulative necrosis of the liver at all dose levels, minimal to mild congestion of the adrenal gland in females at all dose levels and in males at 60 mg/kg, mild to marked necrosis and mineralisation of the adrenal gland in females at all dose levels, mineralisation of the stomach and minimal focal or multifocal congestion of the thymus at all dose levels.

Minimal to mild mineralisation of the lamina propria in the glandular portion of the stomach was present in both male and female rats dosed with ≥ 6 mg/kg for 1 month. The multifocal mineralisation in the stomach persisted after the 2-months recovery period in males at all doses and females at ≥ 20 mg/kg. Data is presented describing the mechanism of formation of mineralisation changes in the stomach. Minimal to mild mineralisation of the lamina propria in the glandular portion of the stomach as a manifestation of azotemia/uremia is a consequence of bilateral membranoproliferative glomerulonephritis. The mineralisation changes in the stomach are probably of secondary in significance to the underlying renal alteration and resulting systemic uremia. This mechanism is common to experimental animals and humans. 'Gastric mucosal calcinosis' is occasionally connected to epigastric pain and/or dyspepsia and was mostly identified as a post mortem finding in people.

In the 13-week rat study (20017484) dose levels of 1, 3 and 15 mg/kg were applied. Main group animals were terminated on Day 93 after 7 doses of luspatercept and recovery animals were terminated on Day 162. In addition to the mature male and female rats used in this study, male and female young adult rats (approximately 8 weeks of age at first dosing) were dosed at 15 mg/kg. Again, a NOAEL could not be established based on findings of minimal cortical necrosis of the zona fasciculate of the adrenal gland, and minimal to mild membrano-proliferative glomerulonephritis in the kidney at all dose levels. Other effects included decreased heart, liver, kidney and lung weights at 3 and 15 mg/kg and minimal to mild mid-zonal hepatocellular vacuolation at all dose levels (not considered adverse). Additional effects in young adult rats included minimal increases in leukocyte, lymphocyte, monocyte, and basophil counts, minimal increases in ALP, decreases in prostate weight and increased adrenal gland weight in females.

Though not considered adverse by the investigators, young adult rats had minimally larger changes in haematology parameters than mature adult rats, including increases in total leukocyte, lymphocyte, monocyte and basophil counts at 15 mg/kg. At the end of the recovery period (Day 162), leukocyte counts were still minimally increased in young adult females that had been dosed at 15 mg/kg.

During the 3-month study, one adult female rat in the high-dose (15 mg/kg) group was found dead during Week 11, with a diagnosis of disseminated pleiomorphic lymphoma. According to the investigators, the death of this animal was not attributed to treatment considering the spontaneous occurrence of this condition in rats of this age and strain, and the absence of any similar finding in remaining animals receiving ACE-536. However, although occurring spontaneously, a relation to the IMP cannot be ruled out.

In the 4-week study in *Cynomolgus* monkeys (WHH00067), animals were dosed subcutaneously at 0.4, 2, 10 and 30 mg/kg and a separate group was dosed intravenously at 10 mg/kg for the main necropsy on Day 29. Additionally, recovery groups were dosed at 10 mg/kg (SC and IV) and 30 mg/kg for necropsy on Day 99. At all doses of ACE-536 through the two routes of administration, there were no adverse clinical signs, effects on food consumption, body weight, or organ weights, nor were there any abnormal electrocardiographic, neurologic, physiologic, gross, or microscopic pathology findings due to the administration of ACE-536. Statistically significant increases in RBC, HgB, and HCT were seen in both males and females on Days 13 and 28 that showed signs of recovery by Day 99 and were consistent with the expected ACE-536 pharmacologic effect. Increases in these measures of red blood cell mass were dose dependent and reached a plateau at 2 mg/kg. The NOAEL for this study was considered 30 mg/kg when dosed via SC injection and 10 mg/kg when dosed via IV injection.

Animals in the 13-week study (20017483) were dosed at 1, 6 mg/kg and 30 mg/kg. The main study animals were necropsied on Day 99 and the control and high dose recovery animals on Day 162. Administration of ACE-536 at dosages ≥ 1 mg/kg resulted in generally time- and dose-dependent minimal to moderate mean increases in indicators of circulating erythrocyte mass consistent with the expected pharmacodynamic effects of ACE-536. At the end of the recovery period, most of the

haematology changes were comparable to the control values. Increases in ALP in males at dosages ≥ 1 mg/kg and in single females at ≥ 6 mg/kg and increases in ferritin were not considered adverse as they were not statistically significant compared to controls and exhibited recovery at Day 162.

ACE-536-related macroscopic changes on Day 99 comprised dark discoloration in the renal cortex which correlated histologically with haemorrhage, and thin cortex which correlated with interstitial fibrosis in the kidneys of two males at 30 mg/kg. Test article-related histopathologic changes were identified in the kidneys of animals at a dosage of ≥ 1 mg/kg, which persisted at the Day 162 recovery necropsy. However, these findings were only considered adverse in relation with increased BUN and creatinine values at ≥ 6 mg/kg.

There was a dose-related decrease in absolute thymus weight and ratios to brain and body weight in males at ≥ 6 mg/kg and in females at ≥ 1 mg/kg. The decrease in thymus weight was associated with the microscopic finding of thymic involution. Thymic involution is a common incidental physiologic change in *Cynomolgus* monkeys associated with aging, stress, malnutrition, and many other conditions. However, the increased incidence amongst dosed animals in this study indicates a direct or indirect association with ACE-536 administration. Thymic involution is a normal physiologic process, and as such, was not considered adverse.

According to the Applicant, the no-observed-adverse-effect-level (NOAEL) for this study was determined to be 1 mg/kg based on increases in BUN and creatinine and the nature and incidence of the kidney histopathology findings. It is acknowledged that increases in BUN and creatinine reflect a functional deficit and were only present at ≥ 6 mg/kg, however, the histologic changes in the kidney consisting of membrano-proliferative glomerulonephritis at ≥ 1 mg/kg were not considered adverse. While morphologic alterations should be considered adverse when occurring in an organ sensitive to such changes, in this instance, considering the minimal severity, the lack of functional consequence, and the well-recognised reserve capacity of the kidney in the face of these scattered alterations, it is acceptable that within the scope of this study, the histologic changes in the kidney at the lowest dose were assessed as non-adverse.

In the 6-months toxicity study (20039148) in *Cynomolgus* monkeys ACE-536 was administered at dosages of 0.3, 1, and 6 mg/kg (5/sex/group). The main study animals were necropsied on Day 197 and the recovery animals on Day 281. Minimal to mild increases in indicators of circulating erythrocyte mass were observed at dosages of ≥ 0.3 mg/kg and were consistent with the expected pharmacodynamic effects of ACE-536. ACE-536 at a dosage of 6 mg/kg in males was associated with minimal increases in alkaline phosphatase. An explanation for the change was not evident since there were no histopathologic findings in the liver or increases in other markers of hepatobiliary injury. Ferritin was increased in males and females at 6 mg/kg. Following a 3-months recovery period, all clinical chemistry and urine chemistry parameters were comparable to control and/or similar or trending toward pre-study values, except alkaline phosphatase in 1 male at the 6 mg/kg dosage.

Effects related to renal changes comprised dose-dependent minimal increase in creatinine in males and females at ≥ 1 mg/kg, minimal increases in BUN at 6 mg/kg and histopathology changes in the kidney consisting of membrano-proliferative glomerulonephritis at ≥ 1 mg/kg in males and females. This was accompanied by other effects, such as accumulation of tubular protein, interstitial or tubular haemorrhage, interstitial fibrosis/fibroplasia, increased extracellular matrix, vacuolization of interstitial cells and/or degeneration/atrophy of tubules in the medulla, near the corticomedullary junction, as well as interstitial mixed inflammatory cell infiltrates. The renal changes were considered adverse at a dose of ≥ 1 mg/kg in both sexes resulting in a LOAEL of 1 mg/kg. Although the ACE-536-related microscopic findings identified at the terminal euthanasia in the kidney were resolving at the end of the recovery phase, most of them were still present to some extent.

At terminal necropsy (Day 197), ACE-536-related microscopic findings were identified in the choroid plexus of the brain at ≥ 1 mg/kg. Microscopic findings included one or more of the following: vascular degeneration, deposition of pigment (hemosiderin), deposition of eosinophilic proteinaceous material, a mixed inflammatory cell infiltrate (small numbers of neutrophils, macrophages, and lymphocytes including plasma cells), and an infiltrate of foamy (vacuolated) macrophages. These changes were further investigated by a second pathologist, who concluded that none of the microscopic changes should be considered adverse for various reasons which were mainly based on comparison to findings with other drug formulations observed in other studies.

The NOAEL for this study was determined to be 0.3 mg/kg based on the nature and incidence of the kidney histopathology findings at dosages ≥ 1 mg/kg. At the NOAEL dose of 0.3 mg/kg, the combined male and female mean Cmax was 5.4 µg/mL and the mean AUC0-336hr was 1228 hr*µg/mL.

It was concluded that the ACE-536-related microscopic findings in the choroid plexus of the brain at ≥ 1 mg/kg at terminal necropsy should not be considered adverse. However, the reasoning provided mainly refers to comparison with other cases with completely different drug products and is not based on a scientific discussion related to luspatercept and its mechanism of action. During the scientific advice procedure of June 2015 (EMEA/H/SAH/044/1/2015/PA/III), the Applicant was advised to generate an integrated risk assessment on choroid plexus findings. Such an assessment has not been provided and the argumentation given in the pathology report is not considered sufficient to judge on the (non-)adversity of the choroid plexus findings. The Applicant was thus asked to discuss the choroid plexus findings in more detail. The provided literature data suggests that the findings are unlikely to be related to the known mechanism of action of luspatercept; however, the data was obtained in rodents, not in monkeys. The actual aetiology of the observed lesions in monkeys remains undetermined, but it is acknowledged that they were considered non-adverse.

As mentioned above, the following safety pharmacology parameters were assessed in the 4-weeks and 13-weeks toxicity studies in *Cynomolgus* monkeys: heart rate, blood pressure, respiratory rate, body temperature, neurological examinations, and electrocardiograms [ECGs]. All changes in physiological measurements (heart rate, respiratory rate, blood pressure, and body temperature) were considered incidental and not related to ACE-536 administration. There were also no changes in neurological function associated with the administration of ACE-536. Also, no abnormal electrocardiographic findings were attributable to the administration of ACE-536; in one animal of the high dose group in the 13-week study, a second-degree AV block was observed. However, a relationship to luspatercept was likely incidental.

Genotoxicity

No stand-alone studies on genotoxicity have been conducted.

Carcinogenicity

Although stand-alone carcinogenicity studies are not considered appropriate due to the immunogenicity of luspatercept in rodents, product-specific assessment of carcinogenic potential would be expected of a product designed to modulate cell maturation and/or proliferation, such as luspatercept. In this regard, the effects of RAP-536 on Lewis lung carcinoma growth were studied (report PPR113). The results of this study are considered inconclusive and are not adequate to conclude on the presence or absence of a tumorigenic potential of luspatercept.

Furthermore, the three hematologic malignancies detected at the highest dose level of luspatercept in the definitive juvenile toxicity study in rats (Report WIL-961003) prompt a concern for potential

malignancies in patients. The Applicant argued that a causal relationship of the tumours observed in the juvenile rat study to luspatercept has not been definitively established. However, the lack of malignancies in the control group and lower dose groups makes an influence of luspatercept high dose in this study more likely. In this context, it is also interesting to note that increases in total leukocyte, lymphocyte, monocyte and basophil counts were observed in the repeat-dose toxicity studies in rats, especially in young adult rats, where leukocyte counts were partly still increased at the end of the recovery period.

Haematological malignancies were observed in 3 out of 44 rats of the highest dose group examined in the definitive juvenile toxicity study (Report WIL-961003). As this incidence of malignant haematopoietic neoplasms was identified at a comparably short experimental duration (all identified approximately within half a year of study duration), the incidence of malignant haematopoietic neoplasms in a typical two years rat carcinogenicity study might be expected to be considerably higher. However, the Applicant argues that the mechanism of luspatercept's carcinogenicity is specific to the developing haematopoietic system in immature rats and that luspatercept would not necessarily lead to a higher incidence of tumours in adult rats.

Reproduction Toxicity

While no adverse effects of luspatercept on male fertility were observed, significant reductions in average numbers of corpora lutea and decreased mean ovarian weights and uterus with cervix weights, a decreased mean number of implantation sites and viable embryos and a decrease in FSH were identified at 15 mg/kg each. Based on these observations, a general NOAEL for toxicity to female fertility in rats when exposed to luspatercept was claimed at 3 mg/kg.

Adverse effects in rat and rabbit EFD studies ranged from decreased foetal weight, decreased average litter size and live litter size, increased average number of resorptions and percent post-implantation loss, maternal toxicity and skeletal variations as well as increased incidences of especially skeletal-, but also gross- and visceral malformations. For the endpoints "reduced foetal weight" in rats and "increased incidence of the skeletal variation angulated hyoid ala" in rabbits, no NOAEL could have been determined in the pivotal EFD studies (these treatment related effects were already observed at the lowest tested dose).

Gross and visceral malformations in the pivotal rat EFD study (Report 20040548) were mainly observed in foetuses of treatment groups (four out of five observed malformed foetuses). These malformations were characterised by a strikingly low incidence in the control group data of the Testing Facility (e.g. agnathia, no tongue present, situs inversus with 1 out of 21065 rat foetuses each); hence, the increased incidence of such malformations in treatment group foetuses could point towards a teratogenic potential of luspatercept. This assumption was strongly supported by the pivotal rabbit EFD study (Report 20040550), in which skeletal malformations of the ribs and vertebrae were detected in ten foetuses of ten litters exclusively from treatment groups. One can calculate the likelihood that all these ten foetuses fall into a treatment group exclusively by chance by the following approach:

Sum of all live foetuses examined: 798; sum of all live foetuses of treatment groups examined: 583

The probability that all 10 foetuses developed malformations of the ribs and vertebrae in treatment groups completely by chance is hence low (4.2%, below the p value significance level of 5%), indicating that the maternal administration of luspatercept was most likely the rationale for the increased incidence of malformations in rabbit foetus treatment groups. As the target of Reblozyl (i.e. TGF-β superfamily ligand binding) is evolutionarily highly conserved among animals, the embryo/foetal toxicity and teratogenicity of Reblozyl observed in the EFD studies are most likely also relevant in humans.

In the PPND study, adverse effects observed at all tested dose levels (lowest one being 3 mg/kg) were significantly lower F1 pup body weights in both sexes from birth until post-weaning (in F1 males until the mating period at all doses) and adverse kidney findings. The latter were membrano-proliferative glomerulonephritis, and/or minimal to mild tubular atrophy/hypoplasia as well as minimal to mild vessel ectasia occasionally associated with haemorrhage. The delay in growth and the adverse kidney findings, in the F1 generation, precluded the determination of a NOAEL for F1 general toxicity. Growth retardation was demonstrated to be long lasting (especially in male pups), and the nephrotoxicity of luspatercept likely irreversibly disrupted general kidney maturation, these effects should additionally be characterised under the category of developmental toxicity (which generally implies long-lasting or even permanent effects).

In the juvenile toxicity studies (WIL-961002 and -3), significant effects were determined at all dose levels (the lowest one being 1 mg/kg, which is below the intended maximum therapeutic dose of 1.75 mg/kg). They comprised impaired use of hind limbs, swollen paws and/or reddened hind limbs, lower mean mating indices, adverse adrenal gland findings, adverse kidney findings with associated findings in urinalysis, decreased heart weights and effects on bone geometry, bone mass and bone density. Furthermore, gastric mucosal lesions were observed at 1 mg/kg in the dose-range finding juvenile toxicity study, and at 3 mg/kg in the pivotal juvenile toxicity study. In the pivotal juvenile rat toxicity study, luspatercept-related adrenal gland, kidney, glandular stomach and adverse hind limb findings persisted throughout the recovery phase at all dose levels. Additionally, malignant haematopoietic neoplasms were observed in high dose groups, as discussed before.

The Applicant hypothesises that the mechanism of the observed carcinogenicity is specific to the developing hematopoietic system in immature rats and that this notion is supported by results of the three-month repeat dose toxicity study in young and mature rats (Report 20017484), where a similar test article-related increased incidence of malignancies was not noted.

Local Tolerance

Local tolerance studies have not been conducted. However, local tolerance parameters were integrated in RDTS and DART studies. Mild adverse skin effects at the injection sites were observed in rabbit EFD studies and followed a dose-response trend, however, at a relatively low incidence. No treatment related injection site reactions were reported in the repeat-dose toxicity studies in rats or *Cynomolgus* monkeys.

Other toxicity studies

To better understand the effect of luspatercept on the progression of renal injury, a 13-week non-GLP study (CC-DISC-TOX-2024) was conducted, in which luspatercept was given to rats that had undergone one of 2 rodent models of renal compromise: 5/6 nephrectomy or unilateral ureteral ligation (UUL). In the 5/6 nephrectomised animals a notable exacerbation of glomerular and tubulointerstitial lesions was observed, which was accompanied amongst others by increased pSmad2 immunostaining. Indeed, literature data indicate that impairment of the TGF-B/Smad signalling pathway may play a role in the pathogenesis of chronic kidney disease, although the exact mechanism is not completely understood (Böttinger & Bitzer 2002, Huang et al. 2008, Meng et al. 2010).

Antigenicity was assessed in ADA analyses in a variety of toxicology studies. In the 13-week repeat-dose toxicity study in rats (study 20017484), 30 of the 47 PK animals had an ADA response. Nineteen of these 47 animals (approximately 40%) exhibited limited exposure or concentrations below the limit of quantitation on Day 85, which resulted in AUC values that were greater than 2 standard deviations (SD) below the mean for animals without an antibody response; therefore these animals were

excluded from pharmacokinetic analysis. The other 11 animals had an ADA response of low titre and their individual AUC values were within 2 SD. These animals were included in the PK analysis on Day 85; however, they had a lower mean AUC0-336 value compared to the ADA negative animals in the same dose groups. These data demonstrate that ADA response reduced the ACE-536 exposure in rats.

No such effect was observed in the repeat-dose toxicity studies in *Cynomolgus* monkeys, where TK analysis confirmed that ACE-536 exposure was maintained, in a dose-dependent manner, following administration of up to 30 mg/kg.

ADAs were also observed in the 13-week renal toxicity study in rats (CC-DISC-TOX-2024); 25% of luspatercept-treated animals and 33% of RAP-536 treated animals were identified as ADA-positive. Drug concentrations in post-last dose samples were reduced in most (but not all) of the ADA-positive animals, compared to the ADA-negative animals, indicating reduced exposure due to immunogenicity.

In DART studies, the incidence of ADA formation towards luspatercept was low. However, the formation of ADA considerably reduced luspatercept sera levels. Immunotoxicity parameters were integrated in the pivotal juvenile rat toxicity study (Report WIL-961003). In a TDAR assessment, no immunosuppression by up to 10 mg/kg luspatercept was detected. Immunophenotyping in the same study indicated increased T, B and NK cell plasma levels, likely related to inflammatory processes associated with the pronounced organ toxicity of luspatercept observed in this study.

To better understand the effect of luspatercept on the progression of renal injury, a 13-week non-GLP study was conducted in which luspatercept (0.5 or 5 mg/kg, once Q2W) or its murine homologue RAP-536 (10 mg/kg, once Q2W) was given to rats representing a rodent model of renal compromise: 5/6 nephrectomy or unilateral ureteral ligation (UUL). Administration of luspatercept or RAP-536 to 5/6 nephrectomised animals caused notable exacerbation of glomerular and tubulointerstitial lesions, increased pSmad2 and aSMA immunostaining and elevated urinary albumin, TIM-1, and/or osteopontin levels when compared to vehicle-treated nephrectomised or respective sham-operated luspatercept or RAP-536 treatment groups. In the UUL arm of the study, there was no notable difference in the severity of obstructive nephropathy between vehicle and luspatercept/RAP-536 treatment groups.

Furthermore, different TGF-β growths factors that can effectively be bound by luspatercept (BMP6) participate in bone and cartilage development (Cao and Chen, 2005). Hence, binding of these ligands may be a rationale for the observed adverse effects of luspatercept on hind limbs, bone geometry, bone mass and bone density, but might also determine the increased incidences of skeletal malformations and variations identified during EFD.

Immunohistochemistry of kidney sections from control and select luspatercept-treated animals from the 3-month rat study and the 3- and 6-month monkey studies revealed immune component deposition (increased IgG, IgM, and/or C3-containing granular deposits in intramembranous and/or mesangial locations) in areas of affected glomeruli in most of the affected animals. However, there was a lack of association of kidney findings with ADA seropositivity in rats in the renal toxicity study. Therefore, a direct drug effect on renal function was presumed. Indeed, literature data indicates that impairment of the TGF-B/Smad signalling pathway may play a role in the pathogenesis of chronic kidney disease. Although Smad2 and Smad3 were found to be overexpressed in a mouse model of kidney disease, inhibition of Smad2 in kidney cells led to more severe fibrosis, suggesting an antagonistic mechanism of Smad2 and Smad3 in the progression of renal fibrosis (Böttinger & Bitzer 2002, Huang et al. 2008, Meng et al. 2010).

Finally, factors of the TGF-β superfamily also act on the function and ontogeny of the thymus and adrenal gland (Jurberg et al, 2015; Vinson 2016), thus a correlation of TGF-β inhibition by luspatercept to adverse effects observed in these organs seems possible.

2.3.5. Ecotoxicity/environmental risk assessment

As luspatercept is a protein, it is not expected to have a negative environmental impact.

Even though proteins such as Reblozyl may be renally excreted due to luspatercept-mediated in patients with kidney damages (as demonstrated in the juvenile rat toxicity study, Report WIL-961003), the protein can be expected to be rapidly degraded by microbes into smaller peptides, its amino acids or even completely mineralised.

Therefore, Luspatercept is not expected to pose a risk to the environment.

2.3.6. Discussion on non-clinical aspects

Mechanism of action and primary pharmacodynamic studies

The Applicant's non-clinical pharmacodynamic programme is comprehensive and was designed to characterise the biologic activity of luspatercept with respect to mechanism of action and pharmacodynamic effects *in vitro* and in different *in vivo* models, thereby using healthy animals and animal disease models.

Luspatercept binds to GDF11 and other TGF-β superfamily ligands (e.g., GDF8, activin B) and inhibits signalling downstream of activin receptors by phospho-Smad 2/3. Luspatercept enhances differentiation of late-stage erythroid precursors (normoblasts) in the bone marrow and consequently leads to an increase in the production of mature erythrocytes. As such, binding specificity of luspatercept was determined by *in vitro* ligand binding experiments with ligands of the TGF-β superfamily. However, several TGF-β superfamily ligands possess osteoinductive activity. Although the ligands most implicated in osteoinductive activity do not interact with luspatercept, there was a great redundancy among these ligands. Effects on bone development (most likely indirect) were observed as a result of luspatercept administration to prenatal or neonatal animals. The Applicant acknowledged that luspatercept treatment results in skeletal effects during fetal development, and as such, luspatercept is contraindicated during pregnancy (SmPC, Section 4.3). There was no evidence from the provided pharmacology studies of such an effect on bone in adult animals.

Due to the high degree of homology between species, luspatercept was predicted to bind human, *Cynomolgus* monkey, rabbit, and rodent ligands with similar affinity. As such, all the laboratory animals used in this context may be considered relevant. The pharmacological effect of luspatercept *in vivo* was investigated in healthy mice, rats and *Cynomolgus* monkeys, but also in a selection of rodent models in which anaemia contributes significantly to morbidity, including models of anaemia associated with nephrectomy, acute blood loss, sickle cell anaemia and chemotherapy. In addition, pharmacological studies were performed with luspatercept in a mouse model of MDS (NUP98-HOXD13) and a model of β-thalassemia (hemoglobin beta (Hbb) major-/-, Hbbth1/th1 mice), representing relevant models for the targeted indications. Some of the rodent pharmacology studies used a murine orthologue of luspatercept, referred to as RAP-536.

The pharmacodynamic effects of increases in RBC, haemoglobin (HGB), and haematocrit (HCT) were observed in all tested species (mice, rats and *Cynomolgus* monkeys) and disease models. However, there was no statistically significant increase in the RBC parameters in the murine model of sickle cell disease. Increases in white blood cell counts were consistent with indirect changes related to inflammatory processes, such as the glomerulonephritis documented in these studies. None of the increases was accompanied by abnormal morphology or distribution of white blood cells as indicated by microscopic examination of hematopoietic or lymphoid tissues.

Secondary pharmacodynamics

Studies of the secondary pharmacodynamics of luspatercept have not been conducted, as such studies are generally not warranted for large molecule biotherapeutics.

Safety pharmacology

Stand-alone safety pharmacology studies have not been conducted with luspatercept. However, safety pharmacology parameters were incorporated into the repeat dose general toxicity studies of up to 3 months duration in monkeys. This is in line with the ICH M3(R2), Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

Pharmacokinetics

The PK was characterised for the single-dose intravenous (IV) (monkey) and in subcutaneous (SC) routes of administration (rat, rabbit, and monkey), which is the intended route of administration in humans.

Toxicology studies

The Applicant's non-clinical toxicology program on Reblozyl (luspatercept) is considered to fulfil the study requirements on non-clinical toxicity testing for filing a marketing authorisation of a medicinal product according to the ICH S5(R2), ICH S6(R1) and ICH M3(R2) guidelines. However, a considerable range of adverse toxicological effects was identified that demanded thorough discussion and clarification by the Applicant.

Repeat-dose toxicity was studied in Sprague-Dawley rats in two studies of 1 month and 13 weeks, respectively, and in *Cynomolgus* monkeys in three studies of 4 weeks, 13 weeks and 6 months, respectively. A Q14D treatment regimen was applied in all of these studies. In rats, the toxicity findings included increases in RBC and related parameters, increased platelet volume and decreased platelet count, increase in monocytes, WBC and lymphocytes, increases in several serum parameters, decreased heart, liver, kidney and lung weights, membrano-proliferative glomerulonephritis, minimal to mild mid-zonal hepatocellular vacuolation, hepatocellular coagulative necrosis of the liver, minimal to mild congestion of the adrenal gland, mild to marked necrosis and mineralization of the adrenal gland, and minimal focal or multifocal congestion of the thymus. Additional effects in young adult rats included minimal increases in leukocyte, lymphocyte, monocyte, and basophil counts, minimal increases in ALP, decreases in prostate weight and increased adrenal gland weight in females.

A NOAEL could not be established in the repeat-dose toxicity studies in rats; the LOAEL was 1 mg/kg.

Anti-drug antibody formation was also observed in rats at all dose levels, which had an impact on luspatercept exposure levels in affected animals.

In *Cynomolgus* monkeys, the toxicity findings included increases in indicators of circulating erythrocyte mass consistent with the expected pharmacodynamic effects of ACE-536, increases in ALP and ferritin, increased BUN and creatinine, and histopathologic changes in the kidney mainly consisting of membrano-proliferative glomerulonephritis. ACE-536-related microscopic findings were also identified in the choroid plexus of the brain.

In the 13-week study in monkeys, the NOAEL was determined to be 1 mg/kg despite mild histopathologic kidney findings observed at this dose level.

In the 6-month study in monkeys, the NOAEL was determined to be 0.3 mg/kg based on kidney histopathology findings at dosages $\geq$ 1 mg/kg.

Anti-drug antibody formation was only incidently observed in monkeys and did not impact luspatercept exposure levels.

To better understand the effect of luspatercept on the progression of renal injury, a 13-week non-GLP study was conducted in which luspatercept was given to rats with one of two rodent models of renal compromise: 5/6 nephrectomy or unilateral ureteral ligation (UUL). In the 5/6 nephrectomised animals a notable exacerbation of glomerular and tubulointerstitial lesions was observed, which was accompanied amongst others by increased pSmad2 immunostaining. Indeed, literature data indicates that impairment of the TGF-B/Smad signalling pathway may play a role in the pathogenesis of chronic kidney disease, although the exact mechanism is not completely understood.

Results of these above findings have been reflected in section 5.3 of the SmPC.

Carcinogenicity

Three hematologic malignancies detected at the highest dose level of luspatercept in the definitive juvenile toxicity study in rats (Report WIL-961003) prompt a concern for potential development of malignancies in patients. The Applicant argued that a causal relationship to luspatercept of the tumours observed in the juvenile rat study has not been definitively established. However, the lack of malignancies in the control group and lower dose groups makes an influence of high dose luspatercept in this study more likely. In the response, the Applicant hypothesises that the mechanism of the observed carcinogenicity is specific to the developing hematopoietic system in immature rats and that this notion is supported by the data from the three-month repeat dose toxicity study in young and mature rats (Report 20017484), where a similar test article-related increased incidence of malignancies was not observed. Therefore, the wording of the SmPC was revised in order to properly describe the malignancy findings in section 5.3 and corresponding measures were added to the RMP.

Reproduction Toxicity

In DART studies, luspatercept was toxic with regards to female fertility in the rat at the highest tested dose of 15 mg/kg (Report 20040551); however, the observed effects were demonstrated to be reversible. Adverse effects of luspatercept to embryo-foetal development in rats and rabbits comprised a decreased foetal weight, decreased average litter size and live litter size, increased average number of resorptions and percent post-implantation loss, maternal toxicity and skeletal variations as well as an increased incidence of especially skeletal-, but also gross- and visceral malformations. Regarding the latter, significant increases in foetuses of treatment groups relative to control groups having skeletal variations were observed in rats and rabbits. In rats (Report 20040547), multiple gross and visceral malformations of a strikingly low background incidence (e.g. 1 out of 21065 rat foetuses) were mainly observed in foetuses of the treatment groups. In the pivotal rabbit EFD study (Report 20040549), malformations of the ribs and vertebrae were detected in ten foetuses of ten litters exclusively from treatment groups, constituting a low probability that these effects were observed by chance (4.2%). As the target of luspatercept (TGF-β superfamily ligand binding) is evolutionarily highly conserved among animals, the embryo/foetal toxicity and teratogenicity of luspatercept observed in the EFD studies are most likely also relevant in humans.

Because of these adverse effects, the Applicant agreed to a contraindication for luspatercept in pregnancy, which should be an effective means for risk mitigation. Section 5.3 of the SmPC wording was amended to adequately communicate the reproductive toxicity of luspatercept.

Throughout peri- and postnatal development (Report ACE-536-TOX-2485), adverse effects observed in rats at all dose levels (lowest one 3 mg/kg) were significantly lower F1 pup body weights that maximally lasted from birth until the mating period of F1 males as well as adverse kidney findings (especially membrano-proliferative glomerulonephritis, tubular atrophy/hypoplasia and vessel ectasia).

In the juvenile toxicity studies (WIL-961002 and -3), significant adverse effects were determined at all dose levels (the lowest one being 1 mg/kg). They comprised impaired adverse hind limb findings, lower mean mating indices, adrenal gland findings, kidney findings with associated findings in

urinalysis, decreased heart weights, effects on bone geometry, bone mass and bone density and gastric mucosal lesions. In the pivotal juvenile rat toxicity study, luspatercept-related adrenal gland, kidney, glandular stomach and adverse hind limb findings persisted throughout the recovery phase at all dose levels. As the target of Reblozyl (TGF-β superfamily ligand binding) is evolutionarily highly preserved among animals, the developmental toxicity of Reblozyl observed in the juvenile toxicity study is most likely also relevant in humans. However, it is unclear whether the pathogenesis mechanism of haematopoietic malignancies in juvenile rats might potentially also be relevant in young children (not included in the current MAA), in which the haematopoietic system is still developing. In human paediatrics, the incidence of e.g. acute lymphoblastic leukaemias shows a peak at approximately 5 years of age, it may therefore be speculated that the microenvironment and/or leukaemia-initiating cells undergo a transition in their susceptibility to oncogenic transformation during early childhood (Copley and Eaves 2013, DOI: 10.1038/emm.2013.98). Furthermore, the pathogenesis of haematopoietic malignancies is considered to differ between young children and adults (Copley and Eaves 2013, DOI: 10.1038/emm.2013.98). One reason for this may be the high rate of haematopoietic stem cell (HCS) replication in young children. HCS replication decreases from ≈42/year immediately after birth to ≈2.5/year at the age of 3 years, whereas between the ages of 3 and 13 years the rate of HSC replication is relatively stable at ≈2.5/year and after the age of 13 years decreases and remains at ≈0.7/year (Sidorov et al. 2009, DOI: 10.1016/j.exphem.2008.11.009). A second reason for the different pathogenesis of haematopoietic malignancies in young children and adults may be a different expression pattern of the oncogenes Lin28b and Hmga2 and the tumour suppressor let-7 miRNA in these two populations. In early HSCs, both, Lin28b and Hmga2 are expressed at higher levels, and let-7 miRNA at lower ones compared to expression levels in adult HSCs (Copley and Eaves 2013, DOI: 10.1038/emm.2013.98). These observations demonstrate that the molecular aetiology of haematopoietic malignancies in humans is (to a certain degree) age dependent, as similarly speculated by the Applicant in the discussion of the observed haematopoietic malignancies in juvenile rats. Considering that the MoA of luspatercept is evolutionarily highly preserved, it is conceivable that the pathogenetic mechanism of the observed malignancies in the juvenile toxicity study might also be relevant in young children with developing haematopoietic system.

Additionally, it is unclear whether the nephrotoxicity that was commonly observed in most non-clinical studies (which frequently persisted throughout recovery phases, also in the juvenile toxicity study), could also be relevant in young patients in which the renal system is still developing. As the kidney development in humans lasts until ≈3.5 years of age (GFR reaches adult levels after 1-2 years of age, glomeruli reach adult size at ≈3.5 years of age; Gomez et al. 1999), administration of luspatercept to very young infants may also be problematic in this respect. Finally, the other adverse effects observed in young rats (adverse adrenal gland findings, problems in bone growth, lower heart weights etc.), could similarly be problematic in young children exposed to luspatercept. Relevant information has been added to section 4.2, 5.1 and 5.3 of the SmPC.

Environmental risk assessment

As luspatercept is a protein, it is not expected to have a negative environmental impact.

2.3.7. Conclusion on the non-clinical aspects

The non-clinical data package is considered acceptable.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant. The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 2: Key Design Features of Pivotal Study ACE-536-MDS-001 and Supportive Studies A536-03 and A536-05 in MDS

Design Feature	Study ACE-536-MDS-001 (MEDALIST)	Study A536-03	Study A536-05
Study Start (first subject first visit)	09 Feb 2016	21 Jan 2013	09 Oct 2014
Status (data cutoff date)	Ongoing (08 May 2018)	Ongoing (09 Aug 2017)	Ongoing (13 Oct 2017)
Study Design	Phase 3, double-blind, randomized, placebo-controlled, multicenter	Phase 2, open-label, multiple ascending dose, multicenter	Phase 2, open-label, multicenter extension
Study Population	Subjects with IPSS-R very low, low, or intermediate risk RS+ MDS who required RBC transfusions for anemia and are refractory to (nonresponse or response that is no longer maintained), intolerant of, or ineligible for (serum EPO > 200 U/L) ESA treatment.	All cohorts: Subjects with IPSS low or intermediate-1 risk MDS and anemia Expansion cohort 1: high transfusion burden (HTB; defined as ≥ 4 units/8 weeks) and low transfusion burden (LTB; defined as < 4 units/8 weeks) Expansion cohort 2A: RS+ LTB subjects with < 4 weeks of exposure to ESAs and serum EPO level ≤ 200 U/L Cohort 2B: non-RS and ≤ 6 units RBC/ 8 weeks; ESA exposure < 4 weeks and ≥ 4 weeks	Eligible subjects from Study A536-03
Dose	Starting dose level of 1.0 mg/kg SC Q3W; dose titrations to 1.33 or 1.75 mg/kg SC Q3W or dose reductions to 0.8, 0.6, or 0.45 mg/kg SC Q3W were permitted per protocol	Ascending-dose cohorts: 0.125 mg/kg SC Q3W to 1.75 mg/kg SC Q3W planned; dose reductions were permitted per protocol within each cohort Expansion cohorts: Starting dose level of 1.0 mg/kg SC Q3W; dose reductions and titrations were permitted per protocol; maximum dose of 1.75 mg/kg	Starting dose level based on last dose level in Study A536-03 for subjects without treatment interruption Starting dose level of 1.0 mg/kg for subjects with treatment interruption Dose reductions and titrations were permitted per protocol; maximum dose of 1.75 mg/kg

Duration of Treatment	Primary analysis: 24 weeks for all subjects and 48 weeks for subjects eligible to continue treatment after the Week 25 visit Treatment continues beyond Week 48 as long as there is clinical benefit and absence of disease progression per IWG-MDS criteria. Post-treatment follow-up of 42 days for all AEs and 3 years from last dose for OS, progression to AML, other malignancies/pre-malignancies (including progression to higher-risk MDS per IPSS-R criteria [ie, high or very high risk]), and subsequent MDS therapies	5 cycles; Post-treatment follow-up 3 months from last dose for subjects not entering A536-05[a]	Up to 5 years; Post-treatment follow-up 3 months from last dose
Primary Endpoint	Proportion of subjects who are RBC transfusion free over any consecutive 56-day (ie, 8-week) period during Week 1 to Week 24	Proportion of subjects with modified erythroid response, defined as: Hgb increase of ≥ 1.5 g/dL from baseline for ≥ 14 days (in the absence of RBC transfusions) in LTB subjects Reduction of either ≥ 4 units or ≥ 50% of units of transfused RBCs compared with pretreatment during any 8-week window in HTB subjects	None (primary objective was long-term safety and tolerability of luspatercept)
Key Secondary Endpoint	Proportion of subjects who are RBC transfusion free over any consecutive 84-day (ie, 12-week) period during Week 1 to Week 24 and during Week 1 to Week 48	None	None
Number of Subjects by Arm Entered/ Completed	Luspatercept: 153/78 (completed 48 weeks) Placebo: 76/12 (completed 48 weeks)	Luspatercept: : 107/95	Luspatercept: 70/12 (45 ongoing)
Subject Demographics (Sex, Mean Age [range], and Race [%])	M 144/F 85 71 y (26 – 95 y) White 69.9% / Black or African American 0.7% / Other 0.7% / Not collected or reported 28.8%	M 65/F 42 72 y (27 – 90 y) White 100%	M 45/F 25 72.5 y (29 – 90 y) White 100%

AE = adverse event; AML = acute myeloid leukemia; EPO = erythropoietin; ESA = erythropoiesis-stimulating agent; F = female; Hgb = hemoglobin; HTB = high transfusion burden (defined as baseline RBC transfusion burden of ≥ 4 units/8 weeks); IPSS = International Prognostic Scoring System; IPSS-R = International Prognostic Scoring System – Revised; IWG = International Working Group; LTB = low transfusion burden (defined as baseline RBC transfusion burden of < 4 units/8 weeks); M = male; MDS = myelodysplastic syndromes; OS = overall survival; Q3W = every 3 weeks; RBC = red blood cell; RS+ = ring sideroblast positive; SC = subcutaneously; y = years.

a A protocol amendment after the cutoff date for the CSR extended follow-up to 3 years.

Table 3: Key Design Features of Pivotal Study ACE-536-B-THAL-001 and Supportive Studies A536-04 and A536-06 (Beta-Thalassaemia)

Design Feature	Study ACE-536-B-THAL-001	Study A536-04	Study A536-06
Study Start (first subject first visit)	02 May 2016	11 Feb 2013	20 Oct 2014
Status (data cutoff date)	Ongoing (11 May 2018)	Complete (11 Nov 2015)	Ongoing (31 Aug 2017)
Study Design	Phase 3, multicenter (65 sites in 15 countries), randomized 2:1 (luspatercept:placebo), DB, PC, parallel-group	Phase 2, multicenter (8 sites in 2 countries) , single-arm, OL, ascending dose	Phase 2 multicenter (8 sites in 2 countries), single-arm, OL extension
Primary Comparison(s)	Luspatercept vs placebo	NA	NA
Subject Population	Age ≥ 18 y; documented diagnosis of β-thalassemia or HgbE/ β-thalassemia; regularly transfused (6 to 20 RBC units in the 24 weeks prior to randomization); ECOG ≤ 1	Age ≥ 18 y; documented diagnosis of β-thalassemia intermedia (dose escalation cohorts) or β-thalassemia major or intermedia (expansion cohort); prior splenectomy or spleen size < 18 cm; anemia (mean Hgb < 10.0 g/dL or TD)	Completed participation in Study A536-04
Dose	Starting dose 1.0 mg/kg SC Q3W Dose delay, reduction, and titrated permitted for subsequent cycles based on change in Hgb and safety. Dose range: 0.45 to 1.25 mg/kg or Placebo SC Q3W	0.2, 0.4, 0.6, 0.8, 1.0, 1.25, and 1.5 mg/kg SC Q3W for ascending-dose cohorts[a] (1.5 mg/kg cohort not enrolled per SRT decision) Expansion cohort:[b] 0.8 to 1.25 mg/kg; Starting dose determined by the Sponsor and SRT	Last dose level of Study A536-04 for subjects continuing treatment; Starting dose of 0.8 mg/kg for subjects with a treatment interruption Titration permitted for subsequent cycles based on change in Hgb and safety assessment 1.25 mg/kg maximum dose
Duration of Treatment	48 weeks (primary analysis of DB Treatment Period) Up to 48 additional weeks in Long-term Treatment Period Up to 5 years in Open-label Phase	3 months	60 months
Primary Endpoint(s)	Erythroid response, defined as ≥ 33% reduction from baseline in transfusion burden (units RBCs/time) with a reduction of at least 2 units, from Week 13 to Week 24	Erythroid response based on Hgb increase and no transfusion in NTD subjects or reduction in RBC units transfused compared to pretreatment in TD subjects	AEs and safety laboratory assessments; erythroid response

Key Secondary Endpoint	Erythroid response, defined as ≥ 33% reduction from baseline in transfusion burden (units RBCs/time) with a reduction of at least 2 units, from Week 37 to Week 48 Erythroid response, defined as ≥ 50% reduction from baseline in transfusion burden (units RBCs/time) with a reduction of at least 2 units as from Week 13 to Week 24 and from Week 37 to Week 48 Mean change from baseline in RBC transfusion burden from Week 13 to Week 24 (units/12 weeks)	None	None
Number of Subjects by Arm Entered/Completed	Luspatercept + BSC: 224/200 (completed 48 weeks) Placebo: + BSC: 112/96 (completed 48 weeks)	Luspatercept: 64/56	Luspatercept: 51/14 (30 ongoing)
Subject Demographics (Sex, Median Age [range], Race [%])	M 141/F 195 30.0 y (18 – 66 y) Asian: 34.8% Black or African American: 0.3% White: 54.2% Not collected or reported: 3.0% Other: 7.7%	M 33/F 31 38.5 y (20 – 62 y) Asian: 1.6% Black or African American: 1.6% White: 96.9%	M 29/F 22 37.0 y (22 – 62 y) Asian: 2.0% Black or African American: 2.0% White: 96.1%

AE = adverse events; BSC = best supportive care; CSR = clinical study report; DB = double-blind; ECOG = Eastern Cooperative Oncology Group; F = female; Hgb = hemoglobin; M = male; NTD = nontransfusion-dependent; OL = open-label; PC = placebo-controlled; Q3W = once every 3 weeks; RBC = red blood cell; SC = subcutaneous; SRT = Safety Review Team; TD = transfusion-dependent; y = years

a Enrollment of subsequent cohorts and the expansion cohort was based on SRT review of Hgb response and safety from the prior cohort. Dose reductions were permitted in the dose escalation cohorts; dose reductions and titrations were permitted in the expansion cohort for subjects meeting predefined criteria.

b The expansion cohort was planned for 30 subjects and consisted of 29 subjects, including 10 subjects who were NTD and 19 subjects who were TD (including a minimum of 6 thalassemia major subjects with onset of regular transfusions before 4 years of age, and a minimum of 6 subjects with onset of regular transfusions after 4 years of age).

2.4.2. Pharmacokinetics

The PK parameters of luspatercept have been characterised in a small study in healthy subjects as well as in three clinical studies in patients with β-thalassaemia and three clinical studies in patients with MDS.

Dose levels of 0.0625, 0.125, and 0.25 mg/kg were evaluated in the first in human study A536-02 conducted in healthy subjects.

Tested dose levels ranged from 0.125 to 1.75 mg/kg in the phase II study A536-03 in MDS. In the pivotal trial ACE-536-MDS-001 the starting dose was 1 mg/kg SC Q3W. Dose titration to a maximum dose of 1.75 mg/kg (i.e., 2 serial intrasubject dose escalations to 1.33 and then 1.75 mg/kg) was allowed.

Tested dose levels ranged from 0.2 to 1.25 mg/kg in the phase II study A536-04 in β-thalassemia. In the pivotal trial ACE-536-B-THAL-001 the starting dose was 1 mg/kg SC Q3W, with one dose titration to 1.25 mg/kg allowed.

In total, 7 clinical studies contributed PK data. Furthermore, a Population PK Analysis was conducted for each indication.

Absorption

Luspatercept is slowly absorbed after SC injection and reaches Cmax after approximately 7 days in both disease populations. AUC and Cmax values appear to be approximately dose proportional over the dose range evaluated in the phase I study as well as in both phase II studies where ascending doses up to 1.75 mg/kg were tested.

Steady state at the tested 3-weekly dosing interval is reached after 3 doses. Accumulation is moderate with an accumulation ratio of 1.5. Interindividual variability of AUCss is moderate (36% and 38% for β-thalassaemia and MDS, respectively).

Distribution

Based on population PK analysis for the pivotal Phase 3 study, the mean apparent volume of distribution of the central compartment (V1/F) was 7.08 L in subjects with β-thalassemia and 9.68 L for subjects with MDS. The small volume of distribution indicates that luspatercept is confined primarily in extracellular fluids, consistent with its large molecular mass.

Elimination

Luspatercept is expected to be catabolised into amino acids by general protein degradation processes in multiple tissues, and thus its elimination is not dependent on a single organ. The mean apparent clearance was 0.437 L/day in patients with β-thalassemia and 0.516 L/day in subjects with MDS. The mean half-life in serum was 11 and 13 days, in β-thalassemia and MDS, respectively.

Linearity/non-linearity

The increase of luspatercept Cmax and AUC in serum is approximately proportional to increases in dose from 0.125 to 1.75 mg/kg. Luspatercept clearance was independent of dose or time.

When administered every three weeks, luspatercept serum concentration reaches the steady state after 3 doses, with an accumulation ratio of approximately 1.5.

Dose proportionality and time dependencies

In β-thalassaemia patients as well as in MDS patients with no or only one dose modification, trough levels appeared to be stable over the observed duration of exposure.

Special populations

Luspatercept has not been specifically investigated in special patient populations, such as the elderly or patients with hepatic or renal impairment. The studied MDS patient population was old, with a median age of 71.0 years, with approximately 80% of subjects ≥ 65 years of age and > 36% of subjects ≥ 75 years of age.

Table 4: Elderly patients included in the clinical studies

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
PK Trials	113	93	0

Population PK analyses found limited influence of renal or hepatic impairment on the PK parameters of luspatercept.

Body weight exerts a statistically significant and clinically relevant influence on the exposure to luspatercept. Simulations were performed on the population PK data to identify the consequences of fixed dose, BW-based dosing and modified BW-based dosing which capped the highest dose on predicted AUCss and Cmaxss. For lower bodyweights, the fixed dose resulted in higher exposure while both BW-based approaches were able to replicate levels expected in patients with average weight. For higher bodyweights, the fixed dose resulted in lower exposure, while both BW-based approach produced comparable levels to patients with average weight, supporting the BW-based dosing recommendation.

With regards to race and gender, no significant effect was observed. Increasing age suggested a trend towards decreased elimination in MDS patients.

Pharmacokinetic interaction studies

No drug-drug interactions have been conducted.

Data from population PK analyses with regards to concomitant iron chelating products showed no apparent influence on the PK parameters of luspatercept.

Analyses of binding and neutralising antibodies showed that while binding antibodies did not influence PK, neutralising antibodies had the ability to render trough levels of luspatercept unmeasurable.

Bioequivalence

The applicant supports the comparability of the different drug products used in the clinical development plan with a population PK analysis using PK data from those MDS patients treated with a

starting dose of 1 mg/kg and evaluable PK data for the first treatment cycle. Data from 192 subjects fulfilled these requirements and were included into the analysis. The bioequivalence criteria of 80%-125% for the proportion of the 90% confidence intervals for Cmax and AUC for the three different presentations tested were fulfilled, thus demonstrating adequate comparability of PK parameters between the different drug products for a single dose of 1 mg/kg.

In the population PK analysis for MDS, the different presentations were assessed as a covariate and were found to have not statistically significant effect on steady state parameters or elimination kinetics, thus supporting the finding of the more sensitive single dose BE evaluation.

2.4.3. Pharmacodynamics

Mechanism of action

Luspatercept is a recombinant fusion protein that binds select transforming growth factor-beta superfamily ligands. By binding to specific endogenous ligands (e.g., growth differentiation factor-11, activin B) luspatercept inhibits Smad2/3 signalling, resulting in erythroid maturation through differentiation of late-stage erythroid precursors (normoblasts) in the bone marrow. Smad2/3 signalling is abnormally high in disease models characterized by ineffective erythropoiesis, i.e., myelodysplastic syndromes (MDS) and β-thalassemia, and in the bone marrow of MDS patients.

The role of the TGF-beta superfamily in regulating erythropoiesis *in vivo* is not completely understood and information provided by the Applicant with regard to the pharmacodynamic effects of luspatercept in humans is sparse. However, in the non-clinical dossier mouse models for β-thalassaemia as well as MDS established the effect of luspatercept on red blood cell count, haemoglobin and haematocrit. Mechanistic studies demonstrated the ability of luspatercept to induce an erythroid maturation independent of erythropoietin.

Primary and Secondary pharmacology

Primary pharmacodynamics

In a phase I trial in healthy volunteers, doses of 0.0625 up to 0.25 mg/kg luspatercept were evaluated in 24 subjects. All investigated PD endpoints (haemoglobin, red blood cell count, reticulocyte count, haematocrit) showed a dose dependent increase, which slowly returned to values near baseline over the 18 week observation period.

In the clinical phase II ascending dose trials in patients with β-thalassaemia and MDS, haemoglobin levels showed a response approximately 7 days after the first injection of luspatercept. The increase from baseline levels was sustained during the treatment period. Unfortunately, for transfusion dependent patients with thalassaemia as well as MDS patients with a high transfusion burden, the efficacy outcomes were described as surrogates for PD endpoints. Therefore, a PD evaluation using haemoglobin levels (excluding levels within 14 days of a transfusion) instead of efficacy endpoints was requested for these two populations. The PD effect of luspatercept treatment on haemoglobin values for the phase II trials has been summarised by the Applicant: The analyses provided are supportive of a dose-dependent haemoglobin increase and are consistent with the results observed in the phase 3 studies. However, due to the small numbers of patients, the lack of a control arm and a dynamic transfusion administration scheme with potential impact on the haemoglobin increase, no robust conclusion can be drawn from this analysis.

The dose groups in the phase II ascending dose trials in both indications were evaluated for reduction of transfusion requirements as a correlate for a desired PD response. However, numbers of patients in these dosing groups were small (n~3), therefore no relevant differences could be identified between these dosing brackets.

A major negative influence of ADAs on the effects of luspatercept was not observed in the majority of cases.

Secondary pharmacodynamics

For large proteins, an effect on ventricular repolarisation is not expected. To substantiate the lack of this effect, an exposure-response analysis examined the influence of repeated doses of luspatercept up to the highest recommenced dose of 1.75 mg/kg Q3W on the QTc interval. Both, the original analysis as well as a sensitivity analysis including all outliers, found that the upper bound of the two-sided 90% confidence interval for the QTc was below 10 ms, proposed as a relevant margin for risk estimation by ICH E14. Furthermore, a categorical analysis of all patients with available QTc data demonstrates that MDS patients receiving the highest dose have QTc changes comparable to placebo patients.

In order to gain a better understanding of potential safety concerns, the Applicant was asked to elaborate on potential off-target effects caused by binding to TGF-β ligands other than the desired activin B receptor ligand. In response, the Applicant has provided a literature overview with regards to the effects of inhibition of GDF11, GDF8, activin B and BMP-6. Due to the manifold effects of those ligands, no specific off-target effect can be singled out, with the exception of renal toxicity associated with BMP-6 inhibition. The clinical studies did not reveal a risk for the decline of renal function for a treatment duration of up to 18 months.

Additionally, the Applicant has provided an overview over all known factors contributing to kidney injury in the non-clinical studies, i.e. inhibition of reno-protective ligands, immune complex deposition and accumulation of renotoxic ligands. Clinical safety data does not show a comparable effect on renal function, as the subjects experiencing kidney EOIs had a predisposing event or intervention and kidney function recovered while on treatment.

2.4.4. Discussion on clinical pharmacology

The PK parameters of luspatercept have been adequately characterised.

The PD effects of luspatercept were characterised in a phase I trial in healthy volunteers and further explored in patients with β-thalassaemia and MDS. The highly complex mode of action was not completely elucidated, however, and possible off-target effects and interactions were only inferred by discussing the known effects of inhibition of single TGF-β ligands.

The Applicant was asked to discuss the potential for PD interactions with regards to medicinal products used in MDS and β-thalassaemia. The Applicant has appropriately discussed the potential for PD drug-drug interactions in both disease population in their response to the D120 LoQ. A conceivable enhancing effect of ESAs will very likely be not relevant for patients with MDS or beta-thalassaemia due to the reasons lined out above, i.e. patients having high endogenous EPO, not responding well to ESAs.

Stopping rules are detailed in section 4.2 of the SmPC and will prevent unnecessary drug exposure in those subjects who experience a loss of efficacy due to any reason, including immunogenicity, therefore monitoring for the development of ADA is not considered mandatory.

No drug-drug interactions have been conducted; these are not expected for large proteins as they are not subject to metabolisation by cytochrome P450 (CYP450) enzymes.

However, considering that no subjects with severe renal or hepatic impairment have been included in the studies and consequently in the population PK model, the SmPC appropriately mentions that no specific dose recommendation can be made in this patient population. The Applicant´s plan to further monitor renal function in ongoing and future clinical trials is strongly endorsed.

Overall, the clinical pharmacology of luspatercept has been sufficiently characterised and there are no outstanding issues.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology of luspatercept is considered acceptable.

2.5. Clinical efficacy MDS

2.5.1. Dose response study(ies)

Dose response studies (phase 2)

The dose recommendations are derived from phase I and II studies and based on the dose-exposure-response relationship as well as safety- and efficacy considerations, from observed and modelled data (see pharmacology section, table 6).

Dose-Exposure-Response Relationship

Luspatercept exposure and other key factors that might impact efficacy and dose escalation were evaluated by the maximum dose level administered in the primary treatment phase of Week 1 to 24 (Table 8). Luspatercept AUCss for the starting dose (1 mg/kg) was similar among subjects with or without dose escalation, and the time-averaged AUC during the first 24 weeks (AUCavg24) increased in subjects who had dose escalation. Higher baseline RBC-transfusion burden, higher baseline serum EPO, and higher baseline IPSS-R score were more frequent in subjects with dose escalation, especially in those with escalation to 1.75 mg/kg.

Table 5: Summary of luspatercept exposure and key factors related to efficacy by maximum dose level (ACE-536-MDS-001)

Parameter	Statistics	Maximum Dose Level in Week 1 to Week 24		
		1 mg/kg (N = 51)	1.33 mg/kg (N = 40)	1.75 mg/kg (N = 62)
AUC_{ss} at 1 mg/kg (day•µg/mL)	Median (90 PI)	149 (86, 250)	152 (99, 247)	145 (76, 205)
AUC_{avg24} (day•µg/mL)	Median (90 PI)	137 (76, 246)	173 (91, 266)	195 (106, 293)
Baseline RBC-T burden (units/24 week)	Median (90 PI)	15 (9, 36)	17 (8, 32)	18 (12, 34)
Baseline RBC-T burden ≥ 6 units/8 weeks	n (%)	16 (31.4)	17 (42.5)	32 (51.6)
Baseline EPO (U/L)	Median (90 PI)	99 (25, 800)	131 (29, 487)	151 (26, 717)
Baseline EPO 200-500 U/L	n (%)	9 (17.6)	10 (25.0)	16 (25.8)
Baseline EPO > 500 U/L	n (%)	5 (9.8)	2 (5.0)	8 (12.9)
Baseline IPSS-R: Intermediate/high	n (%)	4 (7.8)	12 (30.0)	10 (16.1)

AUC = area under the concentration-time curve; AUC_{ss} = AUC at steady state; AUC_{avg24} = average AUC from Week 1 to Week 24; EPO = erythropoietin; IPSS-R = International Prognostic Scoring System-Revised; N = number of subjects for each dose escalation status; n = number of subjects for each parameter; 90 PI = 90% percentile interval (5^{th} to 95^{th} percentiles); RBC-T = red blood cell transfusion.

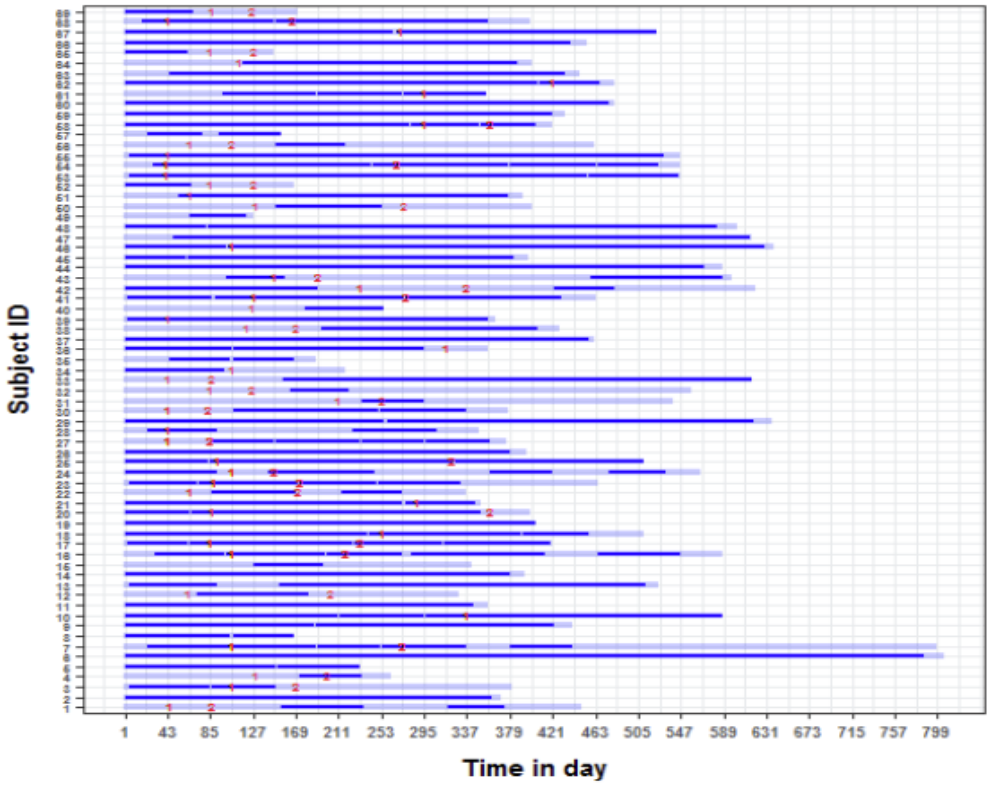
To understand if dose escalation improved response durability, the relation between dose escalation and multiple response episodes was summarised (see Table 9) and inspected in a swimmer plot (Figure 5).

Table 6: Number (%) responders by dose level and response episode sequence during the entire treatment phase (ACE-536-MDS-001)

RBC-TI ≥ 8 weeks Episode	Dose Level prior to the Starting Time of Response Episode			
	0.8 mg/kg	1.0 mg/kg	1.33 mg/kg	1.75 mg/kg
Episode 2 (N = 37)	4 (10.8)	22 (59.5)	5 (13.5)	6 (16.2)
Episode 3 (N = 13)	None	2 (15.4)	5 (38.5)	6 (46.2)
Episode 4 (N = 7)	None	None	1 (14.3)	6 (85.7)
Episode 5 (N = 2)	None	None	None	2 (100.0)

N = number of subjects; RBC-TI = red blood cell transfusion independence.
Responders are defined as subjects who achieved red blood cell transfusion independence ≥ 8 consecutive weeks during the first 48-week treatment period. All available records as of the cutoff date are included for responders.
Source: Ad-hoc analyses for luspatercept Module 2.7.2-MDS (data on file).

Figure 2: Swimmer plot of response episodes in relation to dose escalation time during the entire treatment phase in subjects who achieved red blood cell transfusion independence ≥8 weeks in week 1 to week 48 (ACE-536-MDS-001)



1 = first time for dose escalation to 1.33 mg/kg; 2 = first time for dose escalation to 1.75 mg/kg; ID = subject identifier.
All available records as of the cutoff date are included for responders.
Light blue bars represent the treatment duration and blue bars represent the duration of response episodes.
Source: Ad-hoc analyses for luspatercept Module 2.7.2-MDS (data on file).

Exposure-efficacy response relationship

Two sets of exposure-efficacy analyses were therefore conducted: one included all luspatercept-treated subjects (N = 153) and the other included only the subjects without dose escalation (N = 51). The analysis with all subjects provided the real-world "exposure-response" relationship under the titration dosing regimen and was more suitable for identifying risk factors for efficacy, but it was less sensitive

for determining an exposure-dependent event due to the selection bias. The analysis with subjects who did not have any dose escalation was more sensitive to exposure-dependent events, but it might inflate the response rate and affect identification of risk factors by excluding non-responders who underwent dose escalation. Although both approaches have limitations, the combination of both allowed a reasonable assessment of the effective exposure (dose).

For RBC-TI ≥ 8 weeks in Week 1 to Week 24, no significant exposure (AUCavg24)-dependent trend was observed in both univariate and multivariate analyses, while for RBC-TI ≥ 12 weeks in Week 1 to Week 24, in the more sensitive analysis including subjects without dose escalation, the probability of achieving RBC-TI ≥ 12 weeks was found to be significantly associated with AUCavg24 (odds ratio [OR] = 2.05 per 50 day· μg/mL increase in AUCavg24), but the response plateaued at the 2nd AUCavg24 quartile.

Although the E-R analyses demonstrated a significant treatment effect on RBC-TI and indicated an exposure-driven trend for RBC-TI ≥ 12 weeks, the E-R curves for efficacy endpoints tested were generally nearly flat (plateaued at 1st or 2nd AUCavg24 quartile) for the following reasons:

- only the more effective doses were studied (1 to 1.75 mg/kg),
- luspatercept serum exposure (AUCavg24) largely overlapped between 1 to 1.75 mg/kg due to individual variability,
- when including all subjects, the E-R trend was obscured to some degree by the titration to-response regimen at which dose escalations (thus higher AUCavg24) occurred more frequently in non-responders and
- placebo-treated subjects were excluded from E-R models.

In addition, the response as measured by RBC-TI was not sensitive to small changes in exposure, and transfusion decisions were made based on multiple clinical factors, which could vary among subjects. Therefore, the dosing regimen with the option of escalation to 1.75 mg/kg allowed most subjects to reach an efficacious exposure accounting for individual variability in sensitivity to the drug.

The nearly flat E-R curve with responses at most AUC quartiles greater than those observed with placebo suggests that the maximum effective exposure of luspatercept was reached in most subjects for the tested efficacy endpoints under the current titration dosing regimen.

The multivariate analysis suggested that the probability of achieving RBC-TI was reduced by high baseline RBC-T burden (≥ 6 units/8 weeks), high baseline EPO (> 500 U/L), and older age (OR = 0.56-0.59 for a 10-year increase in age). On the other hand, the probability of achieving RBC-TI ≥ 12 weeks was found to be greater in subjects with baseline total bilirubin > 1.5 x upper limit of normal (ULN; originally defined as moderate/severe hepatic impairment by National Cancer Institute-Organ Dysfunction Working Group [NCI-ODWG] criteria).

In a Kaplan-Meier analysis stratified by luspatercept serum exposure level (≤ median AUCavg48 vs. > median AUCavg48) in early responders (Week 1 - 24), there was no significant difference in the RBC-TI duration between low and high AUCavg48 groups.

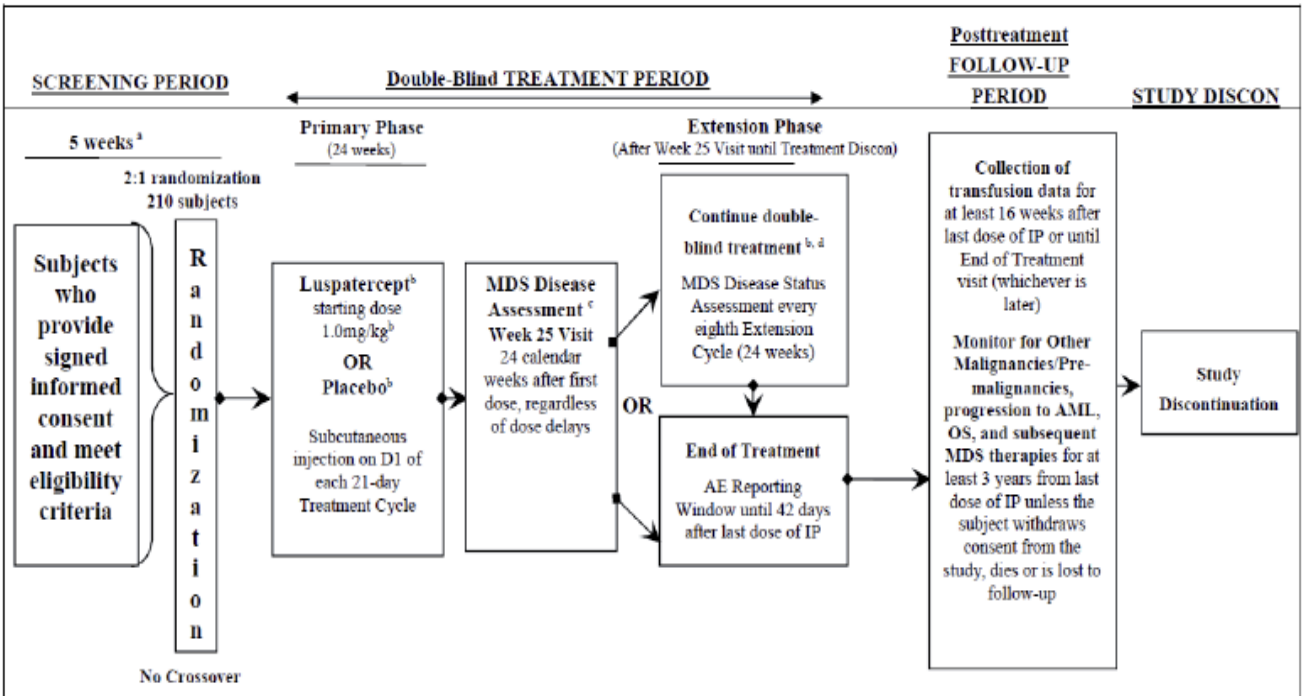
2.5.2. Main studies

Study ACE-536-MDS-001: A phase 3 double-blind, randomized study to compare the efficacy and safety of Luspatercept (ACE-536) versus placebo for the treatment of anaemia due to IPSS-R very low, low or intermediate

risk myelodysplastic syndromes in subjects with ring sideroblasts who require red blood cell transfusion

Methods

Figure 3: Overall study design for the pivotal phase 3 study ACE-536-MDS-001



AE = adverse event; AML = acute myeloid leukemia; D1 = Day 1; Discon = discontinuation; Hgb = hemoglobin; IP = investigational product; IWG = International Working Group; MDS = myelodysplastic syndromes; OS = overall survival.

a Historical documentation of RBC transfusion dependence should have been available (RBC units transfused and pretransfusion Hgb values) for at least 16 weeks prior to randomization.

b Dose may have been titrated up to a maximum of 1.75 mg/kg

c After completion of the Week 25 Visit MDS disease assessment by the investigator, subjects who experienced clinical benefit and had not experienced disease progression per IWG-MDS criteria for altering natural history of MDS (Cheson, 2006), may have continued double-blind treatment with IP beyond the Week 25 Visit in the Extension Phase of the Treatment Period until they met the protocol discontinuation criteria.

d The MDS disease assessment was to be repeated by the investigator at Extension Cycle 8, Day 1 and Day 1 of every eighth Extension Cycle thereafter (ie, Extension Cycle 8, 16, 24+, etc, or every 24 weeks in the event of dose delays) until the subject was discontinued from treatment. For subjects to continue double-blind treatment in the Extension Phase of the Treatment Period, each MDS disease assessment had to confirm continued clinical benefit and absence of disease progression per IWG-MDS criteria for altering natural history of MDS (Cheson, 2006).

Study Participants

Patients were recruited from 65 centres located in the US (11 sites), Canada (4 sites), EU (48 sites) and Turkey (2 sites).

Inclusion criteria

- Patient is at least 18 years of age
- Patients has documented diagnosis of MDS according to WHO/FAB classification that met IPSS-R classification (Greenberg, 2012)of very low-, low-, or intermediate-risk disease, and the following:
    - o Ring sideroblasts ≥ 15% of erythroid precursors in bone marrow or ≥ 5% (but < 15%) if SF3B1 mutation was present
    - o Less than 5% blasts in bone marrow
    - o Peripheral blood white blood cell (WBC) count < 13,000/µL
- Subject was refractory or intolerant to, or ineligible for, prior ESA treatment, as defined by any 1 of the following:
    - o Refractory to prior ESA treatment: Documentation of nonresponse or response that was no longer maintained to prior ESA-containing regimen, either as a single agent or in combination (eg, with G-CSF). The ESA regimen must have been either:
        - ☐ Recombinant human erythropoietin ≥ 40,000 IU/week for at least 8 doses or equivalent; or
        - ☐ Darbepoetin-α ≥ 500 µg q3w for at least 4 doses or equivalent
    - o Intolerant to prior ESA treatment: Documentation of discontinuation of prior ESA-containing regimen, either as a single agent or in combination (eg, with G-CSF), at any time after introduction due to intolerance or an AE
    - o ESA ineligible: Low chance of response to ESA based on endogenous serum EPO level > 200 U/L for subjects not previously treated with ESAs
- If previously treated with ESAs or G-CSF/granulocyte-macrophage colony-stimulating factor (GM-CSF), both agents must have been discontinued ≥ 4 weeks prior to the date of randomization
- Required RBC transfusions, as documented by the following criteria:
    - o Average transfusion requirement of ≥ 2 units/8 weeks of packed RBCs confirmed for a minimum of 16 weeks immediately preceding randomization
    - o Haemoglobin levels at the time of or within 7 days prior to administration of a RBC transfusion must have been ≤ 10.0 g/dL in order for the transfusion to be counted towards meeting eligibility criteria. Red blood cell transfusions administered when Hgb levels were > 10 g/dL and/or RBC transfusions administered for elective surgery did not qualify as a required transfusion for the purpose of meeting eligibility criteria
    - o No consecutive 56-day period that was RBC transfusion free during the 16 weeks immediately preceding randomization
- Eastern Cooperative Oncology Group (ECOG) score of 0, 1, or 2

Exclusion Criteria

- Prior therapy with disease-modifying agents for underlying MDS disease (e.g., immunomodulatory drugs [IMiDs such as lenalidomide], HMAs, or immunosuppressive therapy)

- Subjects who previously received HMAs or lenalidomide may have been enrolled at the investigator's discretion contingent that the subject received no more than 2 doses of HMA or no more than 1 calendar week of treatment with lenalidomide. The last dose must have been ≥ 5 weeks from the date of randomization

- Previously treated with either luspatercept or sotatercept (ACE-011)
- Myelodysplastic syndromes associated with del(5q) cytogenetic abnormality
- Secondary MDS, ie, MDS that was known to have arisen as the result of chemical injury or treatment with chemotherapy and/or radiation for other diseases
- Known clinically significant anaemia due to iron, vitamin B12, or folate deficiencies, or autoimmune or hereditary haemolytic anaemia, or gastrointestinal bleeding
- Prior allogeneic or autologous stem cell transplant
- Known history of diagnosis of AML
- Use of any of the following within 5 weeks prior to randomization:
    - Anticancer cytotoxic chemotherapeutic agent or treatment
    - Corticosteroid, except for subjects on a stable or decreasing dose for ≥ 1 week prior to randomization for medical conditions other than MDS
    - Iron chelation therapy, except for subjects on a stable or decreasing dose for at least 8 weeks prior to randomization
    - Other RBC hematopoietic growth factors (eg, interleukin [IL]-3)
    - Investigational drug or device, or approved therapy for investigational use. If the half-life of the previous study drug was known, the use of it within 5 times the half-life prior to randomization or within 5 weeks, whichever is longer, was excluded
- Uncontrolled hypertension, defined as repeated elevations of diastolic blood pressure (DBP) ≥ 100 mmHg despite adequate treatment
- Absolute neutrophil count (ANC) < 500/μL (0.5 × 109/L)
- Platelet count < 50,000/μL (50 × 109/L)
- Estimated glomerular filtration rate or creatinine clearance < 40 mL/min
- Aspartate aminotransferase (AST)/serum glutamic oxaloacetic transaminase (SGOT) or alanine aminotransferase (ALT)/serum glutamic pyruvic transaminase (SGPT) ≥ 3.0 × upper limit of normal (ULN)
- Total bilirubin ≥ 2.0 × ULN
    - Higher levels were acceptable if these could have been attributed to active RBC precursor destruction within the bone marrow (i.e., ineffective erythropoiesis) or in the presence of known history of Gilbert Syndrome
    - Subjects were excluded if there was evidence of autoimmune haemolytic anaemia manifested as a corrected reticulocyte count of > 2% with either a positive Coombs test or over 50% indirect bilirubin

- Prior history of malignancies, other than MDS, unless the subject had been free of the disease (including completion of any active or adjuvant treatment for prior malignancy) for ≥ 5 years. However, subjects with the following history/concurrent conditions were allowed:
    - Basal or squamous cell carcinoma of the skin
    - Carcinoma in situ of the cervix
    - Carcinoma in situ of the breast
    - Incidental histologic finding of prostate cancer (T1a or T1b using the tumor, nodes, metastasis clinical staging system)
- Major surgery within 8 weeks prior to randomization. Subjects must have been completely recovered from any previous surgery prior to randomization

MDS disease assessment

Screening MDS diagnosis confirmation required bone marrow biopsy, bone marrow aspirate, and peripheral blood samples. Samples may have been reviewed locally but must also have been sent to the central laboratory for analysis.

Cytomorphology assessment:

Bone marrow and peripheral blood samples were prepared locally and sent to the central laboratory for analysis to confirm MDS diagnosis and baseline WHO prior to randomization.

If the central reviewer and local pathologist disagreed on the diagnosis of a subject, a third reviewer at the central laboratory may have been consulted to provide an adjudication assessment. The central laboratory may also have requested the site to send in samples reviewed by the local pathologist for further assessment.

Cytogenetics analysis:

The central laboratory conducted cytogenetic analysis throughout the study. The central laboratory provided standardized analysis and reporting for all subjects. Bone marrow samples were sent to the central laboratory for processing and cytogenetic analysis prior to randomization.

Treatments

Subjects were assigned to treatment as per one of the following regimens:

- Experimental group: Luspatercept at a starting dose level of 1.0 mg/kg SC injection q3w (administered on Day 1 of each 21-day treatment cycle); or
- Control group: Placebo (volume equivalent to experimental group) SC injection q3w (administered on Day 1 of each 21-day treatment cycle)

Luspatercept or placebo was administered as an SC injection to subjects by the study staff at the clinical site. Subjects were required to undergo assessments of Hgb, blood pressure, and weight prior to each IP administration.

Subcutaneous injections were given in the upper arm, thigh, and/or abdomen. Calculated doses requiring reconstituted volume greater than 1.2 mL were divided into separate similar volume injections across separate sites, using the same anatomical location, but on opposite sides of the body (e.g., left thigh and right thigh). The maximum volume per SC injection should not have exceeded 1.2

mL. The maximum total dose per administration did not exceed 168 mg, which resulted in 3.36 mL maximum total volume after reconstitution.

The first dose of IP (either luspatercept or matching placebo) was to be administered within 3 days after randomization and could have been on the same day of randomization.

Subjects received IP on Day 1 of each 21-day treatment cycle.

In both treatment groups, best supportive care (BSC) may have been used in combination with the IP when clinically indicated per investigator. Best supportive care included, but was not limited to, treatment with RBC transfusions; antibiotic, antiviral, and/or antifungal therapy; and nutritional support as needed. Best supportive care for this study excluded the use of ESAs.

Permitted concomitant medication

Granulocyte colony stimulating factors (i.e., G-CSF, GM-CSF) were allowed only in cases of neutropenic fever or as clinically indicated per product label.

Concurrent corticosteroids used for medical conditions other than MDS were allowed provided subject was on a stable or decreasing dose for ≥ 1 week prior to randomization.

Administration of attenuated vaccines (e.g., influenza vaccine) was allowed if clinically indicated, per investigator's discretion.

Iron chelation therapy (ICT)

Subjects who were using ICTs at the time of randomization were to be on a stable or decreasing dose for at least 8 weeks. Concurrent treatment with ICTs during the Treatment Period was allowed at the discretion of the investigator and was recommended to be used per product label.

Prohibited concomitant medications

Best supportive care for this study specifically excluded cancer surgery, immunotherapy, biologic therapy, radiotherapy, and systemic chemotherapy where the goal was to eradicate or slow the progression of the disease.

The following concomitant medications were specifically excluded during the course of the study:

- Cytotoxic, chemotherapeutic, targeted or investigational agents/therapies
- Azacitidine, decitabine, or other HMAs
- Lenalidomide, thalidomide, and other IMiDs
- Erythropoietin stimulating agents and other RBC hematopoietic growth factors (eg, IL-3).
- Granulocyte colony stimulating factors (ie, G-CSF, GM-CSF), except in cases of neutropenic fever or as clinically indicated per product label.
- Hydroxyurea
- Androgens, unless to treat hypogonadism
- Oral retinoids (topical retinoids were permitted)
- Arsenic trioxide
- Interferon

Decision criteria for Rescue RBC transfusions

Concurrent treatment for anaemia with blood transfusions was allowed, at the discretion of the investigator, for low Hgb levels, symptoms associated with anaemia (e.g., hemodynamic or pulmonary compromise requiring treatment), or comorbidity. For any RBC transfusions received during the study, information on Hgb values just prior to transfusion was collected. Each subject had a "pretransfusion Hgb threshold" for requiring transfusion during the study, which was to be determined based on transfusion history. Baseline pretransfusion Hgb threshold was the mean of all documented pretransfusion Hgb values during the 16 weeks prior to Dose 1 Day 1.

During treatment, if the pretransfusion Hgb level was increased by ≥ 1.0 g/dL (at the time of a next anticipated transfusion event) compared with the pretransfusion Hgb threshold for that subject, transfusion should have been delayed by a minimum of 7 days and/or the number of units transfused should have been reduced by 1 or more RBC units. Subjects may have been transfused at the investigator's discretion for symptoms related to anaemia or other requirements (e.g., infection).

Selection of Doses in the Study

The starting dose level was 1.0 mg/kg and the maximum dose level 1.75 mg/kg. Dose reduction was also possible.

Increase:

If all criteria below were met, the dose may have been increased by 1 dose level, starting as soon as Cycle 3 Day 1 and assessed by the investigator prior to every subsequent treatment cycle.

o Subject had ≥ 1 RBC transfusion event (for pretransfusion Hgb of < 9 g/dL) during the 2 most recent prior treatment cycles (~ 6 weeks)

o The 2 most recent prior treatment cycles assessed must have been at the same dose level

o Subject must not have met protocol dose delay and/or reduction criteria in the 2 most recent treatment cycles (exception of dose delay required due to influence of RBC transfusions).

Reduction or delay:

Dose delay and/or reduction or discontinuation may have been required due to increased Hgb or AEs in either treatment group (luspatercept or placebo).

Objectives

Primary objective:

- To evaluate Red Blood Cell-transfusion independence (RBC-TI) of luspatercept compared with placebo for the treatment of anaemia due to IPSS-R very low-, low-, or intermediate-risk MDS in subjects with ring sideroblasts who required RBC transfusions.

Secondary objectives:

- To assess the safety and tolerability of luspatercept compared with placebo
- To evaluate the effect of luspatercept on reduction in RBC transfusions, increase in Hgb, duration of RBC-TI, improvement in HRQoL (ie, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire [EORTC QLQ-C30]), increase in neutrophils, increase in platelets, decrease in serum ferritin, decrease in ICT use, and time to RBC-TI compared with placebo

- To evaluate population PK and exposure-response relationships for luspatercept in MDS subjects
- To determine the effect of luspatercept on HRQoL (ie, QoL-E) compared with placebo (exploratory)

Outcomes/endpoints

Table 7: Study endpoints

Endpoint	Name	Description	Timeframe
Primary Endpoint	RBC-TI ≥ 8 weeks	Proportion of subjects who were RBC transfusion free over any consecutive 56-day period	Week 1 through Week 24
Secondary Endpoints	RBC-TI ≥ 12 weeks	Proportion of subjects who were RBC transfusion free over any consecutive 84-day period	Week 1 through Week 24 Week 1 through Week 48
	RBC-TI ≥ 8 weeks	Proportion of subjects who were RBC transfusion free over any consecutive 56-day period	Week 1 through Week 48
	Reduction in RBC units transfused over 16 weeks	Mean change in total RBC units transfused over a fixed 16-week period	Week 9 through Week 24 Week 33 through Week 48
	mHI-E per IWG (Cheson, 2006)	Proportion of subjects who achieved mHI-E over any consecutive 56-day period	Week 1 through Week 24 Week 1 through Week 48
	Mean Hgb increase ≥ 1.0 g/dL	Proportion of subjects who achieved Hgb increase from baseline of ≥ 1.0 g/dL over any consecutive 56-day period in the absence of RBC transfusions	Week 1 through Week 24 Week 1 through Week 48
	Duration of RBC-TI	Maximum duration of RBC-TI for subjects who achieved RBC-TI of ≥ 8 weeks	Week 1 through Week 24 Week 1 through EOT
	HRQoL	Change in EORTC QLQ-C30 scores	Week 1 through Week 48 Baseline through EOT
	HI-N per IWG (Cheson, 2006)	Proportion of subjects who achieved HI-N over any consecutive 56-day period	Week 1 through Week 24 Week 1 through Week 48
	HI-P per IWG (Cheson, 2006)	Proportion of subjects who achieved HI-P over any consecutive 56-day period	Week 1 through Week 24 Week 1 through Week 48
	Mean decrease in serum ferritin	Change in serum ferritin	Week 9 through Week 24 Week 33 through Week 48
	Mean decrease in ICT use	Change in mean daily dose of ICT	Week 9 through Week 24 Week 33 through Week 48
	Time to RBC-TI	Time from first dose to first onset of RBC-TI ≥ 8 weeks	Week 1 through Week 24 Week 1 through Week 48
	Progression to AML	Number and percentage of subjects progressing to AML; time to AML progression	Randomization through at least 3 years post last dose; Week 1 through Week 48
Secondary Endpoints (continued)	OS	Time from date of randomization to death due to any cause	Randomization through at least 3 years post last dose; Week 1 through Week 48
	Safety	Type, frequency, severity of AEs, and relationship of AEs to luspatercept/placebo	Screening through 42 days post last dose; Week 1 through Week 48
	A population PK model Exposure-response relationship	A population PK model that described the PK exposure data of luspatercept and associated variability Exposure-response relationship for the primary efficacy endpoint, AEs of interest, and selected secondary endpoints	Randomization through 1 year post first dose
	ADAs	Frequency of ADAs and the effect on efficacy or safety or PK	Randomization through 1 year post first dose

ADA = antidrug antibody; AE = adverse event; AML = acute myeloid leukemia; CRP = C-reactive protein; EORTC QLQ-C30 = European Organization for Research and Treatment of Cancer Quality of Life Questionnaire; EOT = end of treatment; GDF = growth differentiation factor; Hgb = hemoglobin; HI-N = hematologic improvement - neutrophils; HI-P = hematologic improvement – platelets; HRQoL = health-related quality of life; HRU = healthcare resource utilization; ICT = iron chelation therapy; IP = investigational product; IWG = International Working Group; MDS = myelodysplastic syndromes; mHI-E = modified hematologic improvement - erythroid; OS = overall survival; PK = pharmacokinetic; QoL-E = psychometric questionnaire assessing HRQoL in MDS patients; RBC = red

blood cell; RBC-TI = red blood cell transfusion independence; SF3B1 = splicing factor 3B subunit 1; TGF-β = transforming growth factor-β.

Rationale for the Efficacy Endpoints Related to Red Blood Cell Transfusion Independence

In order to mitigate the potential bias should subjects in the control group (placebo group) drop out early due to the lack of a rapid response, the primary efficacy analysis in this study was the proportion of subjects achieving RBC-TI with a duration of ≥ 8 weeks measured at 24 weeks.

After completion of the MDS disease assessment by the investigator at the Week 25 Visit, subjects who exhibited clinical benefit with no evidence of disease progression per IWG-MDS criteria for altering natural history of MDS (Cheson, 2006) were to continue the double-blind treatment. The proportion of subjects achieving RBC-TI with a duration of ≥ 8 weeks at 48 weeks was assessed as a secondary endpoint to capture potential late responders. In addition, the proportion of subjects achieving RBC-TI with a duration of ≥ 12 weeks was assessed as a key secondary endpoint, representing extended duration of benefit achieved with IP.

MDS Disease Assessment (week 25 visit)

Treatment response was assessed locally by the investigator in accordance with IWG 2006 criteria for MDS (Cheson, 2006) with modifications for the erythroid response criteria through transfusion assessments, haematology laboratory parameters, peripheral blood smear, bone marrow aspirates and/or biopsies, and cytogenetics.

Central laboratory results from bone marrow and peripheral blood samples (e.g., cytomorphology, cytogenetics analysis) were required as part of the MDS disease assessment.

In order for the subjects to remain on double-blind treatment beyond the first 24 calendar weeks, the following criteria must have been confirmed upon the completion of the MDS disease assessment by the investigator:

- Evidence of clinical benefit (e.g., decrease in RBC transfusion requirement compared with baseline requirement or Hgb increase compared with baseline); and
- Absence of disease progression per IWG-MDS criteria for altering natural history of MDS (Cheson, 2006)

Efficacy data were also reviewed by an external unblinded DMC and external blinded steering committee at specified time points detailed in each committees' respective charters.

Sample size

A total sample size of 210 (140 in experimental arm [luspatercept (ACE-536)], 70 in control arm [placebo]) will have 90% power to detect the difference between a response (RBC-TI ≥ 8 weeks Week 1 through Week 24) rate of 0.30 in the experimental arm (luspatercept [ACE-536]) and a response rate of 0.10 in the control arm (placebo). The sample size calculation is based on one sided alpha of 0.025, test statistics on difference of proportions using pooled estimate of variance and 10% dropout rate.

An interim analysis to assess futility on the primary and key secondary endpoint will be performed when approximately 105 subjects have completed the Primary Phase of the Treatment Period (first 24 weeks of double-blind treatment) or discontinued before reaching 24 weeks of double-blind treatment (50% information for primary endpoint). There will be no plan to claim luspatercept superiority based on efficacy results so the type I error rate remains at 0.025 one sided for the final analysis.

Randomisation

Randomization occurred by a central randomization procedure using integrated response technology (IRT). Authorized site personnel must contact the IRT for randomization, study drug assignment at the beginning of each cycle, to register dose reductions or titrations, and treatment discontinuation. Confirmation of each call will be sent to the investigational site and Celgene. After randomization, no crossover between the treatment arms will be permitted at any point during the study.

Subjects were randomized with a ratio of 2:1 to either treatment with luspatercept or placebo. Randomisation was additionally stratified by RBC Transfusion burden at baseline (≥ 6 RBC units/8 weeks and < 6 RBC units/8 weeks [mean of the two consecutive 8 weeks periods immediately prior to randomization)]) and by IPSS-R at baseline (Very low/low and Intermediate).

Randomization, drug dispensing, dose reduction/titration, and drug discontinuation were accomplished by an IRT system. Authorized site personnel must have contacted the IRT for randomization, IP assignment at the beginning of each cycle, registering dose reductions or titrations, and treatment discontinuation. Confirmation of each call was to be sent to the investigational site and Celgene.

Blinding (masking)

All subjects, study site staff, and Celgene representatives, with the exception of designated individuals (e.g., the pharmacist at the investigational site, the bioanalytical laboratory), remained blinded to all treatment assignments until all subjects completed the study or at the time the study was unblinded (per DMC recommendation) and the database was locked.

The designated site individual (e.g., the pharmacist) at the investigational site used a syringe (that exactly matched the syringe used for reconstituted luspatercept) and sterile normal saline (0.9% sodium chloride for injection) to prepare a matching placebo. Thus, the designated site individual at the investigational site was unblinded and gave investigators and their staff luspatercept and placebo in a blinded manner.

The blind was not to be broken during the course of the study unless, in the opinion of the investigator, it was absolutely necessary to safely treat the subject. If it was medically imperative to know what IP the subject was receiving, IP had to be temporarily discontinued if, in the opinion of the investigator, continuing IP could have negatively affected the outcome of the subject's treatment.

Statistical methods

Analysis sets

The following analysis populations were planned for this study:

- Intent-to-treat (ITT) population: all randomized subjects, regardless of whether or not the subject met the eligibility criteria or received IP. All efficacy analyses were conducted for the ITT population. Subjects were analysed based on randomized treatment group.
- Safety population: all subjects who were randomized and received at least 1 dose of IP. The safety population was used for all safety analyses. Subjects were analysed according to IP they actually received.
- Health-related QoL evaluable population: all subjects in the ITT population who completed the EORTC QLQ-C30 assessment at baseline and at least 1 postbaseline assessment visit.

- Pharmacokinetic population: all subjects who received at least 1 dose of luspatercept and had measurable luspatercept serum concentrations

Handling of Dropouts, Missing Data and Intercurrent events

Missing data were imputed and reference was made to Appendix A of the SAP (Appendix 16.1.9) for comprehensive information on handling of missing data. This could however not be found.

For the primary efficacy endpoint, 56-day RBC transfusion independence, the response rate will be calculated using the number of responders divided by number of subjects (responders plus non-responders). Subjects discontinued from the Primary Phase of the Treatment Period without achieving at least 56 days consecutive of RBC transfusion independence will be counted as nonresponders.

Since the Hgb value can be influenced by a RBC transfusion, the Hgb values used in efficacy analyses are required to satisfy the 14/3-day rule below:

14/3-day rule: Only Hgb values that are at least 14 days after a transfusion may be used unless there is another transfusion within 3 days after the Hgb assessment. If this occurs, the second Hgb value may be used (despite being < 14 days after the previous transfusion).

The rationale is that the Hgb value < 14 days from the first transfusion was only somewhat influenced by that transfusion. In efficacy analyses, after applying above 14/3-day rule, the baseline Hgb value is defined as the lowest Hgb value from the central, local laboratory, or pre-transfusion Hgb from transfusion records that is within 35 days on or prior to the first dose of IP if it is available.

Erythropoietin value can also be influenced by a RBC transfusion. Baseline EPO is defined as the highest EPO value within 35 days of the first dose of IP.

Primary efficacy analysis

For the primary efficacy endpoint, 56-day RBC transfusion independence, the response rate was calculated using the number of responders divided by number of subjects (responders plus non-responders). Subjects discontinued from the Primary Phase of the Treatment Period without achieving at least 56 days consecutive of RBC transfusion independence will be counted as nonresponders. The response rates of the subjects who were randomized to luspatercept and the placebo were calculated. In the primary efficacy analysis, the following statistical hypothesis was tested:

H0: P1= P2

Ha: P1> P2

where P1 denotes the true response rate in the luspatercept group, and P2 denotes the true response rate in the placebo group.

The number and percentage of subjects who achieved RBC-TI and corresponding 97.5% upper bound confidence interval (CI) were tabulated and presented by treatment group. The Cochran- Mantel-Haenszel (CMH) test was used to test the difference between the 2 response rates at a 1-sided significance level of 0.025, stratifying for average baseline RBC transfusion requirement ($\geq$ 6 units versus < 6 units of RBC per 8 weeks) and baseline IPSS-R score (very low or low versus intermediate).

The p-value from the stratified CMH chi-square test was to be the confirmatory p-value for the test of the null hypothesis that the proportion of subjects achieving RBC-TI was equal between the 2 treatment groups. If the p-value was less than 0.025, the null hypothesis was rejected, indicating that the true response rate in the luspatercept treatment group was more than the true response rate in the placebo group.

Key Secondary Efficacy Analyses

The key secondary endpoint, proportion of subjects achieving RBC-TI with duration of ≥ 12 weeks, was tested in the same manner as the primary efficacy endpoint using the CMH test.

The analyses for the key secondary endpoint were based on the ITT population. In order to perform hypothesis testing on multiple endpoints while controlling the overall Type I error rate, a sequential testing approach was employed where the order of the endpoints to be tested was prespecified. The primary efficacy endpoint was tested first at the 1-sided 0.025 significance level. If superiority of luspatercept was demonstrated for the primary efficacy endpoint, then the key secondary endpoint was tested, at a 1-sided 0.025 significance level.

Results

Participant flow

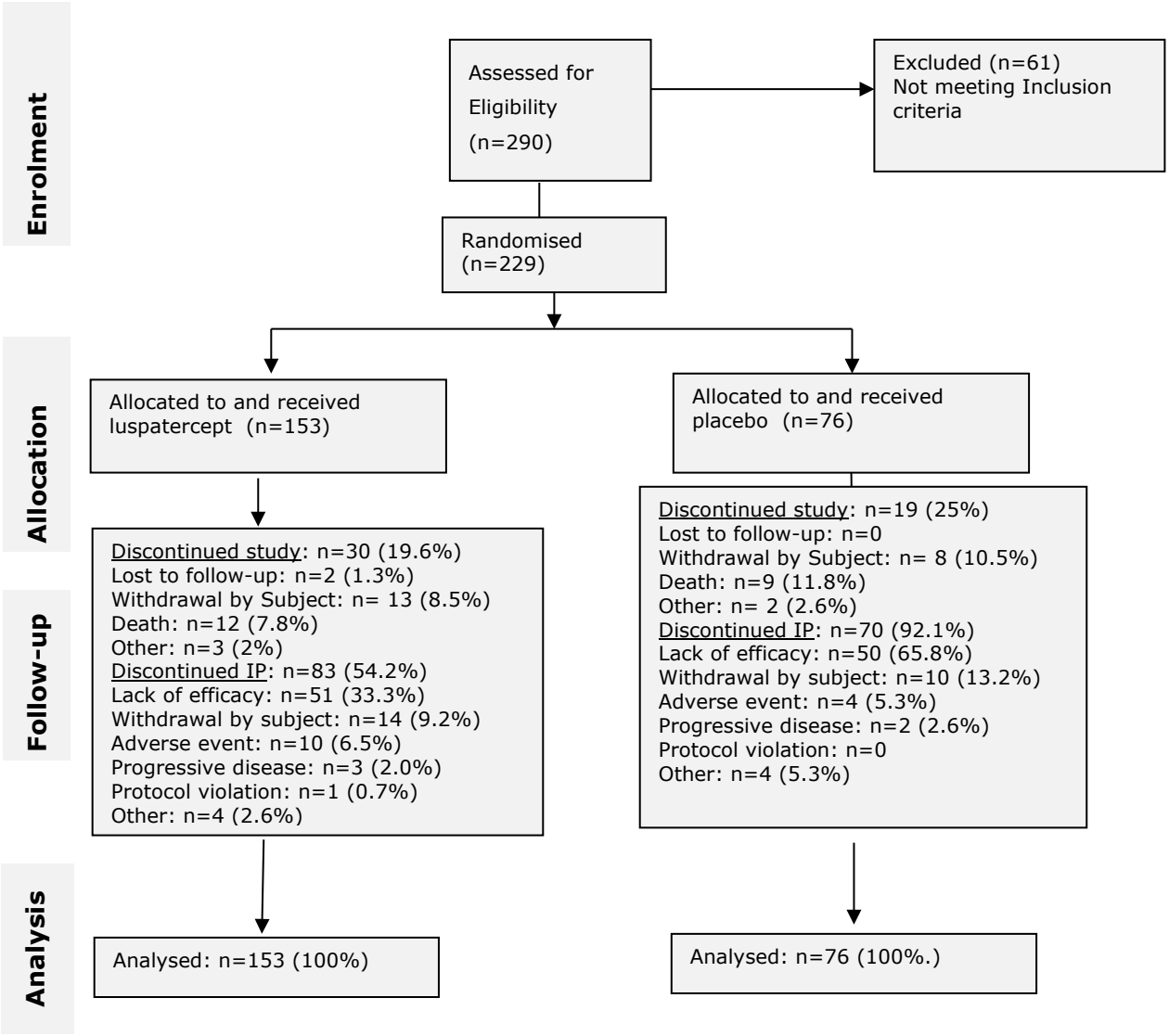


Figure 4

Follow-up period

Post-treatment follow-up of 42 days for all AEs and 3 years from last dose for OS, progression to AML,

other malignancies/pre-malignancies (including progression to higher-risk MDS per IPSS-R criteria

[i.e., high or very high risk]), and subsequent MDS therapies, unless the subject withdrew consent from the study, died, or was lost to follow-up.

Recruitment

The first subject's visit 1 was on 09 February 2016.

Subjects were enrolled at 65 sites in 11 countries. Follow-up was planned to be 3 years. A commitment was provided to extend the follow-up time to 5 years.

Conduct of the study

The original study protocol was finalized on 25th September 2015 and was amended several times (2 global and 3 country-specific amendments):

DE-specific amendment: 31 Mars 2016

- Added clarification related to contraception method, additional requirements related to confirmation of subject's HIV, hepatitis B, hepatitis C status by the investigational site and addition of language explicitly excluding vulnerable subjects (imprisoned, institutionalized persons)

First global amendment: 21 September 2016

Summary of key changes (excerpt):

- Added monitoring of other malignancies/pre-malignancies as "important medical events" due to preclinical findings
- Added testing of other exploratory markers (eg, c-reactive protein)
- Included dose modification and treatment discontinuation criteria regarding leukocyte increase and disease progression as per International Working Group (IWG) criteria (Cheson, 2006)
- Accounted for update of World Health Organization (WHO) classification system (Arber 2016) and included French-American-British (FAB) classification system for baseline MDS diagnosis
- Added site guidance regarding collection of transfusion data as additional assurance that all transfusion data is collected throughout the course of the study (including transfusions that may have occurred in between study visits at local institutions)
- Extended collection of transfusion data after treatment discontinuation to 16 weeks after last dose of IP or End of Treatment visit (whichever is later)
- Included upper pre-transfusion Hgb threshold of 10 g/dL to protocol eligibility criterion related to requirement of transfusions
- Added language to allow for the participation of patients with > 2.0 upper limit of normal (ULN) serum bilirubin if in the presence of diagnosed or known Gilbert syndrome
- Revised eligibility criteria to exclude patients with significant cardiac dysfunction based on known local ECHO/MUGA results (patients with known ejection fraction < 35% be excluded)
- Revised eligibility criteria to allow use of experimental agents prior to randomization
- Revised eligibility criteria to allow enrollment of patients who received a prior subtherapeutic course of hypomethylating agent or lenalidomide

- Decreased the ESA/G-CSF/GM-CSF washout window to 4 weeks from date of randomization to avoid unnecessary screen failures

Protocol Amendment 1.1 FR country-specific; France (21 Sep 2016)

- Additionally Updated inclusion criterion related to ESA to modify serum EPO threshold to > 500 U/L in ESA-naïve subjects

Second global amendment: 09 May 2017 [and Protocol Amendment 2.1 FR country-specific; France (19 Apr 2017)]

Summary of key changes (excerpt)

- Removal of "progression to acute myeloid leukemia (AML) or high/very high risk category MDS per IPSS-R" from the dose modification and treatment discontinuation criteria as per Steering Committee request to avoid redundancy as "progression to AML and high/very high risk category MDS per IPSS-R" is covered by the criteria for disease progression per International Working Group (IWG) (Cheson, 2006)

- Clarification on the anti-drug antibodies (ADA) and pharmacokinetic (PK) sample collection in the Follow-up period to maintain the blinding of the study

- Clarification on the timing and allowed time window for the Week 25 Visit

- Modified protocol criteria related to dose modifications (Dose Delay, Dose Reduction and Discontinuation) measures related to potential cases of leukocytosis

- Extended the Posttreatment Follow-up Period from "at least 2 years" to "at least 3 years" from the date of last dose of investigational product (IP)

Baseline data

Demographic characteristics

Table 8: Demographic characteristics (ITT population)

Demographic Characteristic	Luspatercept (N = 153)	Placebo (N = 76)	Total (N = 229)
Age[a] (years)			
Mean (SD)	70.5 (8.68)	70.7 (10.88)	70.6 (9.44)
Median (Min, Max)	71.0 (40, 95)	72.0 (26, 91)	71.0 (26, 95)
Age Category (Years), n (%)			
≤ 64	29 (19.0)	16 (21.1)	45 (19.7)
65 - 74	72 (47.1)	29 (38.2)	101 (44.1)
≥ 75	52 (34.0)	31 (40.8)	83 (36.2)
Gender, n (%)			
Male	94 (61.4)	50 (65.8)	144 (62.9)
Female	59 (38.6)	26 (34.2)	85 (37.1)
Race, n (%)			
Black or African American	1 (0.7)	0	1 (0.4)
White	107 (69.9)	51 (67.1)	158 (69.0)
Not Collected or Reported	44 (28.8)	24 (31.6)	68 (29.7)
Other	1 (0.7)	1 (1.3)	2 (0.9)
Ethnicity, n (%)			
Hispanic or Latino	3 (2.0)	4 (5.3)	7 (3.1)
Not Hispanic or Latino	115 (75.2)	52 (68.4)	167 (72.9)
Not Reported	35 (22.9)	20 (26.3)	55 (24.0)
Weight (kg)			
Mean (SD)	76.2 (15.07)	77.4 (15.78)	76.6 (15.29)
Median (Min, Max)	76.0 (46, 124)	75.0 (51, 153)	76.0 (46, 153)
Weight Category (kg), n (%)			
< 70	53 (34.6)	24 (31.6)	77 (33.6)
70 to < 85	56 (36.6)	32 (42.1)	88 (38.4)
85 to < 100	32 (20.9)	14 (18.4)	46 (20.1)
≥ 100	12 (7.8)	6 (7.9)	18 (7.9)

Demographic Characteristic	Luspatercept (N = 153)	Placebo (N = 76)	Total (N = 229)
BMI[b] (kg/m^2)			
n	152	75	227
Mean (SD)	26.6 (4.19)	27.0 (4.58)	26.7 (4.32)
Median (Min, Max)	26.2 (17, 40)	27.1 (20, 48)	26.6 (17, 48)

BMI = body mass index; ITT = intent-to-treat; max = maximum; min = minimum; SD = standard deviation.
[a] Age was calculated based on the informed consent signing date.
[b] Body mass index was calculated as weight (kg)/(height [m])^2.

Baseline characteristics

Table 9: Baseline Disease Characteristics (ITT population)

Baseline Characteristic	Luspatercept (N = 153)	Placebo (N = 76)	Total (N = 229)
Time Since Original MDS Diagnosis[a] (Months)			
Mean (SD)	57.8 (56.59)	52.7 (42.29)	56.1 (52.24)
Median (Min, Max)	44.0 (3, 421)	36.1 (4, 193)	41.8 (3, 421)
Time Since Original MDS Diagnosis Categories[a], n (%)			
≤ 2 Years	40 (26.1)	19 (25.0)	59 (25.8)
> 2 to 5 Years	62 (40.5)	34 (44.7)	96 (41.9)
> 5 Years	51 (33.3)	23 (30.3)	74 (32.3)
Ring Sideroblasts, n (%)			
≥ 15%	153 (100.0)	76 (100.0)	229 (100.0)
MDS WHO Classification, n (%)			
MDS RARS	7 (4.6)	2 (2.6)	9 (3.9)
MDS RCMD[b]	145 (94.8)	74 (97.4)	219 (95.6)
Other[c]	1 (0.7)	0	1 (0.4)
IPSS-R Classification Risk Category, n (%)			
Very Low, Low	127 (83.0)	63 (82.9)	190 (83.0)
Very Low	18 (11.8)	6 (7.9)	24 (10.5)
Low	109 (71.2)	57 (75.0)	166 (72.5)
Intermediate	25 (16.3)	13 (17.1)	38 (16.6)
High	1 (0.7)	0	1 (0.4)
Serum EPO[d] (U/L)			
n	152	76	228
Mean (SD)	279.6 (361.33)	284.5 (433.84)	281.2 (386.05)
Median (Min, Max)	156.9 (12, 2454)	130.8 (29, 2760)	153.2 (12, 2760)
Serum EPO (U/L) Categories, n (%)			
< 100	51 (33.3)	31 (40.8)	82 (35.8)
100 to < 200	37 (24.2)	19 (25.0)	56 (24.5)
200 to 500	43 (28.1)	15 (19.7)	58 (25.3)
> 500	21 (13.7)	11 (14.5)	32 (14.0)
Missing	1 (0.7)	0	1 (0.4)
SF3B1, n (%)			
Mutated	141 (92.2)	65 (85.5)	206 (90.0)
Nonmutated	12 (7.8)	10 (13.2)	22 (9.6)
Missing	0	1 (1.3)	1 (0.4)
ECOG Performance Status, n (%)			
0	54 (35.3)	33 (43.4)	87 (38.0)
1	91 (59.5)	32 (42.1)	123 (53.7)
2	8 (5.2)	11 (14.5)	19 (8.3)

ECOG = Eastern Cooperative Oncology Group; EPO = erythropoietin; IPSS-R = International Prognostic Scoring System-Revised; ITT = intent-to-treat; max = maximum; MDS = myelodysplastic syndromes; MDS-RS = myelodysplastic syndromes with ring sideroblasts; min = minimum; RAEB = refractory anemia with excess blasts; RARS = refractory anemia with ring sideroblasts; RBC = red blood cell; RCMD = refractory cytopenia with multilineage dysplasia; SD = standard deviation; SF3B1 = splicing factor 3B subunit 1; WHO = World Health Organization.

[a] Time since original MDS diagnosis was defined as the number of years from the date of original diagnosis to the date of informed consent.

[b] All subjects were classified as RCMD-RS as they were required to have ring sideroblasts per inclusion criteria.

[c] Locally diagnosed MDS-RS and multilineage dysplasia (Listing 16.2.4.9).

[d] Baseline EPO was defined as the highest EPO value within 35 days of the first dose of the investigational product.

Source: Table 14.1.2.1.

Table 10: Baseline transfusion burden (ITT population)

Baseline Characteristic	Luspatercept (N = 153)	Placebo (N = 76)	Total (N = 229)
Baseline Transfusion Burden			
RBC Transfusions/Last 8 Weeks			
Mean (SD)	5.9 (2.97)	6.2 (2.99)	6.0 (2.97)
Median (Min, Max)	6.0 (2, 16)	6.0 (0, 16)	6.0 (0, 16)
RBC Transfusions/Last 8 Weeks Categories, n (%)			
≥ 6 Units	78 (51.0)	46 (60.5)	124 (54.1)
< 6 Units	75 (49.0)	30 (39.5)	105 (45.9)
≥ 4 and < 6 Units	47 (30.7)	19 (25.0)	66 (28.8)
< 4 Units	28 (18.3)	11 (14.5)	39 (17.0)
RBC Transfusions/8 Weeks Over 16 Weeks			
Mean (SD)	5.5 (2.76)	5.8 (2.95)	5.6 (2.82)
Median (Min, Max)	5.0 (1, 15)	5.0 (2, 20)	5.0 (1, 20)
RBC Transfusions/8 Weeks Over 16 Weeks Categories, n (%)			
≥ 6 Units	66 (43.1)	33 (43.4)	99 (43.2)
< 6 Units	87 (56.9)	43 (56.6)	130 (56.8)
≥ 4 and < 6 Units	41 (26.8)	23 (30.3)	64 (27.9)
< 4 Units	46 (30.1)	20 (26.3)	66 (28.8)
Hemoglobin[a] (g/dL)			
N	153	76	229
Mean (SD)	7.7 (0.84)	7.6 (0.77)	7.7 (0.81)
Median (Min, Max)	7.6 (6, 10)	7.6 (5, 9)	7.6 (5, 10)

IP = investigational product; ITT = intent-to-treat; RBC = red blood cell; SD = standard deviation.

[a] Baseline hemoglobin was defined as the last value measured on or before the date and time of the first dose of IP. After applying the 14/3 day rule, the baseline hemoglobin was defined as the lowest hemoglobin value from the central laboratory that was within 35 days on or prior to the first dose date. If a qualified central laboratory value was not available, the lowest hemoglobin value from the local laboratory that was within 35 days on or prior to the first dose of IP was used. If neither central nor local laboratory values were available, the lowest pretransfusion hemoglobin level from the transfusion records within 35 days of the first dose of IP was used.

Source: Table 14.1.2.1.

Table 11: : MDS treatment history (ITT population)

Disease Characteristic	Luspatercept (N = 153)	Placebo (N = 76)	Total (N = 229)
Prior ESA, n (%)			
Yes	148 (96.7)	70 (92.1)	218 (95.2)
No	5 (3.3)	6 (7.9)	11 (4.8)
Reasons for Prior ESA Discontinuation, n (%)[a]			
Refractory[b]	144 (97.3)	69 (98.6)	213 (97.7)
Intolerant[c]	4 (2.7)	1 (1.4)	5 (2.3)
Time From End of Prior ESA to Start of Study[d] (Months)			
n[e]	148	70	218
Mean (SD)	14.79 (28.824)	11.18 (13.553)	13.63 (24.981)
Median (Min, Max)	5.26 (0.9, 257.9)	5.13 (0.2, 64.9)	5.26 (0.2, 257.9)
Time From End of Prior ESA to Start of Study Categories[d], n (%)[a]			
< 6 Months	82 (55.4)	37 (52.9)	119 (54.6)
6 to 12 Months	21 (14.2)	13 (18.6)	34 (15.6)
> 12 to 24 Months	19 (12.8)	7 (10.0)	26 (11.9)
> 24 Months	26 (17.6)	13 (18.6)	39 (17.9)
Longest Duration of Prior ESA Treatment (Months)			
n[e]	148	70	218
Mean (SD)	17.83 (22.415)	19.51 (20.202)	18.37 (21.697)
Median (Min, Max)	10.48 (1.2, 143.2)	13.17 (1.4, 90.9)	11.81 (1.2, 143.2)
Longest Duration of Prior ESA Treatment, n (%)[a]			
< 6 Months	48 (32.4)	20 (28.6)	68 (31.2)
6 to 12 Months	34 (23.0)	9 (12.9)	43 (19.7)
> 12 to 24 Months	35 (23.6)	21 (30.0)	56 (25.7)
> 24 Months	31 (20.9)	20 (28.6)	51 (23.4)
Prior ICT Use, n (%)			
Yes	71 (46.4)	40 (52.6)	111 (48.5)
No	82 (53.6)	36 (47.4)	118 (51.5)
Prior G-CSF/GM-CSF Usage[f]			
Yes	51 (33.3)	22 (28.9)	73 (31.9)
No	102 (66.7)	54 (71.1)	156 (68.1)

ESA = erythropoiesis-stimulating agent; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; ICT = iron chelation therapy; ITT = intent-to-treat; SD = standard deviation.

[a] Percentages calculated relative to the number of subjects with prior ESA use.

[b] Defined as documentation of nonresponse or response that is no longer maintained to prior ESA-containing regimen.

[c] Defined as documentation of discontinuation of prior ESA-containing regimen at any time after introduction due to intolerance or an adverse event.

[d] Time from end of prior ESA to start of study was defined as the number of months from the date of the end of prior ESA to the date of Cycle 1 Day 1. When Cycle 1 Day 1 was missing, the randomization date was used.

[e] Number of subjects with prior ESA use.

[f] Any drugs with Anatomical Therapeutic Chemical code L or L03 for G-CSF/GM-CSF usage.

Table 12: Baseline Laboratory Characteristics (ITT Population)

Disease Characteristic	Luspatercept (N = 153)	Placebo (N = 76)	Total (N = 229)
Serum Ferritin (µg/L)			
Mean (SD)	1348.0 (971.24)	1503.8 (1242.94)	1399.7 (1068.86)
Median (Min, Max)	1089.2 (64, 5968)	1122.1 (165, 5849)	1101.9 (64, 5968)

The use of prior medications was generally well balanced between the treatment groups. All subjects had at least 1 prior medication. The most common prior medications were antianemic preparations eg, vitamin prophylaxis) (96.1%), drugs for (gastrointestinal) acid related disorders (32.3%), immunostimulants (31.9%), and antithrombotic agents (27.5%).

Numbers analysed

Table 13: Analysis populations

Analysis Population	Number of Subjects		
	Luspatercept (N = 153)	Placebo (N = 76)	Total (N = 229)
ITT Population[a]	153	76	229
Safety Population[b]	153	76	229
HRQoL-evaluable Population[c]	149	76	225
PK Population[d]	153	N/A	153

EORTC QLQ-C30 = European Organization for Research and Treatment of Cancer Quality of Life Questionnaire; HRQoL = health-related quality of life; IP = investigational product; ITT = intent-to-treat; N/A = not applicable; PK = pharmacokinetic.

[a] Included all subjects who were randomized, regardless of whether they received IP.

[b] Included all randomized subjects who received at least 1 dose of IP.

[c] Included subjects from the ITT population who completed the EORTC QLQ-C30 assessment at baseline (ie, Cycle 1 Day 1 Visit or Screening Visit if assessment at the Cycle 1 Day 1 Visit was not collected or available) and at least 1 postbaseline assessment visit.

[d] Included all subjects who received at least 1 dose of luspatercept and had measurable luspatercept serum concentrations.

Outcomes and estimation

Table 14: Results of Primary and Key Secondary Endpoint Analyses (ITT Population)

Rank in multiple testing[a]	Endpoint	Luspatercept (N=153)	Placebo (N=76)	p-value[b]	Common Risk Difference on Response Rate (%) (95% CI)	Odds Ratio (95% CI)[b]
Primary efficacy endpoints		Number of responders, response rates and corresponding 95%CI				
1	RBC-TI ≥ 8 weeks from Week 1 through Week 24[c]	n=58 37.91% (30.20, 46.10)	n= 10 13.16% (6.49, 22.87)	< 0.0001	24.56 (14.48, 34.64)	5.065 (2.278, 11.259)
Key secondary efficacy endpoints		Number of responders, response rates and corresponding 95%CI				
2	RBC-TI ≥ 12 weeks from Week 1 through Week 48[d]	n=51 33.33% (25.93, 41.40)	n=9 11.84% (5.56, 21.29)	0.0003	21.37 (11.23, 31.51)	4.045 (1.827, 8.956)
3	RBC-TI ≥ 12 weeks from Week 1 through Week 24[e]	n=43 28.10% (21.14, 35.93)	n=6 7.89% (2.95, 16.40)	0.0002	20.00 (10.92, 29.08)	5.071 (2.002, 12.844)

IPSS-R = International Prognostic Scoring System-Revised; ITT = intent-to-treat; RBC = red blood cell; RBC-TI = red blood cell transfusion independence.

[a] The primary efficacy endpoint was tested first at the 1-sided 0.025 significance level. In order to preserve the overall alpha level at 0.025 across the RBC-TI endpoints, formal statistical inference for the RBC-TI ≥ 12 weeks analysis (first tested for Week 1 to Week 48 and then Week 1 to Week 24) was to be made only if superiority of luspatercept was demonstrated for the primary efficacy endpoint (RBC-TI of ≥ 8 weeks), at the 1-sided 0.025 significance level.

[b] 2-sided p-value from Cochran-Mantel-Haenszel (CMH) test stratified for average baseline RBC transfusion requirement (≥ 6 units versus < 6 units of RBC per 8 weeks), and baseline IPSS-R score (very low or low versus intermediate).

[c] Defined as the absence of any RBC transfusion during any consecutive 56-day (8-week) period during the Primary Phase of the Treatment Period (first 24 weeks of double-blind treatment).

[d] Defined as the absence of any RBC transfusion during any consecutive 84-day (12-week) period during Week 1 to Week 48.

[e] Defined as the absence of any RBC transfusion during any consecutive 84-day (12-week) period during the Primary Phase of the Treatment Period (first 24 weeks of double-blind treatment).

Only the most relevant secondary endpoint results and subgroup analyses are reported here.

Time to Red Blood Cell Transfusion Independence (defined as the time between first dose date and the date of onset of RBC-TI first observed for subjects who achieved RBC-TI of ≥ 8 weeks during the Treatment Period (Week 1 through Week 24)

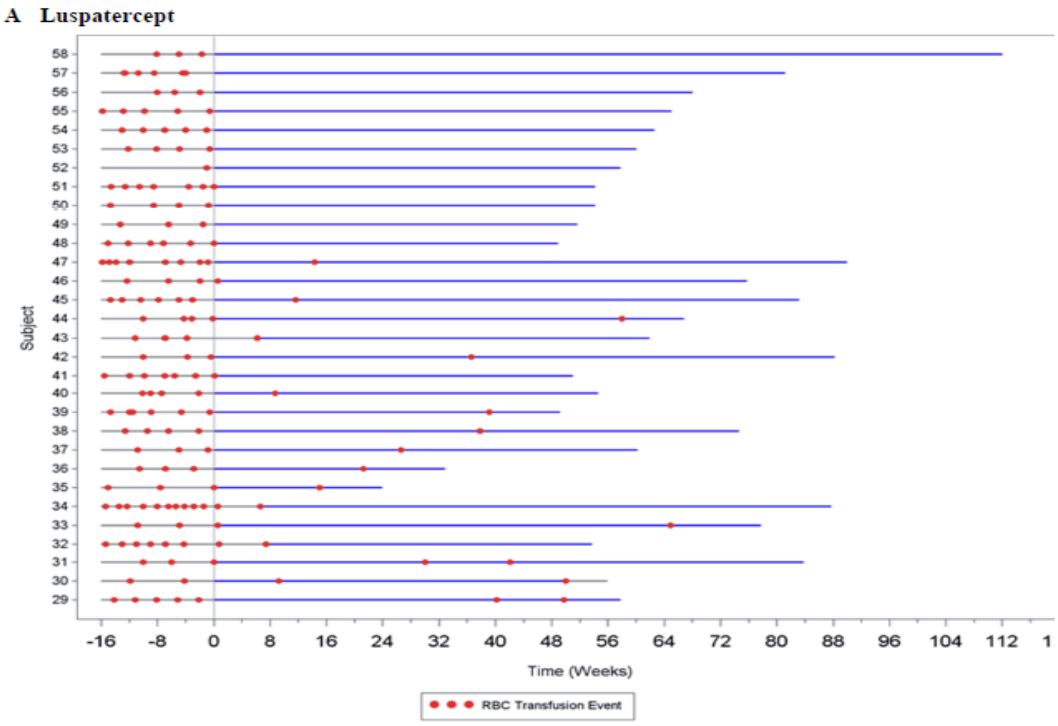
Table 15: Time to RBC-TI of 8 weeks or more for responses achieved during week 1 through week 24 (ITT population)

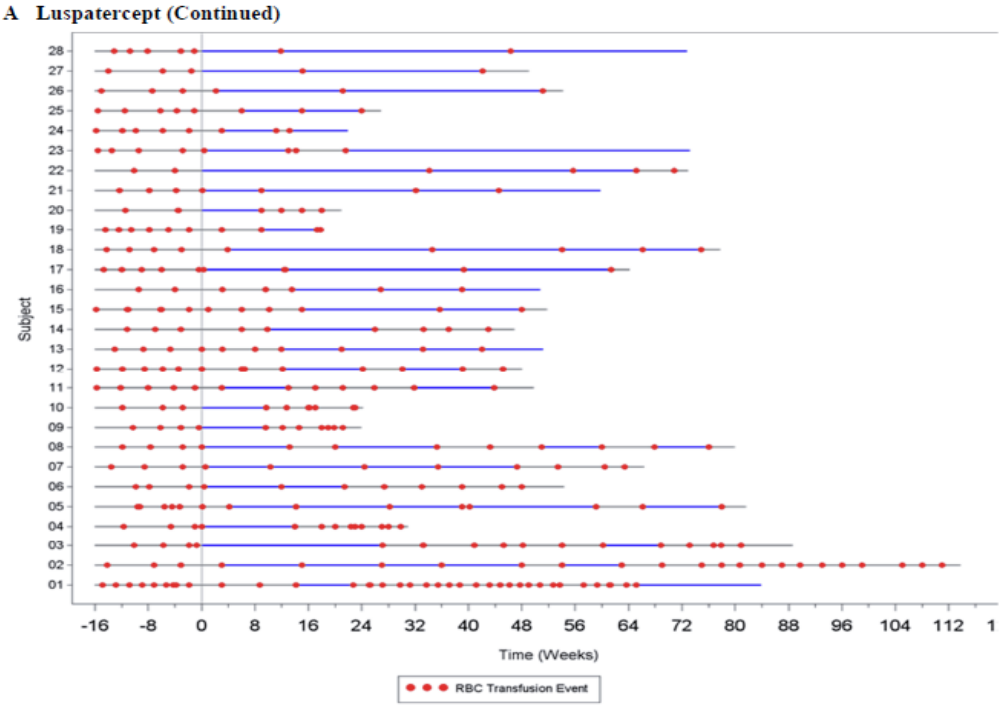
Parameter	Luspatercept (N = 58)	Placebo (N = 10)
Time to RBC-TI ≥ 8 weeks During Weeks 1 through 24 (Days)		
n	58	10
Mean (SD)	17.2 (29.40)	26.0 (31.83)
Median (Min, Max)	1.0 (1.0, 106.0)	17.0 (1.0, 100.0)

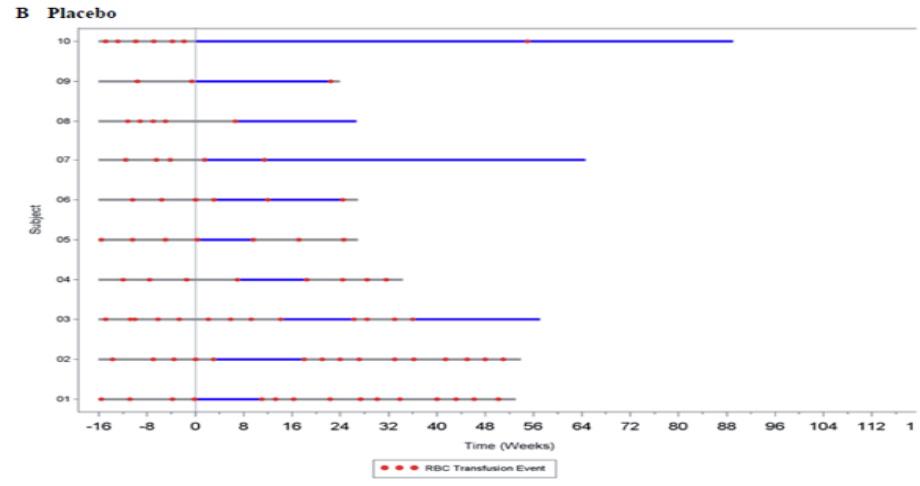
ITT = intent-to-treat; max = maximum; min = minimum; RBC = red blood cell; RBC-TI = red blood cell transfusion independence; SD = standard deviation.
Note: Time to RBC-TI was defined as the time between first dose date and the date of onset of RBC-TI first observed for subjects who achieved RBC-TI of ≥ 8 weeks (ie, Day 1 of 56 days [8 weeks] or more days without any RBC transfusions) during the Primary Treatment Phase (Week 1 through Week 24).

Subjects with more than a single episode of response (Week 1 Through Week 24) (Figure 7)

Figure 5: Swimmer plot of transfusions and RBC-TI of 8 weeks or more responses (week 1 through week 24 during the entire treatment phase (ITT population)







Blue line indicates an RBC-TI ≥ 8-week response.
Note: One luspatercept-treated subject -01 in Figure 4A (Subject ID: 4031002) was inadvertently included in this post hoc analysis, which includes post Week 48 transfusion data. Subsequent to the clinical database lock, additional transfusion data were identified during Weeks 66 to 84 indicating the subject had only one episode of response within Weeks 1 to 24 rather than what is depicted in Figure 4A. As the duration of response was calculated for RBC-TI response occurring within 24 and 48 weeks, the additional transfusions for this subject did not impact the overall duration of response conclusions, nor the primary (RBC-TI ≥ 8 weeks) or key secondary (RBC-TI ≥ 12 weeks) endpoints, as they occurred outside a transfusion-free period and outside of the 24- and 48-week analysis timeframe.

Post hoc analysis of change from baseline in RBC transfusion rates

In Table 18, change in the frequency of postbaseline transfusion events between the 2 treatment groups was analyzed for the overall ITT population regardless of treatment discontinuation by 24-week intervals and overall for Weeks 1 to 48 using a negative binomial regression model for the analysis of count data. Baseline for these analyses is defined as the number of transfusion events during the 24-week interval on or prior to the first dose date. As historical transfusion records in Study ACE-536-MDS-001 were only collected for 16 weeks prior to randomization, the 24-week baseline transfusion frequency was calculated as the event rate in the 16 weeks prior to the first dose multiplied by 1.5. Missing data were imputed using baseline values.

Table 16: Analysis of Postbaseline Transfusion Event Frequency by 24-week Interval and Overall Including All Observed Data regardless of Early Discontinuation with Imputation by Baseline (ITT Population)

Parameter	Luspatercept (N = 153)	Placebo (N = 76)
Transfusion Event Frequency at Baseline Per 24 Weeks[a]		
n	153	76
Mean (SD)	9.00 (4.329)	9.32 (4.720)
Median	7.50	9.00
Min, Max	1.5, 25.5	3.0, 31.5
Transfusion Event Frequency During Week 1-24		
n	153	76
Mean (SD)	6.82 (5.454)	9.66 (4.748)
Median	7.00	10.00
Min, Max	0.0, 25.0	0.0, 24.0
Interval Transfusion Rate (95% CI)[b]	6.26 (5.56, 7.05)	9.20 (7.98, 10.60)
Relative Risk Versus Placebo (95% CI)[b]	0.68 (0.58, 0.80)	
p-value[b]	< 0.0001	
Transfusion Event Frequency During Week 25-48		
n	153	76
Mean (SD)	6.85 (5.736)	9.37 (5.423)
Median	7.00	8.50
Min, Max	0.0, 30.0	0.0, 32.0
Interval Transfusion Rate (95% CI)[b]	6.27 (5.47, 7.19)	8.72 (7.40, 10.28)
Relative Risk Versus Placebo (95% CI)[b]	0.72 (0.60, 0.86)	
p-value[b]	0.0004	

Parameter	Luspatercept (N = 153)	Placebo (N = 76)
Transfusion Event Frequency During Week 1-48		
n	153	76
Mean (SD)	13.67 (10.709)	19.01 (9.702)
Median	14.00	19.00
Min, Max	0.0, 50.0	0.0, 56.0
Interval Transfusion Rate (95% CI)[b]	12.40 (10.86, 14.14)	17.99 (15.30, 21.16)
Relative Risk Versus Placebo (95% CI)[b]	0.69 (0.58, 0.82)	
p-value[b]	< 0.0001	

CI = confidence interval; IPSS-R = International Prognostic Scoring System – Revised; ITT = intent to treat; Max = maximum; Min = minimum; RBC = red blood cell; SD = standard deviation.

[a] Defined as the number of transfusion events during the 24-week interval on or prior to first dose for comparability to the 24-week on-treatment intervals. As transfusion data were collected for 16 weeks prior to randomization, the baseline transfusion frequency was calculated as the event rate in the 16 weeks prior to the first dose * 1.5.
[b] Nominal p-value and 95% CI are from negative binomial regression model with post-baseline transfusion frequency as dependent variable, and with treatment arm, baseline RBC transfusion burden (≥ 6 units versus < 6 RBC units/8 weeks), and baseline IPSS-R (very low/low versus intermediate), and baseline RBC transfusion event frequency per 24 weeks as independent variables.

All observed data from all subjects were included through Day 336. This includes data collected after treatment discontinuation for subjects who discontinued treatment before Day 336. If a subject's transfusion records ended prior to Day 336, then the number of transfusion events is imputed from the last visit date where transfusion (yes/no) was recorded up to Day 336. Imputation is based on the average number of RBC transfusion events in the 24 weeks prior to first dose (calculated on a per day basis).

Post Hoc Analysis of RBC Transfusion Units

The mean difference in RBC transfusion units between treatment groups in the intervals from Week 1 to Week 24 and Week 25 to Week 48 was determined using ANCOVA to compare the treatment difference between groups. This analysis included the parameter of interest in the time interval (ie, number of RBC units transfused) as the dependent variable, treatment group (2 levels) as a factor, and baseline value (ie, transfusion units during the 24-week baseline period) as a covariate. The analysis was stratified by average baseline RBC transfusion requirement (≥ 6 RBC units versus < 6 RBC units/8 weeks) and baseline IPSS-R (very low or low versus intermediate). If a subject's record was curtailed prior to Day 336, then the data were imputed from baseline as follows:

The number of transfusion units was imputed from the last visit date where transfusion (yes/no) was recorded up to Day 336. Imputation was based on the average number of RBC units transfused in the 24 weeks prior to first dose (calculated on a units per day basis).

Table 17: Analysis of RBC Transfusion Units Including All Observed Data Regardless of Early Discontinuation with Imputation by Baseline (ITT Population)

Parameter	Luspatercept (N = 153)	Placebo (N = 76)
RBC Transfusion Units Week 1-24[a]		
n	153	76
Mean (SD)	12.03 (9.896)	17.97 (9.110)
Median	11.00	18.00
Min, Max	0.0, 46.0	0.0, 49.0
LS Mean (SE)	12.6 (0.65)	17.9 (0.84)
95% CI for LS Mean	11.3, 13.9	16.3, 19.6
Treatment Comparison (Luspatercept vs Placebo)[b]		
LS Mean Difference (SE)	-5.3 (0.91)	
95% CI for LS Mean Difference	-7.1, -3.6	
p-value	< 0.0001	
RBC Transfusion Units Week 25-48[a]		
n	153	76

Mean (SD)	12.47 (10.675)	17.05 (10.176)
Median	12.00	16.00
Min, Max	0.0, 49.0	0.0, 60.0
LS Mean (SE)	12.6 (0.74)	16.6 (0.95)
95% CI for LS Mean	11.2, 14.1	14.7, 18.4
Treatment Comparison (Luspatercept vs Placebo)[b]		
LS Mean Difference (SE)	-3.9 (1.02)	
95% CI for LS Mean Difference	-5.9, -1.9	
p-value	0.0002	

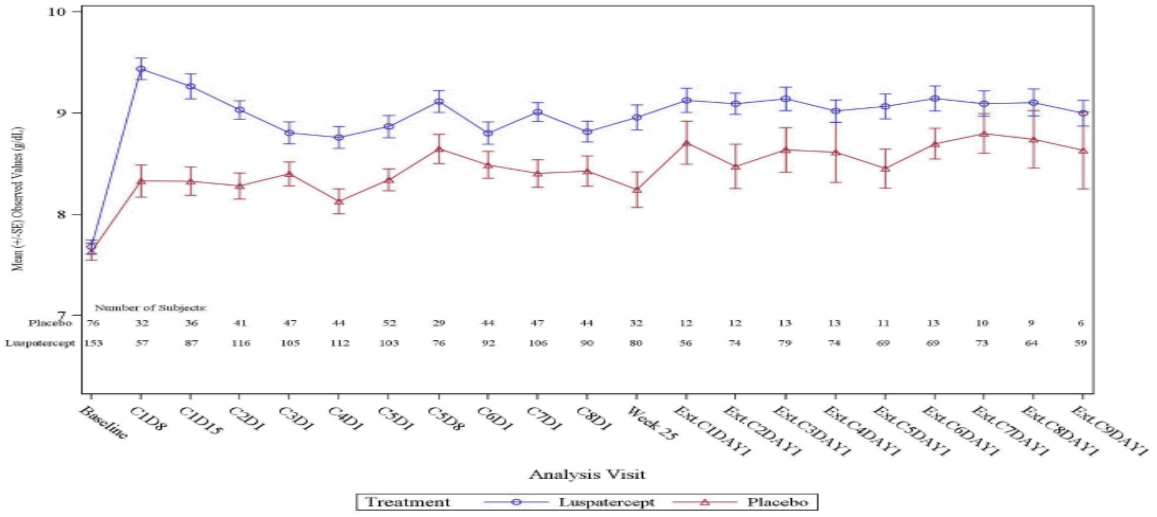
CI = confidence interval; IPSS-R = International Prognostic Scoring System – Revised; ITT = intent to treat; LS = least squares; Max = maximum; Min = minimum; RBC = red blood cell; SD = standard deviation; SE = standard error; vs = versus.

[a] If a subject's record of transfusion was curtailed prior to Day 336 (ie, Week 48), then the number of transfusion units is imputed from the last visit date where transfusion (yes/no) was recorded up to Day 336. Imputation is based on the average number of RBC units transfused in the 24 weeks prior to first dose (calculated on a units per day basis).

[b] Analysis of covariance was used to compare the treatment difference between groups (including nominal p-value), with the number of RBC units transfused in the time interval as the dependent variable, treatment group (2 levels) as a factor, and transfusion units during the 24-week baseline period as a covariate, stratifying by average baseline RBC transfusion requirement (≥ 6 RBC units versus < 6 RBC units/8 weeks), and baseline IPSS-R (very low or low versus intermediate).

Haemoglobin Values over Time

Figure 6: Mean (+/- SE) observed values in haemoglobin by time point from baseline through week 48 (ITT population)



C = cycle; D = day; Ext = extension; IP = investigational product; ITT = intent-to-treat; SE = standard error of the mean.
Note: Baseline is defined (after applying the 14/3-day rule) as the lowest of the pretreatment and/or baseline values from the central, local laboratory, or pre-transfusion hemoglobin from transfusion records that are within 35 days on or prior to the first dose of IP. Hemoglobin values obtained within 14 days after an RBC transfusion unless within 3 days prior to another RBC transfusion are censored from the analysis.
Note: As per protocol, entry into the Extension Phase was restricted to subjects who did not progress and demonstrated evidence of clinical benefit at Week 25; thus, the few placebo-treated subjects who entered the Extension Phase (ie, beyond Week 25) represented a well-performing subset of subjects.

Post hoc analyses on Hb change over time

The mean difference between treatment groups in hemoglobin at Week 24 and Week 48 was determined using analysis of covariance (ANCOVA) to compare the treatment difference between groups. This analysis included hemoglobin value as the dependent variable, treatment group (2 levels) as a factor, and baseline hemoglobin value as a covariate, and was stratified by average baseline RBC transfusion requirement (≥ 6 RBC units versus < 6 RBC units/8 weeks), and baseline IPSS-R (very low or low versus intermediate).

Table 18: Analysis of Haemoglobin Levels (g/dL) Including All Observed Data after Application of 14/3-day Rule regardless of Early Discontinuation with Imputation by Baseline (ITT Population)

Parameter	Luspatercept (N = 153)	Placebo (N = 76)
Hemoglobin (g/dL) at Week 24[a]		
n	153	76
Mean (SD)	9.0 (1.19)	8.6 (1.08)
Median	9.1	8.6
Min, Max	6.3, 12.0	5.9, 11.1
LS Mean (SE)	9.0 (0.11)	8.6 (0.14)
95% CI for LS Mean	8.8, 9.2	8.3, 8.9
Treatment Comparison (Luspatercept vs Placebo)[b]		
LS Mean Difference (SE)	0.4 (0.15)	
95% CI for LS Mean Difference	0.1, 0.7	
p-value	0.0034	
Parameter	**Luspatercept (N = 153)**	**Placebo (N = 76)**
Hemoglobin (g/dL) at Week 48[a]		
n	153	76
Mean (SD)	8.5 (1.28)	7.7 (0.90)
Median	8.4	7.7
Min, Max	5.9, 12.6	4.9, 9.8
LS Mean (SE)	8.5 (0.10)	7.7 (0.12)
95% CI for LS Mean	8.3, 8.7	7.5, 8.0
Treatment Comparison (Luspatercept vs Placebo)[b]		
LS Mean Difference (SE)	0.7 (0.13)	
95% CI for LS Mean Difference	0.5, 1.0	
p-value	< 0.0001	

CI = confidence interval; IPSS-R = International Prognostic Scoring System – Revised; ITT = intent to treat; LS = least squares; Max = maximum; Min = minimum; RBC = red blood cell; SAP = statistical analysis plan; SD = standard deviation; SE = standard error; vs = versus.

[a] Visit weeks for analysis were identified using the Week 25 visit label for the analysis at Week 24 and the Cycle 9

Day 1 visit label for analysis at Week 48. If missing, the data were searched for an event date within the first dose date + 168 (± 14 days) for Week 24, or within the first dose date + 336 (± 14 days) for Week 48. If no hemoglobin values were found in the designated intervals, the hemoglobin value was imputed using the baseline hemoglobin value (as defined in Section 5.1 of the ACE-536-MDS-001 SAP).

[b] Analysis of covariance was used to compare the treatment difference between groups (including nominal p-value), with the hemoglobin value as the dependent variable, treatment group (2 levels) as a factor, and baseline hemoglobin value as a covariate, stratifying by average baseline RBC transfusion requirement (≥ 6 RBC units versus < 6 RBC units/8 weeks), and baseline IPSS-R (very low or low versus intermediate).

All central laboratory hemoglobin values were included in the analysis after applying the 14/3 rule to take into account RBC transfusions as intercurrent events with a potential impact on hemoglobin levels.

Modified Hematologic improvement – Erythroid (mHI-E) (Table 21; excerpt of the originally provided)

For subjects with baseline RBC transfusion burden of ≥ 4 units/8 weeks (HTB), mHI-E was defined as a reduction in RBC transfusion of at least 4 units/8 weeks.

For subjects with baseline RBC transfusion burden of < 4 units/8 weeks (LTB), mHI-E was defined as a mean increase in haemoglobin of ≥ 1.5 g/dL for 8 weeks in the absence of RBC transfusions.

Table 19: Analysis of subjects who achieved mHI-E (ITT population)

Parameter	Luspatercept (N = 153)	Placebo (N = 76)
Week 1 Through Week 24		
mHI-E, n (%)[a] (95% CI)	81 (52.9) (44.72, 61.05)	9 (11.8) (5.56, 21.29)
p-value[b]	< 0.0001	
RBC Transfusion Reduction of 4 Units/8 Weeks, n (%)[c]	52/107 (48.6)	8/56 (14.3)
Mean Hemoglobin Increase of ≥ 1.5 g/dL for 8 Weeks, n (%)[d]	29/46 (63.0)	1/20 (5.0)

CI = confidence interval; ITT = intent-to-treat; IWG = International Working Group; mHI-E = modified hematologic improvement – erythroid.

[a] Defined as the proportion of subjects who met mHI-E criteria per the IWG (Cheson, 2006) sustained over any consecutive 56-day (8-week) period during the Treatment Period: for subjects with baseline RBC transfusion burden of ≥ 4units/8 weeks, a reduction of at least 4 units RBC transfusion/8 weeks; for subjects with baseline RBC transfusion burden of < 4units/8 weeks, mean increase of hemoglobin of at least 1.5 g/dL in the absence of transfusions for at least 8 weeks.

[b] P-value from Cochran-Mantel-Haenszel test to compare luspatercept treatment group to placebo group.

[c] Percentage based on number of subjects with baseline RBC transfusion burden of ≥ 4 units/8 weeks.

[d] Percentage based on number of subjects with baseline RBC transfusion burden of < 4 units/8 weeks.

Of note, no conclusions can be drawn on the haematologic improvement in terms of neutrophils and platelets (as defined by the IWG MDS heaemtologic response criteria), since an insufficient number of patients had low baseline levels.

Change in Mean Daily Dose of Iron Chelation Therapy (Post hoc analyses using imputation by baseline)

The mean difference between treatment groups in serum ferritin, and mean daily dose of ICT in the intervals from Week 1 to Week 24 and Week 25 to Week 48 was determined using ANCOVA to compare the treatment difference between groups. This analysis included the parameter of interest in the time interval (ie, serum ferritin value, or change in value of ICT) as the dependent variable, treatment group (2 levels) as a factor, and baseline value (ie, baseline serum ferritin, or baseline mean daily dose of ICT) as a covariate. The analysis was stratified by average baseline RBC transfusion

requirement (≥ 6 RBC units versus < 6 RBC units/8 weeks) and baseline IPSS-R (very low or low versus intermediate). If a subject's record was curtailed prior to Day 336, then the data were imputed from baseline as follows:

For serum ferritin, missing values at protocol-defined scheduled visits (ie, no values within the analysis interval) were imputed using the baseline value.

For ICT, the mean daily dose of ICT from the stop date of the last record up to Day 336 was imputed from the baseline mean daily dose.

Table 20: Mean Change from Baseline in Mean Serum Ferritin with Imputation by Baseline (ITT Population)

Parameter	Luspatercept (N = 153)	Placebo (N = 76)
Mean Change From Baseline in Mean Serum Ferritin Averaged Over Weeks 9 Through 24 (µg/L)[a]		
n	153	76
LS Mean (SE)	9.9 (47.09)	190.0 (60.30)
95% CI for LS Mean	-82.9, 102.7	71.2, 308.8
Treatment Comparison (Luspatercept vs Placebo)[b]		
LS Mean Difference (SE)	-180.1 (65.81)	
95% CI for LS Mean Difference	-309.8, -50.4	
p-value	0.0067	
Mean Change From Baseline in Mean Serum Ferritin Averaged Over Weeks 33 Through 48 (µg/L)[a]		
n	153	76
LS Mean (SE)	0.2 (18.57)	46.2 (23.78)
95% CI for LS Mean	-36.4, 36.8	-0.7, 93.0
Treatment Comparison (Luspatercept vs Placebo)[b]		
LS Mean Difference (SE)	-46.0 (25.95)	
95% CI for LS Mean Difference	-97.2, 5.1	
p-value	0.0775	

CI = confidence interval; IPSS-R = International Prognostic Scoring System – Revised; ITT = intent-to-treat; LS = least squares; RBC = red blood cell; SE = standard error; vs = versus.

[a] If a subject did not have a serum ferritin value within the designated postbaseline interval, the serum ferritin is imputed from the baseline value.

[b] Analysis of covariance was used to compare the treatment difference between groups (including nominal p-value), with the change in serum ferritin as the dependent variable, treatment group (2 levels) as a factor, and baseline serum ferritin value as covariates, stratified by average baseline RBC transfusion requirement (≥ 6 units versus < 6 units of RBC per 8 weeks), and baseline IPSS-R (very low or low versus intermediate).

Table 21: Change in Mean Daily Dose (mg) of Iron Chelation Therapy with Imputation by Baseline (ITT Population)

Parameter	Luspatercept (N = 153)	Placebo (N = 76)
Mean Change From Baseline for Mean Daily Dose (mg/day) of ICT Averaged Over Weeks 9 Through 24[a]		
n	153	76
LS Mean (SE)	47.9 (21.32)	70.9 (27.26)
95% CI for LS Mean	5.9, 89.9	17.2, 124.6
Treatment Comparison (Luspatercept vs Placebo)[b]		
LS Mean Difference (SE)	-23.0 (29.73)	
95% CI for LS Mean Difference	-81.6, 35.6	
p-value	0.4396	
Mean Change From Baseline for Mean Daily Dose (mg/day) of ICT Averaged Over Weeks 33 Through 48[a]		
n	153	76
LS Mean (SE)	53.1 (29.61)	93.4 (37.85)
95% CI for LS Mean	-5.2, 111.5	18.8, 168.0
Treatment Comparison (Luspatercept vs Placebo)[b]		
LS Mean Difference (SE)	-40.3 (41.29)	
95% CI for LS Mean Difference	-121.7, 41.1	
p-value	0.3303	

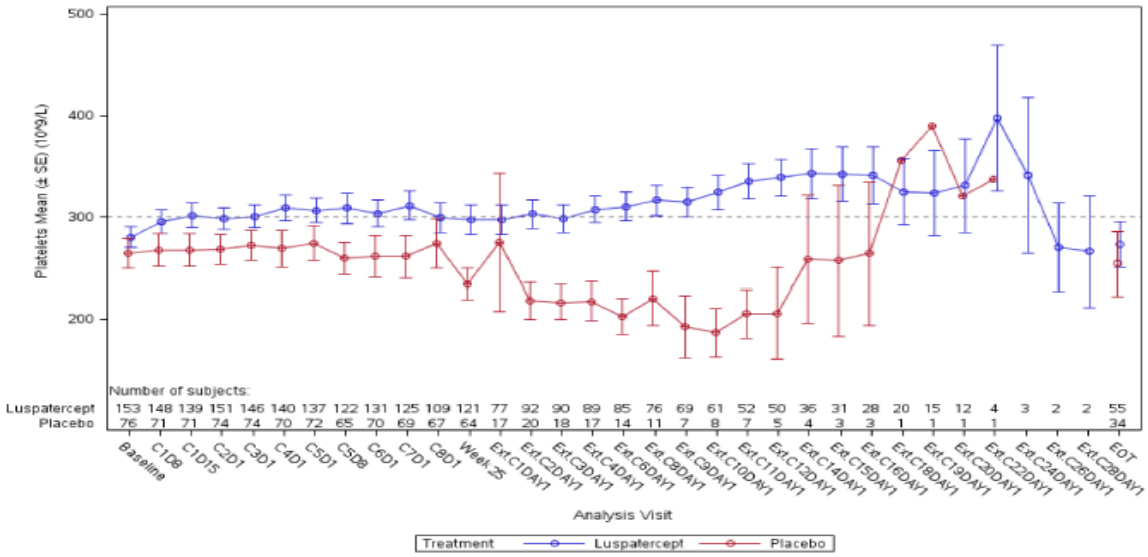
CI = confidence interval; ICT = iron chelation therapy; IPSS-R = International Prognostic Scoring System – Revised; ITT = intent-to-treat; LS = least squares; RBC = red blood cell; SE = standard error; vs = versus.

[a] If a subject's record of ICT was curtailed prior to Day 336 (ie, Week 48), then the mean daily dose of ICT from the stop date of the last record up to Day 336 is imputed from baseline mean daily dose.

[b] Analysis of covariance was used to compare the treatment difference between groups (including nominal p-value), with the change in daily dose as the dependent variable, treatment group (2 levels) as a factor, and baseline ICT value as covariates, stratified by average baseline RBC transfusion requirement (≥ 6 units versus < 6 units of RBC per 8 weeks), and baseline IPSS-R (very low or low versus intermediate).

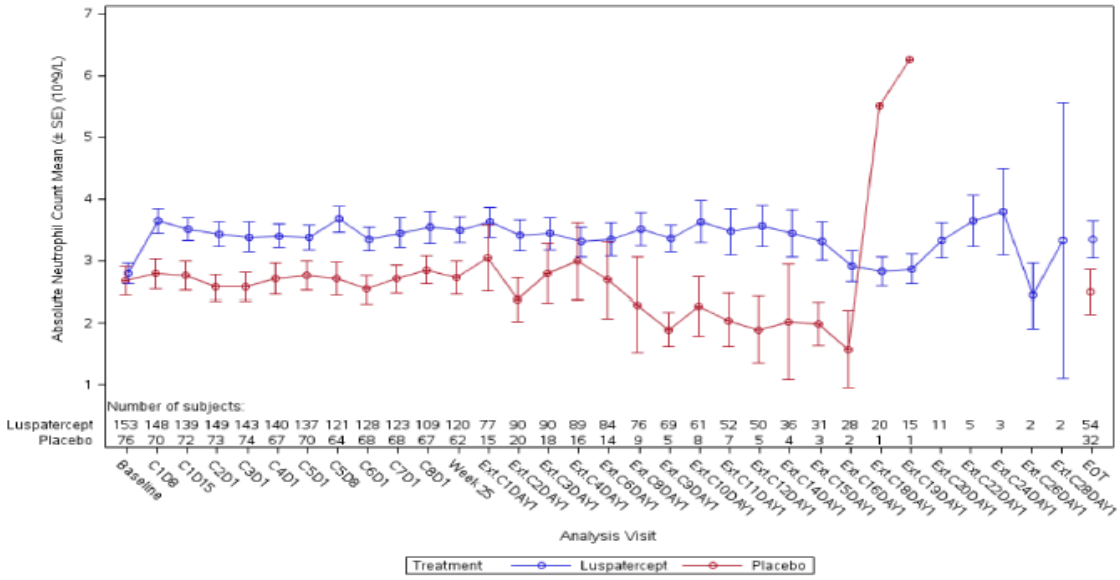
Platelet counts and ANC over time

Figure 7: Platelet Count Over Time in Study ACE-536-MDS-001 (ITT Population)



C = cycle; D = day; EOT = end of treatment; Ext = extension; ITT = intent-to-treat; SE = standard error of the mean.

Figure 8: Absolute Neutrophil Count Over Time in Study ACE-536-MDS-001 (ITT Population)



C = cycle; D = day; EOT = end of treatment; Ext = extension; ITT = intent-to-treat; SE = standard error of the mean.

Survival Analyses

Table 22: Overall Survival (ITT population)

Parameter	Luspatercept (N = 153)	Placebo (N = 76)
Subjects Alive (Censored), n (%)	141 (92.2)	67 (88.2)
Subjects Died, n (%)	12 (7.8)	9 (11.8)
Kaplan-Meier Estimates		
Median (Months) (95% CI)[a]	NE	NE
p-value[b]	0.5427	
Hazard Ratio (95% CI)[c]	0.763 (0.318, 1.829)	
Summary of Follow-up Time (Months)		
n	153	76
Mean (SD)	14.1 (4.62)	14.1 (4.28)
Median (Min, Max)	13.9 (2.8, 26.2)	14.3 (1.7, 21.8)

CI = confidence interval; IPSS-R = International Prognostic Scoring System-Revised; ITT = intent-to-treat; max = maximum; min = minimum; NE = not evaluable; RBC = red blood cell; SD = standard deviation.

[a] Median was from the Kaplan-Meier method stratified by average baseline RBC transfusion requirement (≥ 6 units versus < 6 units of RBC per 8 weeks) and baseline IPSS-R score (very low or low versus intermediate).

[b] P-value from log-rank test to compare luspatercept and placebo.

[c] The hazard ratio was from the Cox proportional hazards model with RBC transfusion requirement (≥ 6 units versus < 6 units of RBC per 8 weeks) and baseline IPSS-R score (very low or low versus intermediate) as covariates.

Note: Overall survival was calculated as the time from randomization date to death of any cause. Overall survival was censored at the last date that the subject was known to be alive for subjects who were alive at the time of analysis and for subjects who discontinued from the study or were lost to follow-up.

Source: Table 14.2.12.1.

Progression to AML and summary of follow-up time

Table 23: Summary of time to AML progression ITT population

Parameter	Luspatercept (N=153) n (%)		Placebo (N=76) n (%)
Subjects without AML Progression (censored) (n %)	150 (98.0)		75 (98.7)
Subjects Progressed to AML (n %)	3 (2.0)		1 (1.3)
Kaplan-Meier Estimates			
Median Time to AML Progression (months) (95% CI) [a]	NA (NA, NA)		NA (NA, NA)
P-value [b]		0.6927	
Hazard Ratio (95% CI) [c]		1.573 (0.163, 15.192)	
Summary of Follow-up Time (months)			
n	153		76
Mean	14.0		13.9
SD	4.73		4.39
Median	13.9		14.3
Q1, Q3	11.6 , 17.5		11.8 , 17.0
Min, Max	1.6 , 26.2		1.7 , 21.8

AML = Acute Myelogenous Leukemia (as per World Health Organization (WHO) classification of >= 20% blasts in peripheral blood or bone marrow). Time to AML progression is defined as the time between randomization date and first diagnosis of AML.
[a] Median is from the Kaplan Meier Method stratified by average baseline Red Blood Cell(RBC) transfusion requirement(>= 6 units vs. < 6 units of RBC per 8 weeks), and baseline IPSS-R score (Very low or low, vs. Intermediate).
[b] P-value from log-rank test to compare Luspatercpet and placebo.
[c] The hazard ratio is from the Cox proportional hazards model with RBC transfusion requirement (>= 6 units vs. < 6 units of RBC per 8 weeks) and baseline IPSS-R (Very low or low, vs. Intermediate) as covariates.
[d] Defined as the time between original diagnosis of MDS and first diagnosis of AML.

Health-related Quality of Life and Healthcare Resource Utilization Endpoints

The main HRQoL questionnaire used was the EORTC QLQ-C30.

The HRQoL-evaluable population consisted of 225 subjects, 149 in the luspatercept group and 76 in the placebo group (only 4 subjects from the luspatercept treatment group were excluded).

Mean scores were comparable at baseline between treatment groups across all domains of the EORTC QLQ-C30. No clinically meaningful changes from baseline were observed for both treatment groups across all the primary domains of interest over the 24-week treatment phase. The distributions of observed change scores across the 5 primary domains of interest (fatigue, dyspnea, global health status, physical functioning, emotional functioning) and the scheduled visits within the 24-week treatment phase showed that HRQoL was maintained over time and comparable between treatment groups (ie, no clinically meaningful changes).

According to the applicant, maintenance of HRQoL is a clinically relevant outcome in this population since HRQoL is expected to worsen over time in MDS patients due to chronic transfusions (Oliva, 2012).

Ancillary analyses

Subgroup analyses

Forest plots were provided for the primary endpoint (week 1-24), key secondary endpoint (week 1-24) and mHI-E (week 1-24) for the following subgroups: age (≤ 64 years, 65 to 74 years, and ≥ 75 years), gender, time since initial diagnosis, ECOG performance status, average baseline transfusion burden (< 4, ≥ 4 and < 6, ≥ 6 units), IPSS-R, baseline serum EPO (< 100, 100 to < 200, 200 to 500, and > 500 U/L), renal (creatinine clearance > 40 to 60 versus ≥ 60 mL/min) (and several more, please see clinical assessment report).

Figure 9: Forest plot of subgroup analysis for RBC-TI of 8 weeks or more from week 1 through week 24 (ITT population)

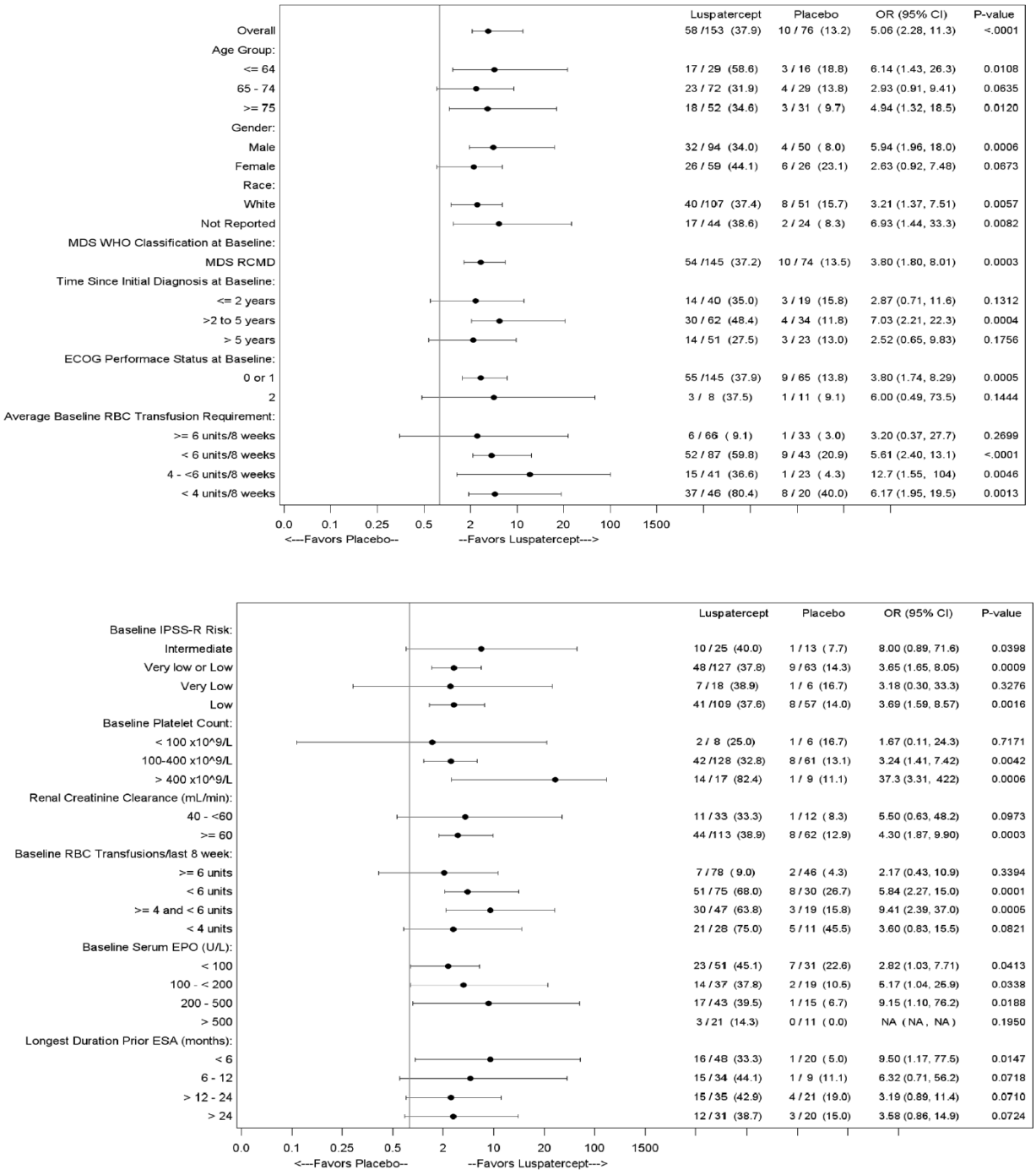
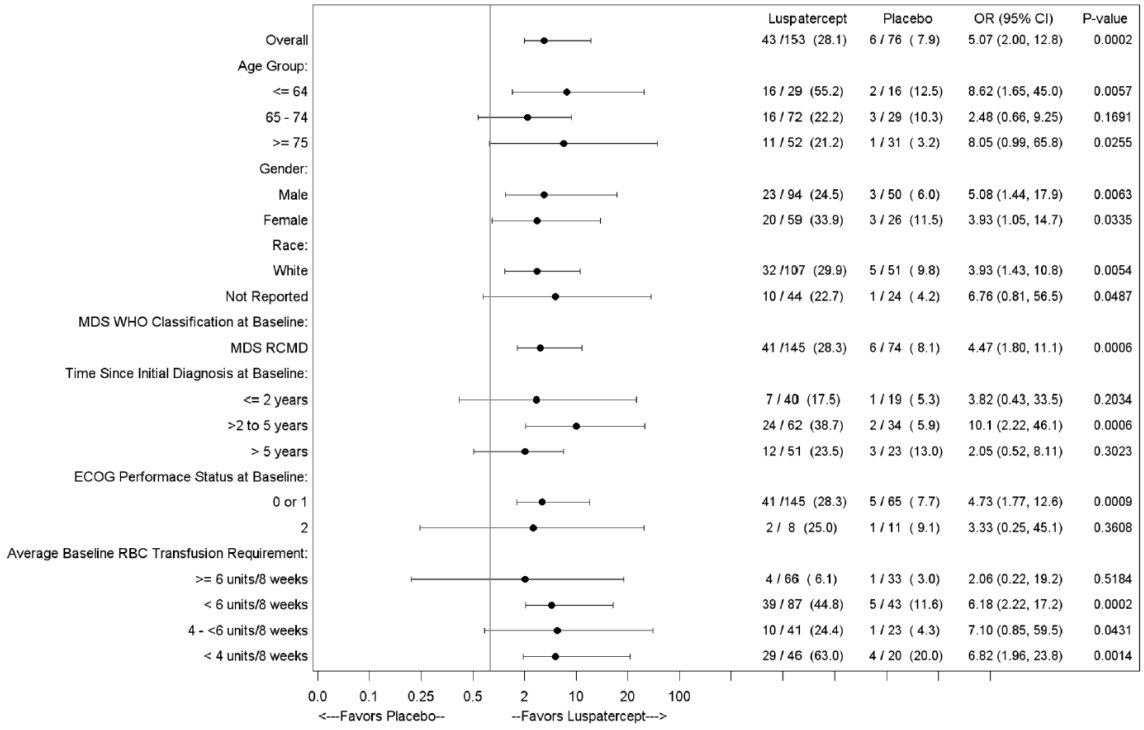

Luspatercept
Placebo
OR (95% CI)
P-value
Overall 58 /153 (37.9) 10 / 76 (13.2) 5.06 (2.28, 11.3) <.0001
Age Group:
<= 64 17 / 29 (58.6) 3 / 16 (18.8) 6.14 (1.43, 26.3) 0.0108
65 - 74 23 / 72 (31.9) 4 / 29 (13.8) 2.93 (0.91, 9.41) 0.0635
>= 75 18 / 52 (34.6) 3 / 31 (9.7) 4.94 (1.32, 18.5) 0.0120
Gender:
Male 32 / 94 (34.0) 4 / 50 (8.0) 5.94 (1.96, 18.0) 0.0006
Female 26 / 59 (44.1) 6 / 26 (23.1) 2.63 (0.92, 7.48) 0.0673
Race:
White 40 /107 (37.4) 8 / 51 (15.7) 3.21 (1.37, 7.51) 0.0057
Not Reported 17 / 44 (38.6) 2 / 24 (8.3) 6.93 (1.44, 33.3) 0.0082
MDS WHO Classification at Baseline:
MDS RCMD 54 /145 (37.2) 10 / 74 (13.5) 3.80 (1.80, 8.01) 0.0003
Time Since Initial Diagnosis at Baseline:
<= 2 years 14 / 40 (35.0) 3 / 19 (15.8) 2.87 (0.71, 11.6) 0.1312
>2 to 5 years 30 / 62 (48.4) 4 / 34 (11.8) 7.03 (2.21, 22.3) 0.0004
> 5 years 14 / 51 (27.5) 3 / 23 (13.0) 2.52 (0.65, 9.83) 0.1756
ECOG Performace Status at Baseline:
0 or 1 55 /145 (37.9) 9 / 65 (13.8) 3.80 (1.74, 8.29) 0.0005
2 3 / 8 (37.5) 1 / 11 (9.1) 6.00 (0.49, 73.5) 0.1444
Average Baseline RBC Transfusion Requirement:
>= 6 units/8 weeks 6 / 66 (9.1) 1 / 33 (3.0) 3.20 (0.37, 27.7) 0.2699
< 6 units/8 weeks 52 / 87 (59.8) 9 / 43 (20.9) 5.61 (2.40, 13.1) <.0001
4 - <6 units/8 weeks 15 / 41 (36.6) 1 / 23 (4.3) 12.7 (1.55, 104) 0.0046
< 4 units/8 weeks 37 / 46 (80.4) 8 / 20 (40.0) 6.17 (1.95, 19.5) 0.0013
0.0 0.1 0.25 0.5 2 10 20 100 1500
<---Favors Placebo-- --Favors Luspatercept--->
Luspatercept
Placebo
OR (95% CI)
P-value
Baseline IPSS-R Risk:
Intermediate 10 / 25 (40.0) 1 / 13 (7.7) 8.00 (0.89, 71.6) 0.0398
Very low or Low 48 /127 (37.8) 9 / 63 (14.3) 3.65 (1.65, 8.05) 0.0009
Very Low 7 / 18 (38.9) 1 / 6 (16.7) 3.18 (0.30, 33.3) 0.3276
Low 41 /109 (37.6) 8 / 57 (14.0) 3.69 (1.59, 8.57) 0.0016
Baseline Platelet Count:
< 100 x10^9/L 2 / 8 (25.0) 1 / 6 (16.7) 1.67 (0.11, 24.3) 0.7171
100-400 x10^9/L 42 /128 (32.8) 8 / 61 (13.1) 3.24 (1.41, 7.42) 0.0042
> 400 x10^9/L 14 / 17 (82.4) 1 / 9 (11.1) 37.3 (3.31, 422) 0.0006
Renal Creatinine Clearance (mL/min):
40 - <60 11 / 33 (33.3) 1 / 12 (8.3) 5.50 (0.63, 48.2) 0.0973
>= 60 44 /113 (38.9) 8 / 62 (12.9) 4.30 (1.87, 9.90) 0.0003
Baseline RBC Transfusions/last 8 week:
>= 6 units 7 / 78 (9.0) 2 / 46 (4.3) 2.17 (0.43, 10.9) 0.3394
< 6 units 51 / 75 (68.0) 8 / 30 (26.7) 5.84 (2.27, 15.0) 0.0001
>= 4 and < 6 units 30 / 47 (63.8) 3 / 19 (15.8) 9.41 (2.39, 37.0) 0.0005
< 4 units 21 / 28 (75.0) 5 / 11 (45.5) 3.60 (0.83, 15.5) 0.0821
Baseline Serum EPO (U/L):
< 100 23 / 51 (45.1) 7 / 31 (22.6) 2.82 (1.03, 7.71) 0.0413
100 - < 200 14 / 37 (37.8) 2 / 19 (10.5) 5.17 (1.04, 25.9) 0.0338
200 - 500 17 / 43 (39.5) 1 / 15 (6.7) 9.15 (1.10, 76.2) 0.0188
> 500 3 / 21 (14.3) 0 / 11 (0.0) NA (NA , NA) 0.1950
Longest Duration Prior ESA (months):
< 6 16 / 48 (33.3) 1 / 20 (5.0) 9.50 (1.17, 77.5) 0.0147
6 - 12 15 / 34 (44.1) 1 / 9 (11.1) 6.32 (0.71, 56.2) 0.0718
> 12 - 24 15 / 35 (42.9) 4 / 21 (19.0) 3.19 (0.89, 11.4) 0.0710
> 24 12 / 31 (38.7) 3 / 20 (15.0) 3.58 (0.86, 14.9) 0.0724
0.0 0.1 0.25 0.5 2 10 20 100 1500
<---Favors Placebo-- --Favors Luspatercept--->
CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; EPO = erythropoietin; ESA = erythropoiesis-stimulating agent; IPSS-R = International Prognostic Scoring System-Revised; ITT = intent-to-treat; MDS = myelodysplastic syndromes; RCMD = refractory cytopenia with multilineage dysplasia; OR = odds ratio; RBC = red blood cell; RBC-TI = red blood cell-transfusion independence; WHO = World Health Organization.

Figure 10: Forest Plot of subgroup analysis for RBC-TI of 12 weeks or more from week 1 through week 24 (ITT population)



Point estimates mostly favoured Luspatercept treatment. The small sample sizes in each group must be kept in mind; however, these anayses are overall in support of the primary endpoint and strengthen the internal validity of the trial.

Analysis of RBC-TI of at least 8 weeks by Transfusion Burden Category at Baseline

Table 24: Analysis of RBC-TI of 8 weeks or more and transfusion reduction by baseline transfusion burden during weeks 1 through 24 (ITT population)

Subjects based on transfusion burden at baseline # RBC units/8 weeks during the 16 weeks prior to treatment	n/N (%) of Subjects		P-value[a]
	Luspatercept	Placebo	
Transfusion Independence			
RBC-TI ≥ 8 weeks	58/153 (37.91)	10/76 (13.16)	< 0.0001
≥ 6	6/66 (9.1)	1/33 (3.0)	0.2699
4 and < 6	15/41 (36.6)	1/23 (4.3)	0.0046
< 4	37/46 (80.4)	8/20 (40.0)	0.0013
Transfusion Reduction			
Reduction of 4 RBC units/8 weeks[b]	52/107 (48.6)	8/56 (14.3)	< 0.0001
≥ 4 and < 6	16/41 (39.0)	1/23 (4.3)	0.0028
≥ 6	36/66 (54.5)	7/33 (21.2)	0.0017

ITT = intent-to-treat; mHI-E = modified hematologic improvement – erythroid; RBC = red blood cell; RBC-TI = red blood cell transfusion independence.

[a] The p-value was from the Cochran-Mantel-Haenszel (CMH) test to compare luspatercept treatment group to placebo group.

[b] For subjects with ≥ 4 units/8 week baseline transfusion burden.

Post hoc analyses on subjects with higher transfusion burden (refined categories) at baseline

Table 25: Summary of Subgroup Analysis Results for RBC-TI in Subjects with Very high Baseline Transfusion Burden in Study ACE-536-MDS-001 (ITT Population)

Response Criteria	**Phase 3 Study ACE-536-MDS-001**			
	Luspatercept Response Rate[a]		**Placebo Response Rate[a]**	
Transfusion Burden Category at Baseline	**n/N (%)**	**(95% CI)**	**n/N (%)**	**(95% CI)**
RBC-TI ≥ 8 Weeks from Week 1 to Week 24				
≥ 6 units/8 weeks over 16 weeks	6/66 (9.1)	(3.41, 18.74)	1/33 (3.0)	(0.08, 15.76)
6 to 8 units/8 weeks	5/43 (11.6)	(3.9, 25.1)	1/19 (5.3)	(0.1, 26.0)
> 8 to 12 units/8 weeks	1/19 (5.3)	(0.1, 26.0)	0/13 (0)	(75.3, 100.0)
> 12 units/8 weeks	0/4 (0)	(39.8, 100.0)	0/1 (0)	(2.5, 100.0)
RBC-TI ≥ 12 Weeks from Week 1 to Week 24				
≥ 6 units/8 weeks over 16 weeks	4/66 (6.1)	(1.68, 14.80)	1/33 (3.0)	(0.08, 15.76)
6 to 8 units/8 weeks	4/43 (9.3)	(2.6, 22.1)	1/19 (5.3)	(0.1, 26.0)

> 8 to 12 units/8 weeks	0/19 (0)	(82.4, 100.0)	0/13 (0)	(75.3, 100.0)
> 12 units/8 weeks	0/4 (0)	(39.8, 100.0)	0/1 (0)	(2.5, 100.0)
RBC-TI ≥ 12 Weeks from Week 1 to Week 48				
≥ 6 units/8 weeks over 16 weeks	7/66 (10.6)	(4.37, 20.64)	2/33 (6.1)	(0.74, 20.23)
6 to 8 units/8 weeks	6/43 (14.0)	(5.3, 27.9)	1/19 (5.3)	(0.1, 26.0)
> 8 to 12 units/8 weeks	1/19 (5.3)	(0.1, 26.0)	1/13 (7.7)	(0.2, 36.0)
> 12 units/8 weeks	0/4 (0)	(39.8, 100.0)	0/1 (0)	(2.5, 100.0)

[a] Response rate (%) was calculated using the number of responders divided by the number of subjects (responders plus nonresponders) for each treatment, multiplied by 100. Subjects discontinued from the Primary Phase of the Treatment Period without achieving at least 56 consecutive days of RBC TI were counted as nonresponders.

Post hoc analysis of transfusion units with baseline imputation for the subgroups Baseline TB <6 and ≥6 units/8 weeks

Table 26: Analysis of RBC Transfusion Units by Baseline Transfusion Burden including all Observed Data regardless of Early Discontinuation with Imputation by Baseline (ITT Population)

Parameter	Baseline Transfusion Burden < 6 Units/8 Weeks		Baseline Transfusion Burden ≥ 6 Units/8 Weeks	
	Luspatercept (N = 87)	Placebo (N = 43)	Luspatercept (N = 66)	Placebo (N = 33)
RBC Transfusion Units Week 1-24[a]				
n	87	43	66	33
Mean (SD)	6.98 (6.566)	13.21 (5.998)	18.70 (9.627)	24.18 (8.777)
Median	5.00	14.00	17.50	23.00
Min, Max	0.0, 32.0	1.0, 26.0	0.0, 46.0	0.0, 49.0
LS Mean (SE)	7.2 (0.58)	12.8 (0.82)	18.9 (0.93)	23.7 (1.32)
95% CI for LS Mean	6.0, 8.3	11.1, 14.4	17.1, 20.8	21.1, 26.4
Treatment Comparison (Luspatercept vs Placebo)[b]				
LS Mean Difference (SE)	-5.6 (1.01)		-4.8 (1.62)	
95% CI for LS Mean Difference	-7.6, -3.6		-8.0, -1.6	
RBC Transfusion Units Week 25-48[a]				
n	87	43	66	33
Mean (SD)	7.24 (6.751)	12.21 (5.792)	19.36 (11.014)	23.36 (11.227)
Median	6.00	12.00	21.00	23.00
Min, Max	0.0, 28.0	0.0, 27.0	0.0, 49.0	0.0, 60.0
LS Mean (SE)	7.5 (0.57)	11.8 (0.82)	19.6 (1.13)	22.9 (1.60)
95% CI for LS Mean	6.3, 8.6	10.1, 13.4	17.4, 21.9	19.7, 26.0
	Baseline Transfusion Burden < 6 Units/8 Weeks		Baseline Transfusion Burden ≥ 6 Units/8 Weeks	

Parameter	Luspatercept (N = 87)	Placebo (N = 43)	Luspatercept (N = 66)	Placebo (N = 33)
Treatment Comparison (Luspatercept vs Placebo)[b]				
LS Mean Difference (SE)	-4.3 (1.00)		-3.3 (1.96)	
95% CI for LS Mean Difference	-6.3, -2.3		-7.1, 0.6	

CI = confidence interval; ITT = intent to treat; LS = least squares; Max = maximum; Min = minimum; RBC = red blood cell; SD = standard deviation; SE = standard error; vs = versus.

[a] If a subject's record of transfusion was curtailed prior to Day 336 (ie, Week 48), then the number of transfusion units is imputed from the last visit date where transfusion (yes/no) was recorded up to Day 336. Imputation is based on the average number of RBC units transfused in the 24 weeks prior to first dose (calculated on a units per day basis).

[b] Analysis of covariance was used to compare the treatment difference between groups, with the number of RBC units transfused in the time interval as the dependent variable, treatment group (2 levels) as a factor, and transfusion units during the 24-week baseline period as a covariate.

Table 27: Analysis of RBC Transfusion Units by IPSS-R Risk Category Including All Observed Data Regardless of Early Discontinuation with Imputation by Baseline (ITT Population)

Parameter	IPSS-R Risk Category Very Low or Low		IPSS-R Risk Category Intermediate	
	Luspatercept (N = 127)	Placebo (N = 63)	Luspatercept (N = 25)	Placebo (N = 13)
RBC Transfusion Units Week 1-24[a]				
n	127	63	25	13
Mean (SD)	11.87 (9.882)	16.98 (9.120)	12.56 (10.223)	22.77 (7.672)
Median	12.00	17.00	9.00	22.00
Min, Max	0.0, 46.0	0.0, 49.0	0.0, 37.0	4.0, 32.0
LS Mean (SE)	12.0 (0.57)	16.7 (0.82)	13.1 (1.22)	21.8 (1.69)
95% CI for LS Mean	10.9, 13.2	15.1, 18.3	10.6, 15.6	18.3, 25.2
Treatment Comparison (Luspatercept vs Placebo)[b]				
LS Mean Difference (SE)	-4.7 (1.00)		-8.7 (2.09)	
95% CI for LS Mean Difference	-6.6, -2.7		-12.9, -4.4	
	IPSS-R Risk Category Very		IPSS-R Risk Category	
	Luspatercept (N = 127)	Placebo (N = 63)	Luspatercept (N = 25)	Placebo (N = 13)
RBC Transfusion Units Week 25-48[a]				
n	127	63	25	13
Mean (SD)	12.50 (10.734)	16.13 (9.920)	11.96 (10.652)	21.54 (10.604)
Median	12.00	15.00	9.00	23.00
Min, Max	0.0, 49.0	0.0, 60.0	0.0, 42.0	0.0, 40.0
LS Mean (SE)	12.7 (0.61)	15.8 (0.87)	12.4 (1.72)	20.7 (2.39)
95% CI for LS Mean	11.5, 13.9	14.1, 17.5	8.9, 15.9	15.8, 25.5
Treatment Comparison (Luspatercept vs Placebo)[b]				

LS Mean Difference (SE)	-3.1 (1.07)	-8.2 (2.95)
95% CI for LS Mean Difference	-5.2, -1.0	-14.2, -2.2

CI = confidence interval; IPSS-R = International Prognostic Scoring System – Revised; ITT = intent to treat; LS = least squares; Max = maximum; Min = minimum; RBC = red blood cell; SD = standard deviation; SE = standard error; vs = versus.

[a] If a subject's record of transfusion was curtailed prior to Day 336 (ie, Week 48), then the number of transfusion units is imputed from the last visit date where transfusion (yes/no) was recorded up to Day 336. Imputation is based on the average number of RBC units transfused in the 24 weeks prior to first dose (calculated on a units per day basis).

[b] Analysis of covariance was used to compare the treatment difference between groups, with the number of RBC units transfused in the time interval as the dependent variable, treatment group (2 levels) as a factor, and transfusion units during the 24-week baseline period as a covariate.

Post hoc analysis using the proposed IWG 2018 haematological response criteria in patients with MDS included in clinical trials (Platzecker et al., 2019)

LTB subjects

In Study ACE-536-MDS-001, 36 (15.7%) subjects are considered to have LTB based on the IWG 2018 definition: 25 (16.3%) subjects in the luspatercept group and 11 (14.5%) in the placebo group.

Table 28: Summary of Hematologic Improvement – Erythroid per IWG 2018 Criteria during the Primary Treatment Phase (Week 1 - 24) for Subjects with Low Transfusion Burden

Endpoint Evaluated from Week 1 to Week 24 Parameter	**Luspatercept (N = 25)**	**Placebo (N = 11)**
HI-E per IWG 2018[a]		
HI-E Responders, n (%)	19 (76.0)	5 (45.5)
95% CI	(54.87, 90.64)	(16.75, 76.62)
Difference in Response Rate (%) (95% CI)[b]	30.55 (-3.31, 64.40)	
p-value[b]	0.0774	
Clinically Meaningful HI-E per IWG 2018[a]		
Clinically Meaningful HI-E Responders, n (%)	9 (36.0)	2 (18.2)
95% CI	(17.97, 57.48)	(2.28, 51.78)
Difference in Response Rate (%) (95% CI)[b]	17.82 (-11.7, 47.37)	
p-value[b]	0.2918	

CI = confidence interval; HI-E = hematologic improvement – erythroid; HTB = high transfusion burden; IWG = International Working Group; RBC = red blood cell.

[a] Defined as the absence of any RBC transfusion for at least 8 weeks (HI-E) or 16 weeks (clinically meaningful HI-E) with the same transfusion policy compared with the 16 weeks prior to treatment and with no decrease in concurrent mean hemoglobin during the Primary Phase of the Treatment Period (first 24 weeks of double-blind treatment).

[b] Unstratified Cochran-Mantel-Haenszel test; nominal p-value.

Note: Low transfusion burden subjects were those who received 3 to 7 units of RBCs in the last 16 weeks prior to first dose, except if the subject received > 3 RBC units in the last 8 weeks prior to first dose (in which case the subject was considered HTB).

HTB subjects

The majority of subjects randomized in Study ACE-536-MDS-001 are considered to have HTB based on the IWG 2018 definition: 192 (83.8%) of subjects; 127 (83.0%) subjects in the luspatercept group and 65 (85.5%) in the placebo group).

Table 29: Summary of Hematologic Improvement – Erythroid per IWG 2018 Criteria during the Primary Treatment Phase (Week 1 - 24) for Subjects with High Transfusion Burden

Endpoint Evaluated from Week 1 to Week 24 Parameter	**Luspatercept (N = 127)**	**Placebo (N = 65)**
Minor HI-E per IWG 2018		
Minor HI-E Responders, n (%)[a]	80 (63.0)	19 (29.2)
95% CI	(53.98, 71.39)	(18.60, 41.83)
Difference in Response Rate (%) (95% CI)[b]	33.76 (19.88, 47.65)	
p-value[b]	< 0.0001	
Clinically Meaningful Minor HI-E per IWG 2018		
Clinically Meaningful Minor HI-E Responders, n (%)[c]	54 (42.5)	7 (10.8)
95% CI	(33.80, 51.60)	(4.44, 20.94)
Difference in Response Rate (%) (95% CI)[b]	31.75 (20.32, 43.18)	
p-value[b]	< 0.0001	
Major HI-E per IWG 2018		
Major HI-E Responders, n (%)[d]	38 (29.9)	5 (7.7)
95% CI	(22.12, 38.68)	(2.54, 17.05)
Difference in Response Rate (%) (95% CI)[b]	22.23 (11.96, 32.49)	
p-value[b]	0.0005	
Endpoint Evaluated from Week 1 to Week 24 Parameter (continued)	**Luspatercept (N = 127)**	**Placebo (N = 65)**
Clinically Meaningful Major HI-E per IWG 2018		
Clinically Meaningful Major HI-E Responders, n (%)[e]	19 (15.0)	1 (1.5)
95% CI	(9.25, 22.37)	(0.04, 8.28)
Difference in Response Rate (%) (95% CI)[b]	13.42 (6.53, 20.31)	
p-value[b]	0.0041	

CI = confidence interval; HI-E = hematologic improvement – erythroid; IWG = International Working Group; LTB = low transfusion burden; RBC = red blood cell.

[a] Defined as a reduction by at least 50% of RBC transfusion units compared to baseline during any consecutive 56-day (ie, 8-week) period during the Primary Phase of the Treatment Period (first 24 weeks of double-blind treatment).

[b] Unstratified Cochran-Mantel-Haenszel test; nominal p-value.

[c] Defined as a reduction by at least 50% of RBC transfusion units compared to baseline during any consecutive 112 day (ie, 16-week) period during the Primary Phase of the Treatment Period (first 24 weeks of double-blind treatment).

[d] Defined as the absence of any RBC transfusion over a period of minimum 8 weeks with the same transfusion

policy compared with the 16 weeks prior to treatment and with no decrease in concurrent mean hemoglobin during the Primary Phase of the Treatment Period (first 24 weeks of double-blind treatment).

[e] Defined as the absence of any RBC transfusion over a period of minimum 16 weeks with the same transfusion policy compared with the 16 weeks prior to treatment and with no decrease in concurrent mean hemoglobin during the Primary Phase of the Treatment Period (first 24 weeks of double-blind treatment).

Note: High transfusion burden subjects were defined as those who did not meet the criteria of LTB, excluding Subject 1011005 who had a baseline transfusion burden of 2 RBC units in the 16 weeks prior to the first dose and was excluded from the analysis.

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 30: Summary of efficacy for trial ACE-536-MDS-001

Title: A phase 3 double-blind, randomized study to compare the efficacy and safety of Luspatercept (ACE-536) versus placebo for the treatment of anemia due to IPSS-R very low, low or intermediaterisk myelodysplastic syndromes in subjects with ring sideroblasts who require red blood cell transfusion.			
Study identifier	ClinicalTrials.gov identifier: NCT02631070; EudraCT number: 2015-003454-41		
Design	Study ACE-536-MDS-001 is an ongoing Phase 3, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of luspatercept and BSC versus placebo and BSC in adults with anemia due to IPSS-R very low, low or intermediaterisk myelodysplastic syndromes in subjects with ring sideroblasts who require red blood cell transfusion at least 2 units/8weeks averaged over 16 weeks). This study consists of: -a screening phase (5 weeks; 16 weeks of transfusion history need to be available) -a double-blind primary phase (week 1-24) and a double-blind extension phase (week 24-48) -follow-up phase of 3 years (ongoing)		
	Duration of DB main phase: Duration of DB extension phase:		24 weeks 24 weeks
Hypothesis	Superiority		
Treatments groups	Luspatercept + BSC		SC injection (q3w) with a starting dose of 1 mg/kg, N=153
	Placebo + BSC		SC injection (q3w) with a starting dose of 1 mg/kg, N=76
Endpoints and definitions	Primary endpoint	RBC-TI $\geqslant$ 8 weeks from Week 1 through Week 24	To determine proportions of subjects that achieved RBC-TI, defined as the absence of any RBC transfusion during any consecutive 56-day (8-week) period during the Primary Phase of the Treatment

	Key secondary endpoint 1	RBC-TI ⩾ 12 weeks from Week 1 through Week 48	To determine proportions of subjects that achieved RBC-TI, defined as the absence of any RBC transfusion during any consecutive 84-day (12-week) period during Week 1 to Week 48.
	Key secondary endpoint 2	RBC-TI ≥ 12 weeks from Week 1 - 24	To determine proportions of subjects that achieved RBC-TI, defined as the absence of any RBC transfusion during any consecutive 84-day (12-week) period during the Primary Phase of the Treatment Period (first 24 weeks of double-blindtreatment).
	Secondary endpoint	Time to RBC- TI	Defined as the time between first dose date and the date of onset of RBC-TI first observed for subjects who achieved RBC-TI of ≥ 8 weeks during the Treatment Period (Week 1 – 24; week 1 - 48)
	Secondary endpoint	Longest single episode of RBC-TI ≥ 8 weeks (Week 1 - 24) (Week 1 - 48) (Responders only)	To determine the duration of RBC-TI, defined as the longest duration of response for subjects who achieved RBC-TI of ≥ 8 weeks during the Treatment Period (Week 1 - 24 and Week 1 - 48)
	Secondary endpoint	Change in RBC Units Transfused Over Fixed 16-Week Periods (Weeks 9 – 24) (Weeks 33 – 48)	To determine the change from baseline in RBC Units Transfused Over Fixed 16-Week Periods, compared to the baseline transfusion units over the 16 weeks immediately preceding the first dose.

	Secondary endpoint	Mean Hemoglobin Increase of at Least 1.0 g/dL	To determine the proportion of subjects with a mean Hgb increase of ≥ 1.0 g/dL from baseline (after applying the 14/3 day rule) that was sustained over any consecutive 56-day (8-week) period in the absence of RBC transfusions during the Treatment Period (Week 1 - 24 and Week 1 - 48).
	Secondary endpoint	Modified Hematologic Improvement – Erythroid (mHI-E)	For subjects with <u>baseline RBC transfusion burden of ≥ 4 units/8 weeks</u>: defined as a reduction in RBC transfusion of at least 4 units/8 weeks. For subjects with <u>baseline RBC transfusion burden of < 4 units/8 weeks:</u> defined as a mean increase in haemoglobin of ≥ 1.5 g/dL for 8 weeks in the absence of RBC transfusions.
	Additional secondary endpoints	-Hematologic improvement – platelets (not evaluated due to insufficient patients) -Hematologic improvement – neutrophils (not evaluated due to insufficient patients) -HRQoL assessments (EORTC QLQ-C30) -Change in mean daily dose (mg) in Iron chelation therapy (ICT) (Weeks 9-24; 33-48) -Change from baseline in Serum Ferritin (Weeks 9-24; 33-48)	
	Post hoc analyses	-Overall survival (OS) (post hoc) -Time to progression to AML (post hoc) - Hemoglobin and change from baseline in hemoglobin by treatment group over time Provided upon CHMP request: - change from baseline in RBC transfusion rates -transfusion units, serum ferritin, Hb change over time, ICT use with imputation by baseline, also separated by stratification factors - A post hoc-analysis using the proposed IWG 2018 haematological response criteria in patients with MDS included in clinical trials (Platzecker et al., 2019) -sensitivity analysis of the primary endpoint that 1.) considers all discontinued subjects non-responders; 2.) considers all subjects discontinuing for reasons potentially related to treatment non-responders -subgroup analyses on subjects with low and high transfusion burden at baseline	
Database lock	Data cut off date: 08 May 2018		
<u>Results and Analysis</u>			
Analysis description	**Primary Analysis**		

Analysis population and time point	ACE-536-MDS-001: IIT population: n=229; PP: N/A Any 8 weeks during the first 24 weeks		
Effect estimate per comparison	Treatment group	Luspatercept + BSC	Placebo + BSC
	Number of subjects	153	76
Primary endpoint			
RBC-TI ⩾ 8 weeks from Week 1-24	Number of responders	58	10
	Response rates	37.91%	13.16%
	95%CI	(30.20, 46.10)	(6.49, 22.87)
	Odds ratio (95% CI) *	5.065 (2.278, 11.259)	
	p-value *	< 0.0001	
Notes	* 2-sided p-value from Cochran-Mantel-Haenszel (CMH) test stratified for average baseline RBC transfusion requirement (≥ 6 units versus < 6 units of RBC per 8 weeks), and baseline IPSS-R score (very low or low versus intermediate).		

Key secondary endpoint analyses			
RBC-TI ⩾ 12 weeks from Week 1 through Week 24	Number of responders	43	6
	Response rates	28.10%	7.89%
	95%CI	(21.14, 35.93)	(2.95, 16.40)
	Odds ratio (95% CI) *	5.071 (2.002, 12.844)	
	p-value	0.0002	
RBC-TI ⩾ 12 weeks from Week 1 through Week 48	Number of responders	51	9
	Response rates	33.33%	11.84%
	95%CI	(25.93, 41.40)	(5.56, 21.29)
	Odds ratio (95% CI) *	4.045 (1.827, 8.956)	

	p-value	0.0003

Secondary analyses			
Time to RBC-TI≥ 8 weeks (Week 1 - 24)	Treatment group	Luspatercept	Placebo
	Number of subjects	58	10
	Mean (SD)	17.2 (29.4)	26.0 (31.83)
	Median (Min, Max)	1. (1.0-106.0)	17.0 (1.0 – 100.0)
Longest single episode of RBC-TI ≥ 8 weeks (Week 1 - 24)	Treatment group	Luspatercept	Placebo
	Number of subjects (Responder	58	10
	Median duration (weeks)	30.6	13.6
	95%CI	20.6-40.6	9.1-54.9
	Subjects who maintained	20 (34.5)	2 (20.0)
	Subjects who lost response, n(%)*	38 (65.5)	8 (80.0)
Notes	Median was from the unstratified Kaplan-Meier method * Subjects who lost response are those who received RBC transfusion following an RBC-TI period		
Change in RBC Units Transfused Over Fixed 16-Week Periods	Treatment group	Luspatercept (week 9-24 and 33-48)	Placebo (week 9-24 and 33-48)
	Number of subjects	128/78	68/12
	median RBC transfusion volume over 16 weeks	6.0 and 4.0	12.0 and 7.5
Notes	The median baseline RBC transfusion volume over the 16 weeks immediately preceding the first dose was 10.0 units in both the luspatercept and placebo treatment groups.		

Mean Hemoglobin Increase of at Least 1.0 g/dL (week 1-24)	Treatment group	Luspatercept	Placebo
	Number of subjects	153	76
	n (%)	54 (35.3)	6 (7.9)
	95%CI	27.75, 43.42	2.95, 16.40
mHI-E (week 1-24)	Treatment group	Luspatercept	Placebo
	n (%)	81 (52.9)	9 (11.8)
	95% CI	44.72, 61.05	5.56-21.29
RBC transfusion reduction of 4 units/8 weeks	n (%)	52/107 (48.6)	8/56 (14.3)
Mean haemoglobin increase of ≥1.5 g/dL for 8 weeks	n (%)	29/46 (63.0)	1/20 (5.0)

Analysis performed across trials (pooled analyses and meta-analysis)

Due to the differences in the subject populations and efficacy endpoints between the Phase 2 and Phase 3 studies, the Phase 2 and Phase 3 data sets were not pooled for integrated efficacy analyses. However, in order to compare efficacy data across studies, results from Study ACE-536-MDS-001 were presented side-by-side with the results from the combined Phase 2 studies.

The combined Phase 2 data were presented by dose group: < 1.0 mg/kg and ≥ 1.0 mg/kg. In addition, a subset of subjects from the Phase 2 studies that approximated the dosing of Study ACE-536-MDS-001 and also matched key inclusion criteria of Study ACE-536-MDS-001 were defined as follows:

- baseline RBC transfusion burden of ≥ 2 units/8 weeks
- RS+
- prior exposure to ESA or endogenous EPO > 200 U/L

This subset of Phase 2 subjects is referred to as the "Phase 3-like Population" of the Phase 2 studies and is presented side-by-side with the Phase 3 data for subjects in the "Phase 3-like population" who received luspatercept ≥ 1.0 mg/kg. The "Phase 3-like Population" was identified for MDS SCE analysis using the primary endpoint and selected secondary endpoints from Study ACE-536-MDS-001, as well

as subgroup analyses. The number of subjects for analysis in the MDS SCE are shown in the table below.

Table 31: Number of subjects for analysis

Population	Phase 2 Studies A5367-03/A536-05			Phase 3 Study ACE-536-MDS-001	
	Luspatercept < 1.0 mg/kg	Luspatercept ≥ 1.0 mg/kg	Luspatercept ≥ 1.0 mg/kg Phase 3-like Population[a]	Luspatercept	Placebo
ITT[b]	12	95	28	153	76
TD Population[c]	10	64	28	153	76

EPO = erythropoietin; ESA = erythropoiesis-stimulating agent; ITT = intent-to-treat; MDS = myelodysplastic syndromes; RS+ = ring sideroblast positive; SAP = statistical analysis plan; SCE = Summary of Clinical Efficacy; TD = transfusion dependent.

[a] The "Phase 3-like Population" is a subset of subjects from the Phase 2 studies that approximated the dosing of Study ACE-536-MDS-001 and matched the following key inclusion criteria for Phase 3: baseline RBC transfusion burden of ≥ 2 units/8 weeks, RS+, and prior exposure to ESA or endogenous EPO > 200 U/L.

[b] Used for summaries of demographic and baseline characteristics

[c] ITT subjects who required RBC transfusion at baseline. This population is used for SCE efficacy analysis.

Duration of treatment and follow-up is another consideration in comparisons between the Phase 3 and Phase 2 studies. The median duration of treatment for the data cut included in this submission was longer in Phase 2: 55 weeks (min, 12; max, 165) in extension Study A536-06) than in Study ACE-536-MDS-001: 49 weeks (min, 6; max 114) in the luspatercept + BSC group and 24 weeks (min, 7; max, 89) in the placebo + BSC group).

Subject disposition across studies:

Table 32: Subject disposition (ITT population)

	Phase 2 Studies A536-03/A536-05			Phase 3 Study ACE-536-MDS-001	
	Luspatercept < 1.0 mg/kg (N = 12) n (%)	Luspatercept ≥ 1.0 mg/kg (N = 95) n (%)	Luspatercept ≥ 1.0 mg/kg "Phase 3-like Population"[a] (N = 28) n (%)	Luspatercept (N = 153) n (%)	Placebo (N = 76) n (%)
Analysis Population (ITT)	12 (100.0)	95 (100.0)	28 (100.0)	153 (100.0)	76 (100.0)
Subjects discontinued treatment	12 (100.0)	56 (58.9)	22 (78.6)	83 (54.2)	70 (92.1)
Adverse Event	0 (0.0)	0 (0.0)	0 (0.0)	10 (6.5)	4 (5.3)
Death	0 (0.0)	2 (2.1)	1 (3.6)	0 (0.0)	0 (0.0)
Lack of Efficacy	0 (0.0)	4 (4.2)	1 (3.6)	51 (33.3)	50 (65.8)
Physician Decision	0 (0.0)	10 (10.5)	1 (3.6)	0 (0.0)	0 (0.0)
Progressive Disease[b]	0 (0.0)	6 (6.3)	0 (0.0)	3 (2.0)	2 (2.6)
Protocol Deviation	0 (0.0)	1 (1.1)	1 (3.6)	1 (0.7)	0 (0.0)
Withdrawal by Subject	0 (0.0)	10 (10.5)	9 (32.1)	14 (9.2)	10 (13.2)
Completed[c]	12 (100.0)	13 (13.7)	4 (14.3)	0 (0.0)	0 (0.0)
Other	0 (0.0)	10 (10.5)	5 (17.9)	4 (2.6)	4 (5.3)
Subjects Discontinued Study	12 (100.0)	50 (52.6)	22 (78.6)	30 (19.6)	19 (25.0)
Death	0 (0.0)	2 (2.1)	1 (3.6)	12 (7.8)	9 (11.8)
Lost to Follow-up	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)	0 (0.0)
Protocol Deviation	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)
Study Terminated by Sponsor	0 (0.0)	1 (1.1)	1 (3.6)	0 (0.0)	0 (0.0)
Withdrawal by Subject	0 (0.0)	8 (8.4)	5 (17.9)	13 (8.5)	8 (10.5)
Completed[d]	12 (100.0)	31 (32.6)	11 (39.3)	0 (0.0)	0 (0.0)
Other	0 (0.0)	7 (7.4)	4 (14.3)	3 (2.0)	2 (2.6)

EPO = erythropoietin; ESA = erythropoiesis-stimulating agent; ITT = intent-to-treat; MDS = myelodysplastic syndromes; RBC = red blood cell; RS+ = ring sideroblast positive; SCE = Summary of Clinical Efficacy.

[a] The "Phase 3-like Population" is a subset of subjects from the Phase 2 studies that approximated the dosing of Phase 3 Study ACE-536-MDS-001 and matched the following key inclusion criteria for Phase 3: baseline RBC transfusion burden of ≥ 2 U/8 weeks, RS+, and prior exposure to ESA or endogenous EPO > 200 U/L.

[b] Progressive disease includes progression to high-risk MDS or progression to acute myeloid leukemia.

[c] Subject completed treatment in Study A536-03 and did not enroll in extension Study A536-05. These subjects were counted as discontinuations for the SCE analyses of Phase 2 studies because the subjects were not receiving treatment at the time of the data cutoff.

[d] Completion of study is defined as follows: 1) subject rolled over into Study A536-05 at end of treatment visit for Study A536-03 or 2) subject completed follow-up visits after discontinuation of treatment.

Baseline characteristics were generally consistent with the phase 3 study population, except for a higher percentage of subjects with intermediate risk MDS (42.9% vs. 16.3%), a higher median EPO level at baseline (271.4 U/L vs. 156.9 U/L), and a greater variety of MDS subtypes based on the WHO classification system. Patients had lower mean baseline Hgb (7.3 g/dL) and a higher proportion of patients with higher baseline burden (≥ 6 units of RBC transfusion/8weeks). In addition, a slightly lower percentage of subjects had used prior ESA therapy compared with Study ACE-536-MDS-001 (78.6% versus > 90%) and the median duration of ESA therapy was slightly shorter (9.6 months versus 11.8 months).

Results

The RBC-TI response rate of 32.1% (9/28) during Week 1 to Week 24 for the "Phase 3-like

Population" was consistent with the response rate in the luspatercept group of Study ACE-536-MDS-001. Four of 9 (44.4%) subjects maintained response at the last evaluation.

An additional subject in the "Phase 3-like Population" achieved response after Week 24, for a total of 10 responders during Week 1 to Week 48. The median duration of the longest single episode of RBC-TI was 41.7 weeks (95% CI: 8.7, NE) for these 10 responders. The longer duration of response for the Week 1 to Week 24 responders in the "Phase 3-like Population" reflects the impact of individual

subjects on the outcome with a small sample size (ie, the additional responder after Week 24 who had a shorter duration of response).

The RBC-TI ≥ 12 weeks response rates for the "Phase 3-like Population" were 25.0% (7/28) during Week 1 to Week 24 and 28.6% (8/28) during Week 1 to Week 48. Both results were consistent with the response rates in the luspatercept group of Study ACE-536-MDS-001.

The Kaplan-Meier estimate of the median duration of the longest single episode of RBC-TI was 94.9 weeks (95% CI: 8.1, not estimable [NE]) for the 9 responders. The longer median duration of response in the "Phase 3-like Population" compared with Study ACE-536-MDS-001 (94.9 weeks versus 30.6 weeks) reflects the earlier start date of the Phase 2 studies, and thus, the longer duration of treatment and follow-up.

Clinical studies in special populations

No studies in patients with renal or hepatic impairment, in elderly or paediatric patients (not included in the label) were conducted in the MDS indication.

Age

Table 33: Baseline characteristics of subjects – continuous data

Characteristics	A536-03 N = 107		ACE-536-MDS-001 N = 153		Total N = 260	
	Mean (CV%)	Median [Min, Max]	Mean (CV%)	Median [Min, Max]	Mean (CV%)	Median [Min, Max]
Age (years)	70.6 (15.3)	72.0 [27.0, 90.0]	70.5 (12.3)	71.0 [40.0, 95.0]	70.5 (13.6)	72.0 [27.0, 95.0]

Starting dose adjustments based on age are not supported by PK, efficacy, safety, and E-R data. Although the population PK analysis suggested that luspatercept CL/F decreased with age, the effect size was modest (exponential coefficient = -0.534) and is not anticipated to influence luspatercept serum exposure substantially. No large difference in luspatercept serum exposure was observed among age groups.

In multivariate exposure-efficacy analyses for the pivotal Phase 3 study, older age appeared to be associated with a reduced chance of achieving RBC-TI, but this effect was not considered critical given that an age effect was not observed in the more robust integrated exposure-efficacy analyses. In addition, a treatment effect in favour of luspatercept over placebo was observed across all age groups in subgroup analyses for efficacy.

Renal impairment:

No clinically significant differences in Cmax.ss and AUCss were found across renal function groups (normal, mild renal impairment, and moderate renal impairment).

No starting dose adjustment is recommended for patients with mild to moderate renal impairment (eGFR 89 – 30 mL/min/1.73 m2). The recommendation is supported by PK, efficacy, safety, and E-R data. Subgroup analyses results from phase 3 data for mild/moderate renal impairment are consistent with the overall treatment effect. No dose recommendation can be made for patients with eGFR < 30 mL/min/1.73 m2 due to the lack of clinical data.

Hepatic impairment:

No clinically significant differences in Cmax.ss and AUCss were found across hepatic function groups defined by NCI-ODWG criteria (normal, mild hepatic impairment, and moderate/severe hepatic impairment). No clinically significant differences in Cmax.ss and AUCss were predicted for subjects with extreme baseline albumin values and those with normal albumin values. Baseline liver enzymes (AST and ALT, up to ~3 x ULN) had also no clinically meaningful effect on luspatercept PK.

In MDS studies, a significant portion of luspatercept treated subjects was defined by the NCI-ODWG criteria to have compromised hepatic function at baseline, including 31.5% (82/260) of subjects with mild hepatic impairment (total bilirubin > 1-1.5 x ULN or AST or ALT > ULN), 8.8% (23/260) with moderate hepatic impairment (total bilirubin > 1.5-3 x ULN, any ALT or AST), and 0.4% (1/260) with severe hepatic impairment (total bilirubin > 3 x ULN, any ALT or AST).

In β-thalassemia studies, more subjects were classified as having severe hepatic impairment (N = 39) by the NCI-ODWG criteria due to their elevated total bilirubin. However, most of the subjects had elevated total bilirubin without increases in liver enzymes, suggesting the bilirubin elevations might not necessarily be of hepatic origin. Total bilirubin increases could be confounded by ineffective erythropoiesis with haemolysis and apoptosis, which are clinical features of MDS and β-thalassemia. Thus, according to the applicant, the NCI-ODWG criteria may not be a desirable measure of hepatic function in the MDS population.

Subjects with a baseline total bilirubin > 1.5 x ULN (or moderate/severe hepatic impairment by the NCI-ODWG criteria) showed better efficacy than those with lower baseline total bilirubin even though their mean exposure was comparable. This observation was supported by the exposure-efficacy analysis in which the probability was statistically greater in achieving RBC-TI ≥ 8 weeks, RBC-TI ≥ 12 week, or mHI-E for luspatercept-treated subjects with a baseline total bilirubin > 1.5 x ULN after accounting for the effect of luspatercept exposure and significant baseline risk factors. The effect of high bilirubin implied that luspatercept may be more efficacious in a subgroup of subjects, in whom the increased bilirubin results from enhanced ineffective erythropoiesis and haemolysis, though further verification may be needed. No robust conclusions can be drawn based on the limited number of cases observed. No dose recommendation can be made for patients with ALT or AST ≥ 3 ULN due to insufficient clinical data.

Supportive studies

Besides the pivotal phase 3 study ACE-536-MDS-001, two phase 2 studies were submitted to support luspatercept for the treatment of MDS; A536-03 and A536-05 (please see dose response study section above). Of note, several subgroups in the broader phase 2 populations were evaluated for erythroid response and RBC transfusion independence. There are indications that patients eg. with RS, SF3B1 mutation, lower baseline EPO or no prior ESA treatment might better respond to luspatercept treatment. Furthermore there are two phase 2 studies and one phase 3 study submitted to support the 'Beta-Thalassaemia' indication, which also evaluate erythroid response.

2.5.3. Discussion on clinical efficacy

The recommended starting dose of Reblozyl is 1.0 mg/kg administered once every 3 weeks.

In patients who are not RBC transfusion-free after at least 2 consecutive doses at the 1.0 mg/kg starting dose, the Reblozyl dose should be increased to 1.33 mg/kg. If patients are not RBC transfusion-free after at least 2 consecutive doses at the 1.33 mg/kg dose level, the Reblozyl dose should be increased to 1.75 mg/kg. The dose should not be increased more frequently than every 6 weeks (2 doses) or beyond the maximum dose of 1.75 mg/kg. The applicant justifies the titration

scheme (amongst others) by keeping the risk of exaggerated Hgb increase (>2g/dL) low, when starting at lower doses (i.e. 1 mg/kg). The transfusion-reducing effect of luspatercept in subjects with MDS relies on its ability to increase Hgb. Since the dosing schedule resulted in sustained Hgb response in LTB subjects, this should also be appropriate for reducing the frequency of RBC transfusions. In the phase 3 study, reasons for dose reductions in the active treatment arm were an increase in Hgb ≥2 g/dL compared to previous treatment cycle (2%), suspected related Grade 3 AE (3.3%) and 'other' (1.3%). Reasons for dose delays were: suspected related Grade 3 AE (2.6%), pre-dose Hgb ≥11.5 g/dL (6.5%), elevated WBC (2%), and Other (35.9%).

Design and conduct of clinical studies

The target population is adult patients with very low- to intermediate-risk myelodysplastic syndromes with ring sideroblasts (RS) associated with transfusion dependent anaemia who are refractory/intolerant or ineligible to ESA therapy.

The application for treatment of MDS-related anaemia includes one pivotal multi-centre, double-blind randomised phase 3 trial (ACE-536-MDS-001) and one open label phase 2 study (multi-dose ascending) with cross-over to an open label extension study (A536-03/05).

The single pivotal study setting might be acceptable considering that the applicant conducted a second phase 3 study in the Beta-Thalassemia indication, for which the mechanism of action is the same, and results coming from this second study are considered supportive for the MDS indication.

The open label phase 2 study recruited 107 subjects, out of which 28 had similar disease and baseline characteristics (baseline RBC transfusion burden of ≥ 2 units/8 weeks, RS+, prior exposure to ESA or endogenous EPO > 200 U/L) as the phase 3 population. The efficacy outcomes observed for the different endpoints were consistent with the phase 3 results.

For the pivotal double-blind phase 3 trial, a total of 290 subjects were screened, of whom 229 were randomized in a 2:1 ratio to the luspatercept (n = 153) or placebo (n= 76) treatment groups. Patients were recruited over 65 centres in the US, Canada, Turkey and Europe. In Europe, patients from 48 centres have been included (Germany, France, Belgium, Netherlands, UK, France, Spain, Italy and Sweden), recruited overall 172 patients, and are considered representative for patients treated in the EU.

Most subjects (95.6%) had a centrally reviewed diagnosis of MDS classified as refractory cytopenia with multilineage dysplasia (RCMD) per the WHO 2008 criteria, which was well-balanced between the luspatercept and placebo groups (94.8% and 97.4%, respectively). Approximately 75% of subjects had an MDS diagnosis of > 2 years before entering the study.

Most subjects (83%) were classified as having an IPSS-R score of very low/low (10.5% were IPSS-R very low and 72.5% IPSS-R low), the remaining were classified as intermediate. The treatment arms were largely balanced in terms of IPSS-R category.

The majority (approximately 86%) of subjects had a serum EPO ≤ 500 U/L at baseline (median baseline EPO = 153.2 U/L). Overall, approximately 60% of the subjects had a baseline EPO < 200 U/L with a slightly higher proportion of subjects in the placebo treatment arm. A slightly higher proportion of subjects in the luspatercept treatment group had EPO > 200 to 500 U/L (28.1 %) compared with the placebo group (19.7%). This imbalance is not considered relevant for the interpretation of the results.

Most included patients were ESA therapy refractory (97.3% for luspatercept and 98.6% for placebo in the phase 3 study). 2.7% (n=4) of patients in the luspatercept group and 1.4% (n=1) in the placebo

group were intolerant. 3.3% (n=5) of patients in the luspatercept and 7.9% (n=6) in the placebo arm were ESA ineligible with elevated serum EPO > 200 U/L and did not previously receive ESA therapy. Appropriate justification based on the mechanism of action and individual response data was provided. There is currently no clear indication that a differential response to treatment is to be expected in the different ESA subgroups.

The median age was 71.0 years (range: 26 to 95 years), with approximately 80% of subjects ≥ 65 years of age and > 36% of subjects ≥ 75 years of age. The majority of subjects were male (62.9%).

The majority of subjects (90.0%) had the SF3B1 mutation: 92.2% and 85.5% for luspatercept and placebo groups, respectively.

The median 8-week RBC transfusion volume over the 16 weeks was 5.0 units in both groups (range 1-15 for the active treatment arm and 2-20 for the placebo arm). The number of subjects available for analysis is limited for subjects with baseline transfusion burden > 8 RBC units/8 week (15% and 18.4% for luspatercept and placebo, respectively), and particularly for subjects with baseline transfusion burden > 12 RBC units/8 weeks (2.6% and 1.3% for luspatercept and placebo, respectively). This has been reflected in section 5.1 of the SmPC.

Of note, the applicant's definition of transfusion dependence (TD) seems to be based on the IWG 2006 response criteria (Cheson et al., Blood 2006), but the additional classification of TD by low and high transfusion burden (2-3 RBC units/8 weeks and ≥4 RBC units/ 8 weeks averaged over two consecutive 8 weeks-periods, respectively) also appears to be in accordance with the 'Proposals for revised IWG 2018 hematological response criteria in patients with MDS included in clinical trials' (Platzecker et al., Blood 2019). The employed endpoints however differ from both IWG 2006 and 2018 MDS response criteria. Post hoc analyses were provided using the definitions of the MDS 2018 criteria.

The mean baseline haemoglobin level was 7.7 g/dL and was similar between treatment groups.

Median baseline serum ferritin (centrally assessed) was 1101.9 µg/L, which was consistent with historical serum ferritin levels from local evaluations that were available in approximately 70% if subjects (median: 1242.5 µg/L).

Low proportions of patients had platelet counts <100*109/L (5.2 and 7.9 % for luspatercept and placebo); the majority of patients had platelet counts of 100 to 400*109/L (83.7 and 80.3%, respectively), and 11.1 and 11.8%, respectively, had platelets >400*109/L. Mean platelet counts at baseline were 259.3 and 251.7 *109/L for luspatercept and placebo, respectively.

Similarly, low proportions of patients had lower ANC at baseline (<0.5 *109/L: 0.7 and 0% for luspatercept and placebo; 0.5- <1*109/L for 9.2 and 13.2%, respectively); the majority of patients had ANC ≥1.0*109/L (90.2 and 86.8% for luspatercept and placebo). Mean ANC were 2.8 and 2.7 * 109/L for luspatercept and placebo.

Taken together, the recruited study population corresponds to the intended target population and the baseline characteristics were overall balanced.

Comparison to placebo is acceptable in view of the target population, for which no medicinal product is approved in the EU.

The indication was amended during the evaluation to refer to patients with loss of/lack of response to prior ESA therapy and now reads: 'who had an unsatisfactory response to or are ineligible for erythropoietin-based therapy' (see section 5.1). The description of the population as 'transfusion-dependent' rather than with 'anaemia requiring transfusions' has also been agreed.

As based on the analyses and discussion provided, there is no indication that patients with a lower percentage of RS (5-15% RS with SF3B1 mutation) would respond differently to treatment than those

investigated in the phase 3 study population (RS>15%), the wording related to RS (without further specification) is accepted.

As a side note, the applicant's plan to include 'and have received or are not eligible for erythropoiesis-stimulating agents' only in regions where ESAs are approved in MDS is not supported, as for centrally authorised medicinal products the indication wording is standardised across EU countries.

Study objectives and endpoints

The goals of therapy comparing lower- and higher risk MDS patients are slightly different: in patients with lower risk disease, treatment is focused on improving cytopenias, given that a large proportion of patients develop anaemia and become transfusion-dependent. Improvement of quality of life, which is impaired not only by the symptoms related to cytopenia, but also by the chronic need of RBC transfusion in transfusion-dependent patients, is also an important treatment goal. In contrast, treatment in higher risk disease patients rather aims at modifying the disease course (partial or complete remission, stability of disease).

In this regard, the primary objective, i.e. the evaluation of transfusion independence, is considered clinically relevant, but should be supported by secondary outcome parameters evaluating RBC transfusion over time, since also a reduction in transfusion units corresponds to a benefit to the patient. A reduction in transfusion frequency not only helps to reduce iron overload and other transfusion-related adverse effects such as hypersensitivity reactions, but should also improve quality of life. Further relevant secondary objectives that were analysed in this study to support the primary assessment were haemoglobin increase (post hoc), serum ferritin levels and QoL endpoints.

The definition of the primary and key secondary endpoints and, more specifically, the time period chosen, i.e. any 8 (12) weeks during 24 and 48 weeks, does not provide any information on long-term benefits. It is evident that the longer the response to treatment, the higher the clinical benefit to the patient.

From a technical perspective, the statistical analysis of the primary efficacy endpoint is considered adequate. However, the responder criterion applied in the definition of the primary endpoint and related analyses are not considered optimal for the evaluation of treatment benefit in terms of a reduction in transfusion burden. Consequently, additional analyses focusing on the rate of RBC transfusions and transfusion units were requested. Results thereof were included in the SmPC.

There is an important limitation to the study design: after 24 weeks of treatment, all patients were permitted to discontinue treatment, if progression of disease or no clinical benefit (defined as decrease in RBC requirements or increase in Hb compared with baseline) was experienced. Consequently, a large proportion of placebo patients were discontinued from treatment after week 24. Statistical evaluations of potential long-term treatment effects beyond 24 weeks are therefore hampered. Moreover, results including data from Weeks 25 to 48 were only presented for subjects who continue treatment and additional analyses were requested for subjects discontinuing treatment but remaining in the study and using conservative imputation methods. Of note, the SmPC states that luspatercept must be discontinued if no clinical benefit is observed after a maximum of two dose increases and overall 18 weeks of treatment, which mitigates concerns about long-term treatment of non-responders.

About 15% of subjects did not complete 24 Weeks of treatment. The proportion of subjects who did not complete 24 weeks of treatment was slightly lower in the placebo group than the luspatercept group. As subjects who discontinued treatment were counted as responders, in case they achieved 8 weeks of transfusion independence prior to discontinuation, additional sensitivity analyses of the primary endpoint were requested.

The transfusion policy employed before and during the study may be different. Considering however the controlled, randomised study setting, no concern is raised. Decision criteria during the study included subjective elements (i.e. a transfusion was to be given in case of symptoms due to anaemia, regardless of the haemoglobin value). This underlines the importance of impeccable blinding techniques, which are however considered to be appropriate for this Phase 3 study. Of note, the GCP inspection that was conducted for the Phase 3 ß-Thalassaemia trial, which had similar blinding techniques, did not reveal any critical findings regarding the blinding of the study. Most of transfusions given at baseline and throughout the study were reported as 'due to anemia' and potential imbalances are not of concern.

Dose may have been increased dependent on patient's response (starting at 1 mg/kg with two incremental steps up to maximally 1.75 mg/kg) or decreased when adverse effects were noted. Corresponding SmPC recommendations were implemented.

Efficacy data and additional analyses

Regarding efficacy results of the pivotal study, the primary and key secondary endpoints (RBC-TI ≥ 8 weeks and ≥12 weeks during Weeks 1 through 24 and Weeks 1 through 48) were met, with statistically significantly higher response rates observed in the luspatercept treatment group compared with placebo.

To substantiate efficacy across the whole range of baseline transfusion burden (LTB defined as 2-3 units/8 weeks; HTB defined as ≥4 units/8 weeks), the applicant analysed the primary endpoint (for weeks 1-24) separately for the TB subgroups, for luspatercept and placebo, respectively. All analyses favoured luspatercept treatment (LTB: responder rates of 80.4 vs. 40%; HTB: responder rates of 19.6% and 3.6%, respectively, for luspatercept and placebo).

The following secondary outcomes support the primary endpoint and inform about long-term efficacy (bearing in mind above noted limitations as regards robustness):

'Change in RBC Units Transfused compared to baseline' was analysed for a fixed 16-Week period (weeks 9 through 24 and weeks 33 through 48): Median change from baseline was -4.0 and 0 units (week 9-24) and -5.0 vs. -2.5 units (weeks 33-48) for luspatercept- and placebo-treated patients, respectively.

Re-analysis of the transfusion data to estimate the ratio in transfusion rates between treatment arms based on a generalised linear model for count data (i.e. negative binomial regression) was presented. Model fit was adjusted for baseline transfusion burden, IPSS-R, and baseline transfusion frequency. This is considered adequate. Results are in support of a reduction of transfusion frequency. Estimates for the rate ratio (i.e. the number of transfusion events with luspatercept treatment divided by the number of transfusion events with placebo during Weeks 1 to 24 as well as weeks 25 to 48) range from 0.58 to 0.80 (95% CI) (week 1 to 24) as well as 0.60 to 0.86 (95%CI) (week 25 to 48) in favour of luspatercept. Since a large proportion of patients, in particular in the placebo arm, discontinued treatment after week 24, the results from the second period need to be cautiously interpreted.

Results from the analysis of the mean difference in transfusion units using an ANCOVA model are quite similar to those presented for the number of transfusion events. These indicate a reduction of the transfused RBC units in the luspatercept group (compared to placebo) by about 3.6 to 7.1 units (95% CI) during Weeks 1 to 24. Considering that the estimated number of RBC units transfused in the placebo arm during this period is about 18, this equates to a rate ratio roughly between 60% to 80%.

When analysed by baseline burden (<6 and ≥ 6 units/ 8 weeks) or IPSS risk category at baseline (very low/low and intermediate), consistent results were observed, with a slightly lower effect difference to placebo in the higher transfusion burden group.

Mean Hb values over time starting from baseline through week 48 showed that active-treated patients achieved sustained higher Hb values compared to placebo, also when analysed by transfusion burden. The mean increase in Hb over time appears a more informative, thus relevant endpoint as compared to the responder analysis. However, all Hb analyses are hampered by a large number of missing values. Further, the suitability of the 14/3 rule that was applied to exclude an influence of transfusions on Hb was questioned and sensitivity analyses excluding all Hb values within 14 days of a transfusion were requested. These results are consistent with the previous analyses. However, the Applicant also states that even the more stringent 14-day rule would be insufficient to exclude an influence of transfusion on Hb. Therefore, respective analyses need to be interpreted with caution and luspatercept´s effect on haemoglobin increase cannot be reliably quantified.

To investigate regional differences in transfusion policy, the proportions of patients having received a transfusion at an hb level of more than 9 g/dL were evaluated. Overall, more patients in the luspatercept than in the placebo arm received at least one transfusion with a pre-transfusion Hb level of more than 9 g/dL (33.3 vs. 23.7%, respectively).

A discussion was requested on responders with RBC-TI of at least 8 weeks who had Hb values below 8 g/dL and for whom RBC-TI could theoretically be questioned as coming at the expense of a decrease in Hb rather than true transfusion independence. Reassuringly, in the luspatercept group, there was no haemoglobin value below the pretransfusion haemoglobin threshold that did not trigger a transfusion, and all subjects had an effective increment in haemoglobin during the RBC-TI period. The Applicant's conclusion that all luspatercept-treated subjects with a Hb value of <8 g/dL can be considered true RBC-TI responders is supported.

With regards to the endpoint 'modified haematologic improvement – erythroid' (mHI-E) (based on 2006 IWG response criteria), a favourable effect of Luspatercept was evident in LTB patients (mean Hb increase of at least 1.5 g/dL in the absence of transfusions) and in HTB patients (a reduction of at least 4 units/8 weeks). The additional analyses using the proposed IWG 2018 hematological response criteria (Platzecker et al., 2019) are supportive of a favourable effect in LTB and HTB patients in the luspatercept compared to the placebo arm.

During the whole treatment period, more than one single response period has been observed in a proportion of responders. 18.9% of luspatercept responders were reported to be transfusion-independent starting from the first dose for at least 48 weeks.

At the latest cut-off date (07 January 2019), 31.4% (48/153) of the initially luspatercept treated patients were still on treatment (22 subjects discontinued since the last cut off), while none of the placebo patients remained on treatment.

Several forest plots of subgroup analyses for the primary and main secondary endpoints mostly show consistent effects with the overall treatment effect.

Regarding analyses on the mean change in daily dose of iron chelation therapy (ICT) and the change in serum ferritin, which were complemented by additional analyses with imputation of missing data by baseline, showed a favourable treatment effect of luspatercept compared to placebo for serum ferritin despite balanced use of ICT in both treatment groups.

Special populations

No studies in patients with renal or hepatic impairment, in elderly or paediatric patients (not included in the label) were conducted in the MDS indication.

The MDS population is an elderly one and the study population was externally valid in this regard (median age of 71 years, ranging from 40 to 95 years). Starting dose adjustments based on age are not supported by PK, efficacy, safety, and exposure/response data.

As regards patients with renal impairment, no clinically significant differences in Cmax.ss and AUCss were found across renal function groups. No starting dose adjustment is recommended for patients with mild to moderate renal impairment (eGFR <90 – 30 mL/min/1.73 m2). Subgroup analyses results from phase 3 data for mild/moderate renal impairment are consistent with the overall treatment effect. No dose recommendation can however be made for patients with eGFR < 30 mL/min/1.73 m2 due to the lack of clinical data.

No clinically significant differences in Cmax.ss and AUCss were found across hepatic function groups defined by NCI-ODWG criteria (normal, mild hepatic impairment, and moderate/severe hepatic impairment). No starting dose adjustment is recommended for these patients. No dose recommendation can however be made for patients with severe hepatic disease (ALT or AST ≥ 3 ULN) due to insufficient clinical data.

2.5.4. Conclusions on the clinical efficacy

In conclusion, the primary endpoint results favour luspatercept and results for the secondary endpoints as well as the post hoc analyses are all in support of the primary endpoint analysis. The clinical data supports the granting of the Marketing Authorisation of Reblozyl in the following indication: "Reblozyl is indicated for the treatment of adult patients with transfusion-dependent anaemia due to very low, low and intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts, who had an unsatisfactory response to or are ineligible for erythropoietin-based therapy (see section 5.1)."

2.6. Clinical efficacy beta-thalassemia

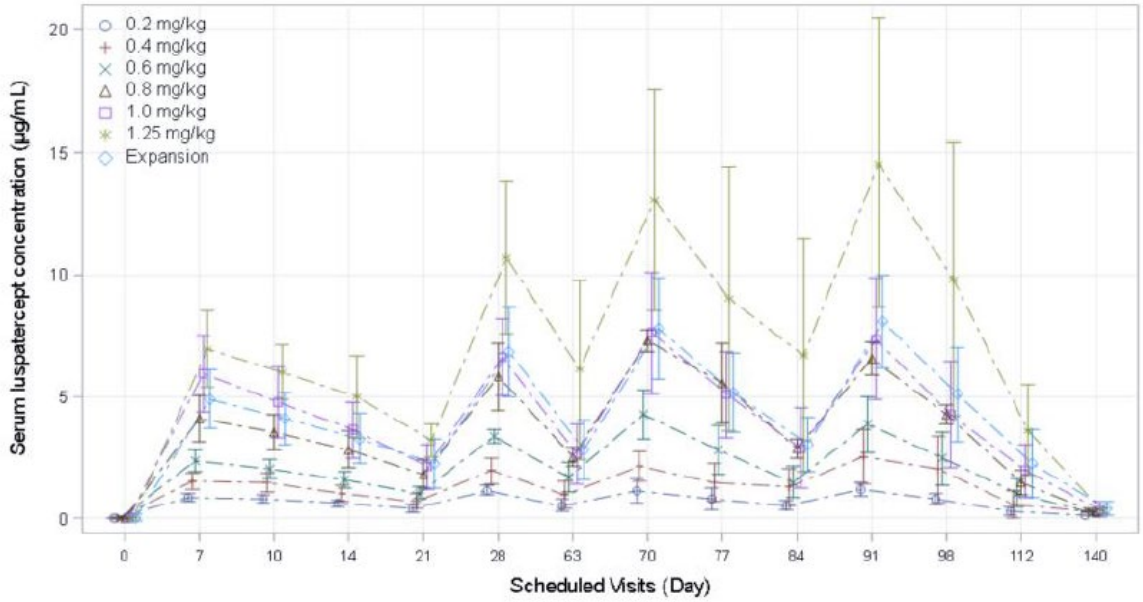
Dose response studies

Dose recommendations for Study ACE-536-B-THAL-001 were derived from phase I and II studies (dose escalating study A536-04 and it's extension study A536-06) and are based on the dose-exposure-response relationship as well as safety and efficacy considerations, from observed and modelled data (see also pharmacology assessment).

Dose-Exposure-Response Relationship

Dose-Exposure

Figure 11: Mean (SD) serum concentration of luspatercept by visit after subcutaneous administration of multiple doses in subjects with beta-thalassemia (PK population)



Exposure-Response

The exposure-dependence of erythroid response over a broader dose range was assessed. The exposure endpoint was the steady-state luspatercept AUC for the starting dose. The erythroid response endpoints included Hgb in non-transfusion dependent ß-thalassemia patients and RBC transfusion burden reduction (units) in transfusion-dependent patients.

Table 34: Hemoglobin increase ≥1.5 g/dL for ≥ 14 days (ITT population, non-transfusion dependent (NTD))

Parameter[a]	0.2 mg/kg (N = 6)	0.4 mg/kg (N = 6)	0.6 mg/kg (N = 5)	0.8 mg/kg (N = 3)	1.0 mg/kg (N = 2)	1.25 mg/kg (N = 1)	Expansion Cohort 0.8 to 1.25 mg/kg (N = 10)	Total (N = 33)
Hemoglobin Increase ≥ 1.5 g/dL for ≥ 14 days								
n (%)	0	0	0	2 (66.7)	1 (50.0)	0	6 (60.0)	9 (27.3)
95% CI	(0.0, 45.9)	(0.0, 45.9)	(0.0, 52.2)	(9.4, 99.2)	(1.3, 98.7)	(0.0, 97.5)	(26.2, 87.8)	(13.3, 45.5)

CI = confidence interval; ITT = intent-to-treat; N = number of subjects per treatment group or overall; n = number of subjects

[a] 95% CI is the exact 95% confidence interval based in the binomial distribution.

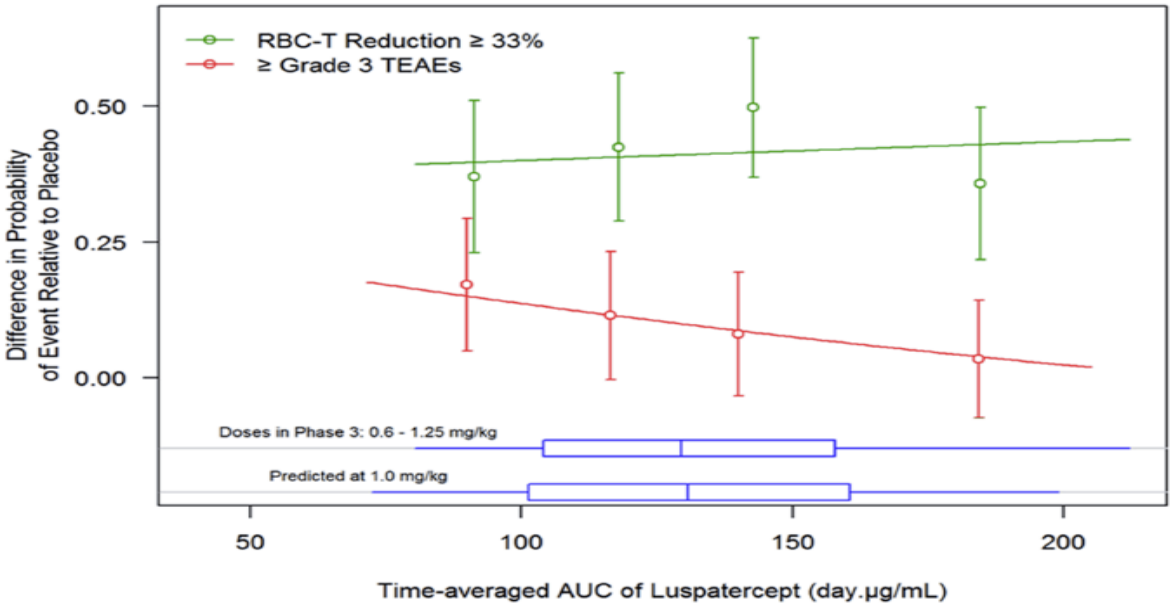
Table 35: : RBC transfusion burden reduction ≥ 20% during rolling 12 weeks (ITT population, transfusion dependent)

Parameter[a]	0.2 mg/kg (N = 0)	0.4 mg/kg (N = 0)	0.6 mg/kg (N = 1)	0.8 mg/kg (N = 3)	1.0 mg/kg (N = 4)	1.25 mg/kg (N = 4)	Expansion Cohort 0.8 to 1.25 mg/kg (N = 19)	Total (N = 31)
RBC Transfusion Burden Reduction ≥ 20% During Rolling 12 Weeks								
n (%)	0	0	1 (100)	2 (66.7)	4 (100)	3 (75.0)	15 (78.9)	25 (80.6)
95% CI			(2.5, 100)	(9.4, 99.2)	(39.8, 100)	(19.4, 99.4)	(54.4, 93.9)	(62.5, 92.5)

CI = confidence interval; ITT = intent-to-treat; N = number of subjects per treatment group or overall; n = number of subjects; RBC = red blood cell

[a] CI based on the binomial distribution

Figure 12: observed and predicted therapeutic margin of luspatercept under titration dosing regimen in subjects with beta-thalassemia



AE = adverse event; AUC = area under the plasma concentration-time curve; AUC_{ave} = average AUC; AUC_{ss} = AUC at steady-state; CI = confidence interval; E-R = exposure-response; RBC-T = red blood cell transfusion; RBC-T reduction ≥ 33% = ≥ 33% reduction in RBC transfusion burden with a reduction of at least 2 units during any consecutive 12-week interval; TEAEs = treatment emergent adverse events.
Note: The symbols and error bars represent the estimated difference in proportion relative to placebo (90% CI) of subjects who experienced the event, grouped by quartiles of the time-averaged AUC of luspatercept in serum and plotted at the median for each AUC quartile group (AUC_{avg48} is used for efficacy and AUC_{ave} to the first AE is used for safety). The lines represent the predicted placebo-adjusted probabilities from the final E-R models; where the models include categorical covariates, the prediction is taken as the weighted average of the predictions for each combination of the categorical covariates, weighted by the relative frequency of each combination in the study population; where the models include continuous covariates, the prediction is taken at the median of the covariates. The horizontal box shows the distribution of the observed AUC_{avg48} in Study ACE-536-B-THAL-001 (Phase 3) or predicted AUC_{ss} at 1.0 mg/kg in A536-04 and ACE-536-B-THAL-001 studies. The interior bar represents the median, the two ends of the box represent the 25th and 75th percentiles, and the whiskers represent the 5th and 95th percentiles.

Dosing Approach

The recommended dosing approach is body weight-based dosing. This approach was used in the early stage of Phase 2 studies and showed erythroid response in the primary treatment phase of Phase 3 studies. The dosing approach was also supported by PK and E-R data.

Body weight is a statistically significant covariate of luspatercept apparent clearance (CL/F) and volume of distribution in population PK analysis for subjects with β-thalassemia. Model-based simulations predicted that the weight-based dosing would perform better than the fixed dosing by limiting the exposure difference between light/heavy subjects and normal weight subjects to within 10% instead of approximately 25% predicted for the fixed dose, with low risk of overexposing or under-exposing subjects with extreme body weight. Further, in the multivariate exposure-safety analysis for β-thalassemia, the proportion of subjects experiencing $\geq$ Grade 3 treatment-emergent adverse events (TEAEs) did not increase with increasing luspatercept serum exposure or body weight.

Dosing Schedule

The recommended dosing schedule, which was used in all Phase 2 and Phase 3 studies, is once every 3 weeks. This schedule is supported by the observed time profile of Luspatercept serum concentrations, Hgb response, and RBC transfusion burden reduction in clinical studies.

Luspatercept is slowly absorbed and eliminated. In a Phase 1 study with healthy volunteers (A536-02), the time to reach the maximum concentration in serum was approximately 1 week and the terminal half-time in serum was approximately 2 weeks (Report A536-02). Thus, a once every 3 weeks dosing schedule was expected to maintain approximately 50% of peak concentration at the end of a dosing interval, and was hence used for subsequent clinical studies.

In the clinical studies, different treatment intervals were possible as dose delays based on clinical parameters were possible.

Starting Dose and Dose Titration

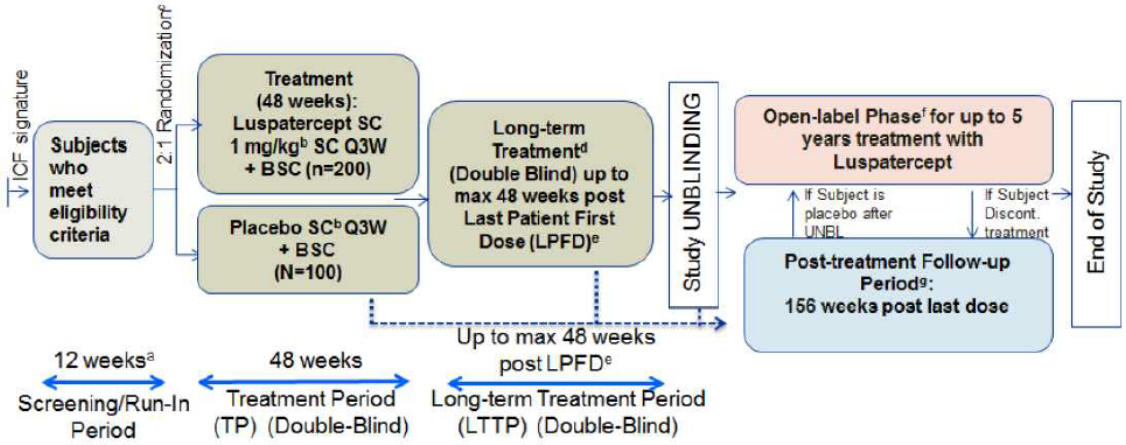
The recommended starting dose is 1 mg/kg, SC, Q3W. If a patient does not achieve a reduction in RBC transfusion after at least 2 consecutive doses (6 weeks) at the 1.0 mg/kg starting dose, it is recommended to increase the luspatercept dose to 1.25 mg/kg; down-titration is based on safety and elevated Hb levels. A titration-to-response regimen was used in the expansion/extension stages of Phase 2 studies as well as in the Phase 3 study.

In the phase III trial, in principle, 5 different dose levels were used; the starting dose (1 mg/kg), down-titration (to 0.8, 0.6 or 0.45mg/kg) due to AEs or excessive Hgb increase and up-titration to 1.25mg/kg.

Main study

ACE-536-B-THAL-001

Figure 13: Overall study design pivotal trial ACE-536-B-THAL-001



BSC = best supportive care; DMC = data monitoring committee; ICF = informed consent form; LPFD = last patient first dose; LTTP = Long-term Treatment Period; Q3W = every 3 weeks; SC = subcutaneous; TP = Treatment Period; UNBL = unblinding.

[a] The historical documentation of transfusion dependence for β-thalassemia subjects (including units transfused and hemoglobin levels measured prior to each transfusion) for 24 weeks prior to subject randomization was to be made available.

[b] Dose may have been titrated up to a maximum of 1.25 mg/kg.

[c] Randomization was 2:1, luspatercept + BSC versus placebo + BSC.

[d] All subjects who completed 48 weeks of the double-blind TP of this study had the opportunity to continue into a double-blind LTTP at the investigator's discretion. Subjects who did not enroll in the double-blind LTTP or who discontinued early were to proceed to the Posttreatment Follow-up Period.

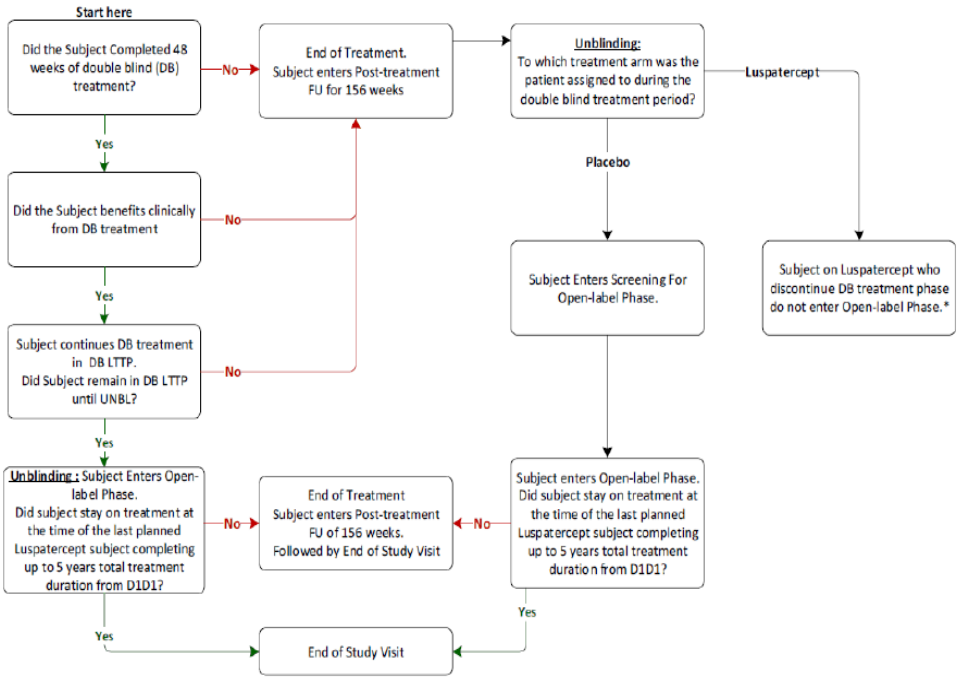
[e] Maximum duration of 48 weeks post LPFD, or when all subjects completed 48 weeks of the double-blind treatment or discontinued before reaching 48 weeks of the double-blind treatment, or in the event that the study was unblinded per DMC recommendation.

[f] Open-label Phase: Subjects who were compliant with the study protocol 48 weeks post Dose 1 Day 1 can enter in the Open-label Phase, unless medically contraindicated and as described in Section 9.1.4.

[g] Early discontinued subjects, ie, subjects who discontinued before completing the double-blind TP (48 weeks) were to be continued to be monitored at Week 9, followed by Weeks 24, 48, 72, 120, and 144 after the last dose up to Week 156, ie, 3 years (Section 9.1.5).

Source: Study protocol (Appendix 16.1.1).

Figure 14: Subject management decision tree:



D1D1 = Dose 1 Day 1; DB = double blind; FU = follow-up; LTTP = Long-term Treatment Period; UNBL = unblinding.
* Refer also to Section 9.1.4.

Methods

Study Participants

Inclusion criteria (excerpt, see AR and study report for more details)

1. Male or female, ≥ 18 years of age at the time of signing the ICF

4. Documented diagnosis of β-thalassemia or HbE/β-thalassemia (β-thalassemia with mutation and/or multiplication of α-globin was allowed)

5. Regularly transfused, defined as 6 to 20 RBC units* in the 24 weeks prior to randomization and no transfusion-free period for > 35 days during that period

* Sites that prescribed transfusions and had the transfusion records only in volumes should have used for conversion of volume to units per the below criteria, in order to obtain number of units within the last 24 weeks to assess the eligibility: 1 unit in the protocol referred to a quantity of packed RBCs of approximately 200 to 350 mL. (1) sites that used transfusion bags within this range or ≥ 350 mL, the conversion in units was to be done by dividing the volume transfused to the subject by 350 mL; (2) sites that used transfusion bags < 200 mL, the conversion in units was to be done by dividing the volume transfused to the subject by 200 mL

6. Performance status: Eastern Cooperative Oncology Group (ECOG) score of 0 or 1

Exclusion Criteria (excerpt, see AR and study report for more details)

4. A diagnosis of HbS/β-thalassemia or α-thalassemia (eg, HbH)

5. Evidence of active hepatitis C virus (HCV), hepatitis B virus (HBV) or known positive human immunodeficiency virus (HIV)

7. Use of chronic anticoagulant therapy was excluded, unless the treatment stopped at least 28 days prior to randomization.

8. Platelet count > 1000 x 109/L

10. Treatment with another investigational drug or device ≤ 28 days prior to randomization

11. Prior exposure to sotatercept (ACE-011) or luspatercept (ACE-536)

12. Used an ESA (erythropoiesis-stimulating agent) ≤ 24 weeks prior to randomization

13. Iron chelation therapy, if initiated ≤ 24 weeks prior to randomization (allowed if initiated > 24 weeks before or during treatment)

14. Hydroxyurea treatment ≤ 24 weeks prior to randomization

17. Major organ damage, including liver-, heart- or lung disease or creatinine clearance < 60 mL/min

18. Proteinuria ≥ Grade 3 according to NCI CTCAE version 4.0

21. History of severe allergic or anaphylactic reactions or hypersensitivity to recombinant proteins or excipients in investigational product (IP)

22. Cytotoxic agents, immunosuppressants ≤ 28 days prior to randomization (ie, anti-thymocyte globulin or cyclosporine)

23. History of malignancy, except for curatively resected non-melanomatous skin cancer, curatively treated cervical carcinoma in situ or other solid tumor with no known active disease in the opinion of the investigator

Prior and Concomitant Therapy

During screening and throughout the study, subjects were permitted to take stable doses of medications for chronic conditions that were not specifically excluded by the protocol. Prior/concomitant medications were collected beginning at the Screening/Run-in Period and included all medications taken within 12 weeks prior to Dose 1 Day 1.

All concomitant treatments, used from 12 weeks prior to first dose of the study drug until 9 weeks post last dose, were to be reported in the eCRF. All prior and concomitant blood and blood products received, and ICTs used from 24 weeks prior to the first dose of study drug until 9 weeks post last dose were to be reported in the eCRF.

Treatments

Luspatercept or placebo was administered as an SC injection to subjects by the study staff at the clinical site, and administration was documented in the subject's source record. Subcutaneous injections were given in the upper arm, thigh, and/or abdomen.

Subjects were assigned to treatment as per one of the following regimens:

- Luspatercept starting dose level 1.0 mg/kg SC once q3w
- Placebo SC once q3w

The study drug was to be administered according to the following criteria:

- Pretreatment/pretransfusion Hb value was to be < 11.5 g/dL and increase of Hb was to be ≤ 2.0 g/dL compared with the predose Hb on Day 1 of the previous treatment dose cycle;
- Any related AEs must have been < Grade 2 according to NCI CTCAE criteria (Appendix C of the study protocol [Appendix 16.1.1]); and
- White blood cell (WBC) count < 3 x baseline. Corrected WBC values were to be used to establish the baseline WBC. Baseline was equal to the highest WBC value between screening WBC and Dose 1 Day 1.

Subjects must have had Hb assessed, and results had to be available prior to each study drug administration. Haemoglobin not influenced by a transfusion was to be considered for dosing, delays, and reduction actions related to luspatercept. Haemoglobin not influenced by a transfusion was initially considered a valid Hb measurement 14 days post-transfusion.

In addition to the above treatments, all subjects received BSC (RBC transfusions; ICTs; antibiotic, antiviral, and antifungal therapy; and/or nutritional support as needed) at the investigator's discretion.

Identity of Investigational Products

Luspatercept was provided by the sponsor. Luspatercept for injection was formulated as a sterile, preservative-free, lyophilised cake/powder, available in 2 strengths. The drug product was packaged in a 3-mL glass vial at the following strengths; a 25 mg/vial (containing 37.5 mg of luspatercept protein) and a 75 mg/vial (contained 87.5 mg of luspatercept protein).

Placebo used in the study was sterile normal saline (0.9% sodium chloride for injection) administered as an SC injection. Sterile, normal saline was prepared in syringes by the investigational site's designated individuals to match the active syringe. The investigator and subject were blinded to treatment assignment.

Selection of Doses in the Study

The dose up-titration criteria were defined as follows:

- Transfusion reduction over at least 2 dose cycles (approximately 6 weeks) was < 33%, compared with the transfusion burden (units/week) at baseline; or
- Transfusion reduction over at least 2 dose cycles (approximately 6 weeks) was ≥ 33%, but ≤ 50% compared with baseline, at the discretion of the investigator. After safety and efficacy data review, the sponsor may allow dose titration following special requests, such as, but not limited to, subjects whose dose had been reduced once and whose response to treatment had been lost.

Table 36: Starting dose level with dose reductions and dose titration

Third Dose Reduction (~ 25%)	Second Dose Reduction (~ 25%)	First Dose Reduction (~ 25%)	Starting Dose Level	First Dose Titration
0.45 mg/kg	0.6 mg/kg	0.8 mg/kg	1.0 mg/kg	1.25 mg/kg

Dose Delay and Dose Reduction

Dose delay of luspatercept from the planned dosing schedule was allowed due to increased Hb or AEs and per the guidelines for dose modifications and dose delay. Dose reduction may have been required based on the change in mean Hb level (Hb not influenced by a transfusion) with respect to the last dose, as well as related Grade ≥3 AEs. If a subject experienced a dose delay due to Hb increase, Hb measurement was to be performed every week. If the dose delay was 15 weeks or longer from the previous dose administered, including cases of elective surgery/hospitalization, the treatment was to be discontinued.

Table 37: dose delay, dose reduction and treatment discontinuation guidelines

Event at the Day of Dosing	Action
Any related AE = Grade 2[a]	Dose was to be delayed[b] until resolved to ≤ Grade 1 or baseline
Any related AE ≥ Grade 3[a]	Dose was to be delayed[b] until resolved to ≤ Grade 1 or baseline, and then dose reduced by 25%[c]
> 2 dose reductions due to related AE[a]	Treatment was to be discontinued
Increase in Hb > 2.0 g/dL compared with predose Hb of Day 1 of the previous treatment dose cycle[d]	Dose was to be reduced by 25%[c] if ΔHb was not influenced by RBC transfusions
Hb ≥ 11.5 g/dL[d]	Dose was to be delayed until Hb was ≤ 11.0 g/dL
WBC	
WBC count[e] ≥ 2 x baseline in the absence of an associated condition (eg, infection or concomitant corticosteroid use)	Treatment was to be continued and WBC count was to be repeated within 1 week • If repeat WBC count remained ≥ 2 x above baseline, investigator was to assess the cause of increase to exclude hematologic malignancy as per standard clinical practice • If hematologic malignancy was confirmed, treatment was to be discontinued[f]
WBC count[e] ≥ 3 x baseline	Dose was to be delayed[b] with weekly WBC monitoring until WBC count was < 3 x baseline • Investigator was to assess the cause of increase per standard clinical practice to exclude hematologic malignancy • If hematologic malignancy was confirmed, treatment was to be discontinued[f]
Grade 3 leukocytosis[g]	Treatment was to be discontinued

ΔHb = changes in Hb; AE = adverse event; Hb = hemoglobin; RBC = red blood cell; WBC = white blood cell.
[a] Possibly, probably, or definitely related.
[b] If dose delay was 15 weeks or longer from the prior dose administration, treatment was to be discontinued.
[c] See Table 3 for dose reductions.
[d] Based on the pretreatment Hb value not influenced by transfusion (ie, ≥ 14 days posttransfusion); Hb was to be rechecked weekly during dose delay.
[e] Central laboratory corrected WBC values were to be used for confirming the investigator decision, which could have been based on the local laboratory result. The baseline WBC was defined as the highest WBC value between screening WBC and study Dose 1 Day 1.
[f] For a full list of events triggering discontinuation, refer to Section 11 of the protocol (Appendix 16.1.1).
[g] Grade 3 leukocytosis (as per Common Terminology Criteria for Adverse Events, ie, WBC above 100,000) was to be based on central laboratory values.

Objectives

Primary Objective

- The primary objective of the study was to determine the proportion of subjects treated with luspatercept + best supportive care (BSC) versus placebo + BSC who achieved erythroid response, defined as ≥ 33% reduction from baseline in transfusion burden (units RBCs/time)with a reduction of at least 2 units, from Week 13 to Week 24.

Secondary Objectives

- To evaluate the proportion of subjects who achieved ≥ 33% reduction from baseline in transfusion burden from Week 37 to Week 48 versus placebo
- To evaluate the proportion of subjects who achieved ≥ 50% reduction from baseline in transfusion burden from Week 13 to Week 24 versus placebo
- To evaluate the proportion of subjects who achieved ≥ 50% reduction from baseline in transfusion burden from Week 37 to Week 48 versus placebo
- To evaluate the mean change from baseline in transfusion burden from Week 13 to Week 24
- To evaluate the mean change from baseline in LIC (liver iron concentration) versus placebo
- To evaluate the mean change from baseline in mean daily dose of ICT (iron chelation therapy) used versus placebo
- To evaluate the mean change from baseline in serum ferritin versus placebo
- To evaluate the effect of luspatercept on osteoporosis/osteopenia, total hip, and lumbar spine measured by bone mineral density (BMD) versus placebo

- To evaluate the mean change from baseline in myocardial iron versus placebo
- To evaluate the mean change from baseline in QoL assessments, such as Transfusion-dependent QoL questionnaire (TranQoL) and 36-item Short Form Health Survey (SF-36) versus placebo
- To evaluate the effect of luspatercept on HRU versus placebo
- To evaluate the proportion of subjects who were transfusion independent for ≥ 8 weeks versus placebo
- To evaluate the duration of reduction in transfusion burden or transfusion independence
- To evaluate the time to erythroid response
- To evaluate the postbaseline transfusion events frequency versus placebo
- To evaluate the population PK of luspatercept in subjects with β-thalassemia
- To evaluate the safety and immunogenicity of luspatercept versus placebo

Exploratory Objectives

- To examine the relationship of baseline and change in serum GDF11 and other related biomarkers with response to treatment with luspatercept
- To examine the effect of luspatercept on changes in HbF

Outcomes/endpoints

Table 38: Study endpoints

Endpoint	Name	Description	Time of Endpoint Measurement			Time Frame
			24 Weeks	48 Weeks	Long-term	
Primary Endpoint	Proportion of subjects with hematologic improvement, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24 compared with the 12-week interval prior to randomization for luspatercept + BSC versus placebo + BSC	Number of RBC units transfused from Week 13 to Week 24, and in the 12 weeks prior to randomization	X	-	-	12 weeks prior to randomization; Week 13 to Week 24
Secondary Efficacy Endpoints	Proportion of subjects with hematologic improvement, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48 compared with the 12-week interval prior to randomization for luspatercept + BSC versus placebo + BSC	Number of RBC units transfused from Week 37 to Week 48, and in the 12 weeks prior to randomization	-	X	-	12 weeks prior to randomization; Week 37 to Week 48
	Proportion of subjects with ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24 compared with the 12-week interval prior to randomization for luspatercept + BSC versus placebo + BSC	Number of RBC units transfused from Week 13 to Week 24, and in the 12 weeks prior to randomization	X	-	-	12 weeks prior to randomization; Week 13 to Week 24

	Proportion of subjects with ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48 compared with the 12-week interval prior to randomization for luspatercept + BSC versus placebo + BSC	Number of RBC units transfused from Week 37 to Week 48, and in the 12 weeks prior to randomization	-	X	-	12 weeks prior to randomization; Week 37 to Week 48
	Mean change from baseline in transfusion burden (RBC units) from Week 13 to Week 24	Change from baseline as continuous variable	X	-	-	12 weeks prior to randomization; Week 13 to Week 24

Randomisation and blinding (masking)

Subjects were randomized with a ratio of 2:1 to either treatment with luspatercept or placebo. Randomisation was additionally stratified by geographical region.

All subjects, study site staff, and Celgene representatives with the exception of designated individuals (e.g., the pharmacist at the investigational site) remained blinded to all treatment assignments until all subjects completed 48 weeks of double-blind treatment or discontinued before reaching 48 weeks of double-blind treatment, whichever was earlier, or at the time the study was unblinded (per DMC recommendation) and the database was locked. The designated site individual (e.g., the pharmacist) at the investigational site used a syringe (that exactly matched the syringe used for reconstituted luspatercept) and sterile normal saline (0.9% sodium chloride for injection) to prepare a matching placebo. Thus, the designated site individual at the investigational site was unblinded and gave investigators and their staff luspatercept and placebo in a blinded manner.

Randomization, drug dispensing, dose reduction/titration, and drug discontinuation were accomplished by an IRT system. Authorised site personnel must have contacted the IRT for randomization, study drug assignment at the beginning of each cycle, registration of dose reductions or titrations, and treatment discontinuation. Confirmation of each call was to be sent to the investigational site and Celgene.

The blind was not to be broken during the course of the study unless, in the opinion of the investigator, it was absolutely necessary to safely treat the subject. If it was medically imperative to know what study drug the subject was receiving, the study drug had to be discontinued if, in the opinion of the investigator, continuing to receive study drug could have negatively affected the outcome of the subject's treatment. The decision to break the blind in emergency situations remained the responsibility of the treating physician. Emergency unblinding was only to be performed by the investigator through the IRT by using an emergency unblinding personal ID number, and the investigator had to call the IRT for unblinded dose information.

Statistical methods

Analysis Populations

The following analysis populations were planned for this study:

- Intent-to-treat population: The intent-to-treat (ITT) population consisted of all subjects, regardless of whether or not the subject received the study drug. All efficacy analyses were conducted for the ITT population and were analysed based on randomization group.
- Safety population: The safety population consisted of all subjects who were randomized and received at least 1 dose of the study drug. Subjects were included in the treatment group corresponding to the study drug they actually received.
- Pharmacokinetic population: The PK population consisted of all subjects who received at least 1 dose of luspatercept and had measurable luspatercept serum concentrations.

- Health-related QoL evaluable population: The HRQoL evaluable population consisted all subjects in the ITT population who completed the HRQoL assessment at screening and at least 1 post Baseline/Screening Visit. The completion of an HRQoL assessment was defined for each health-related QoL measure.
- TranQoL: Completion at a given visit was defined as ≥ 75% of all items being answered (ie, ≥ 27 items of the 36 items or a nonmissing total score).
- SF-36: Completion at a given visit was defined as ≥ 50% of all items being answered (ie, ≥ 18 items of the 36 items).

Analysis of Primary Efficacy Endpoint

The efficacy analysis was performed on the ITT population. The primary efficacy analysis was performed based on 24 weeks of data after all subjects completed the double-blind 24-week Treatment Period or discontinued before reaching 24 weeks of double-blind treatment (for this study, 1 month was considered equal to 4 weeks). A higher response rate in the luspatercept over placebo and a 2-sided p-value < 0.05 was required to confirm the superiority of luspatercept in the efficacy.

The primary efficacy endpoint of this study was defined as subjects with ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24 compared with the 12-week interval on or prior to Dose 1 Day 1 for luspatercept + BSC versus placebo + BSC. For the early discontinued subjects, i.e., who did not complete 24 weeks of double-blind Treatment Period, the transfusion records were collected up to 48 weeks. All the transfusion records up to the last dose + 20 days were used to evaluate primary and secondary efficacy endpoints.

The first day to be used for efficacy analysis was defined as the date of the first dose. A transfusion received on Dose 1 Day 1 was counted in the baseline transfusion burden. The primary endpoint response rate was calculated using the number of responders divided by all subjects in the ITT population. The response rates of the subjects who were randomized to luspatercept and placebo were calculated.

The following statistical hypothesis was tested:

- H0: P1 (response rate in the luspatercept group) = P2 (response rate in the placebo group)
- Ha: P1≠P2

The number and percentage of subjects in the ITT population who achieved the response were calculated for luspatercept and placebo. The difference in proportions between luspatercept and placebo was calculated using the Cochran-Mantel-Haenszel test stratified by the geographical regions defined at randomization as a stratification factor. The odds ratio (OR; luspatercept versus placebo) with corresponding 2-sided (at 0.05 alpha level) 95% confidence intervals and p-value were provided. A higher response rate in the luspatercept + BSC treatment group over the placebo + BSC treatment group with a p-value < 0.05 inferred that luspatercept was significantly superior to placebo.

Analysis of Key Secondary Efficacy Endpoints

The analyses of key secondary efficacy endpoints were performed on the ITT population. The results were presented by treatment groups. The statistical tests were conducted to compare the treatment groups.

The key secondary efficacy endpoints were measured at 24 weeks or 48 weeks from randomization and were statistically tested in a sequential order at the α = 0.05 level.

1. Proportion of subjects with hematologic improvement, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units, from Week 37 to Week 48. Subjects were analysed as described in Section 9.7.1.4.1 for the primary endpoint.

2. Proportion of subjects with hematologic improvement, defined as ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units, from Week 13 to Week 24. Subjects were analysed as described in Section 9.7.1.4.1 for the primary endpoint.

3. Proportion of subjects with hematologic improvement, defined as ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units, from Week 37 to Week 48. Subjects were analysed as described in Section 9.7.1.4.1 for the primary endpoint.

4. Mean change in transfusion burden (RBC units/12 weeks) from Week 13 to Week 24. The mean change in transfusion burden (RBC units) from baseline was analysed using analysis of covariance (ANCOVA) method with stratification factor and baseline transfusion burden as covariates from Week 13 to Week 24. Corresponding 95% Cis associated with the test were provided.

The totality of RBC transfusion burden reduction was evaluated using 24 weeks baseline (sum of 12-week historic data and 12-week run-in data). Baseline of 48 weeks of transfusion burden was calculated based on 2 times 24 weeks transfusion burden baseline data. Descriptive statistics (n, mean, median, standard deviation [SD], and range) for totality of RBC transfusion burden reduction, along with the change from baseline, were summarized for each treatment group by A waterfall plot was provided for the 24-week baseline and postbaseline RBC transfusion burden by treatment group and time point. Each individual subject's RBC transfusion burden was displayed in a single bar.

Multiplicity

After the result from the primary efficacy analysis in the ITT population showed statistical significance, the key secondary efficacy Endpoint 1 was tested next. The key secondary efficacy Endpoint 2 was tested only if the test results for both the primary efficacy endpoint and the key secondary efficacy Endpoint 1 were significant. The key secondary efficacy Endpoint 3 was tested only if the test results for the primary efficacy endpoint and the key secondary efficacy Endpoints 1 and 2 were all significant. The testing procedure above was implemented strictly in order to control the family-wise error rate for primary and key secondary efficacy endpoints at a level of 0.05.

Missing Data Imputation

In case of any missing data for RBC transfusion units records and MRI liver iron content, imputation will be applied for each section.

The imputation for RBC transfusion units was: if at the time of data summary, a subject's efficacy cut-off date is before the end of the 12-week interval or a subject has any invalid transfusion records (i.e., transfusion unit not available) during the specified 12-week interval, this subject will be included in the analysis as a non-responder.

The imputation logic for missing LIC value is stated in section 10.4.1: the value of LIC will be either the value collected from eCRF or the value derived from T2*, R2* or R2 parameters depending on which techniques and software were used for MRI LIC data acquisition.

Results

Figure 15: Participant flow

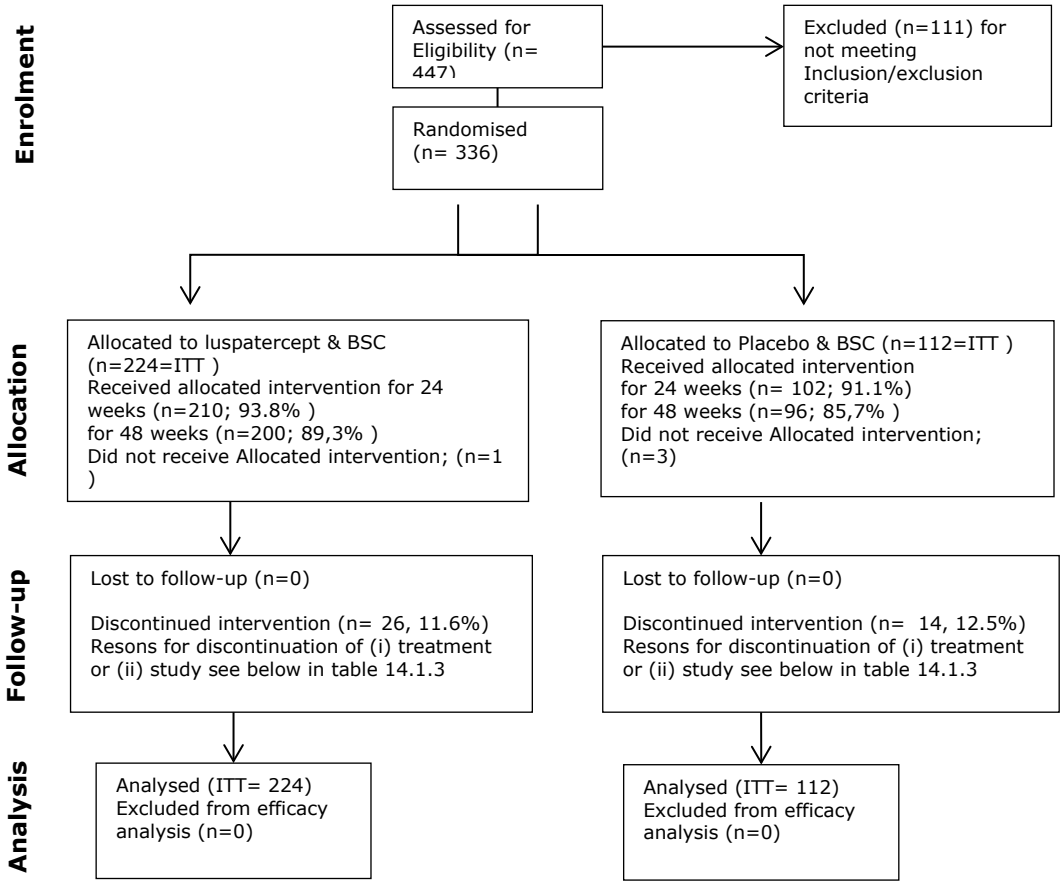


Table 39

Celgene Corporation
Study ACE-536-B-THAL-001

Database Cutoff Date: 11MAY2018

Table 14.1.3
Subject Disposition
ITT Population

	Luspatercept + BSC (N=224) n (%)	Placebo + BSC (N=112) n (%)	Total (N=336) n (%)
Subject Randomized	224 (100.0)	112 (100.0)	336 (100.0)
Subject Received Treatment	223 (99.6)	109 (97.3)	332 (98.8)
Treatment Discontinued	42 (18.8)	24 (21.4)	66 (19.6)
Treatment Ongoing	181 (80.8)	85 (75.9)	266 (79.2)
Completed 24 Weeks of Treatment	210 (93.8)	102 (91.1)	312 (92.9)
Completed 48 Weeks of Treatment	200 (89.3)	96 (85.7)	296 (88.1)
Subject Discontinued Study	26 (11.6)	14 (12.5)	40 (11.9)

Reason for Treatment Discontinuation			
Death	0	0	0
Adverse Event/Adverse Event: Other	10 (4.5)	1 (0.9)	11 (3.3)
Pregnancy	0	0	0
Progressive Disease	0	0	0
Lack of Efficacy	2 (0.9)	8 (7.1)	10 (3.0)
Recovery	0	0	0
Withdrawal by Subject	26 (11.6)	12 (10.7)	38 (11.3)
Non-Compliance with Study Drug	0	0	0
Lost to Follow Up	0	0	0
Study Terminated by Sponsor	0	0	0
Transition to Commercially Available Treatment	0	0	0
Physician Decision	0	0	0
Disease Relapse	0	0	0
Symptomatic Deterioration	0	0	0
Protocol Violation	1 (0.4)	0	1 (0.3)
Adverse Event: Leukocytosis Grade 3	0	0	0
Adverse Event: Hematological Malignancy	0	0	0
Other	3 (1.3)	3 (2.7)	6 (1.8)
Reason for Study Discontinuation			
Death	1 (0.4)	1 (0.9)	2 (0.6)
Adverse Event	4 (1.8)	0	4 (1.2)
Pregnancy	0	0	0
Lack of Efficacy	0	0	0
Recovery	0	0	0
Withdrawal by Subject	13 (5.8)	6 (5.4)	19 (5.7)
Non-Compliance with Study Drug	0	0	0
Lost to Follow Up	0	0	0
Study Terminated by Sponsor	0	0	0
Transition to Commercially Available Treatment	0	0	0
Physician Decision	0	0	0
Disease Relapse	0	0	0
Symptomatic Deterioration	0	0	0
Protocol Violation	0	0	0
Other	2 (0.9)	1 (0.9)	3 (0.9)

Subject discontinued study includes 12 subjects who completed study as per original protocol version 25Aug2015.
\Celgene CGNACE536BTHAL1\Production\TLF\tl40103.sas Extraction Date: 21JUN2018 Run Date: 30NOV2018 18:23

Baseline data

The majority of subjects was female (58.0%), white (54.2%), and ≤ 32 years of age (57.1%); the median age was 30.0 years (range: 18 to 66 years). The median baseline body weight and BMI were 56.4 kg (range: 34 to 94 kg) and 21.97 kg/m2 (range: 13.9 to 38.3 kg/m2), respectively, and were generally well balanced between the 2 treatment groups. Based on the prespecified protocol stratification by geographical regions, 44.6% of subjects were randomized at sites from North America and Europe; 23.2% of subjects were randomized at sites from the Middle East and North Africa and 32.1% of subjects were randomized at sites from the Asia-Pacific region.

Baseline disease characteristics

Table 40: Baseline characteristics (ITT population)

Disease Characteristic	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)	Total (N = 336)
β-thalassemia Diagnosis, n (%)			
β-thalassemia	174 (77.7)	83 (74.1)	257 (76.5)
HbE/β-thalassemia	31 (13.8)	21 (18.8)	52 (15.5)
β-thalassemia Combined With α-thalassemia	18 (8.0)	8 (7.1)	26 (7.7)
Missing[a]	1 (0.4)	0	1 (0.3)
Age When Subject Started Regular Transfusions (Years)			
n	169	85	254
Mean (SD)	5.9 (11.02)	5.7 (9.67)	5.8 (10.57)
Median (Min, Max)	2.0 (0, 52)	2.0 (0, 51)	2.0 (0, 52)

Baseline Transfusion Burden			
12-week Run-in Data (RBC Units/12 Weeks) (Week -12 to Day 1)			
n	224	112	336
Mean (SD)	6.86 (1.998)	6.88 (1.829)	6.87 (1.941)
Median (Min, Max)	6.12 (3.0, 14.0)	6.27 (3.0, 12.0)	6.12 (3.0, 14.0)
12-week Historic Data (RBC Units/12 Weeks) (Week -24 to -12)			
n	224	112	336
Mean (SD)	7.66 (2.110)	7.90 (2.222)	7.74 (2.148)
Median (Min, Max)	8.00 (3.0, 12.0)	8.00 (3.0, 14.0)	8.00 (3.0, 14.0)
12-week Run-in Data (RBC Units/12 Weeks) Categories, n (%)			
≤ 6	112 (50.0)	56 (50.0)	168 (50.0)
> 6	112 (50.0)	56 (50.0)	168 (50.0)
12-week Historic Data and 12-week Run-in Data (RBC Units/24 Weeks)			
n	224	112	336
Mean (SD)	14.52 (3.641)	14.79 (3.516)	14.61 (3.596)
Median (Min, Max)	14.00 (6.0, 24.0[b])	15.00 (6.0, 26.0[b])	14.25 (6.0, 26.0[b])

Splenectomy, n (%)			
Yes	129 (57.6)	65 (58.0)	194 (57.7)
No	95 (42.4)	47 (42.0)	142 (42.3)

β-thalassemia Gene Mutation Grouping, n (%)			
β0/β0	68 (30.4)	35 (31.3)	103 (30.7)
Non-β0/β0	155 (69.2)	77 (68.8)	232 (69.0)
Missing[a]	1 (0.4)	0	1 (0.3)

Pretransfusion Hb Threshold[c] (g/dL)			
24-week (g/dL)			
n	224	112	336
Mean (SD)	9.12 (1.106)	9.05 (1.066)	9.09 (1.092)
Median (Min, Max)	9.31 (4.5, 11.4)	9.15 (5.8, 11.7)	9.27 (4.5, 11.7)

MRI (LIC)[e]			
LIC (mg/g dw)			
n	224	112	336
Mean (SD)	12.04 (14.847)	10.09 (11.499)	11.39 (13.835)
Median (Min, Max)	6.14 (0.8, 125.0)	5.05 (0.2, 53.2)	5.69 (0.2, 125.0)

MRI (Myocardial Iron)			
Myocardial T2* (ms)			
n	224	112	336
Mean (SD)	33.52 (16.170)	34.76 (10.665)	33.93 (14.563)
Median (Min, Max)	34.65 (3.0, 205.9)	36.30 (6.4, 57.5)	35.00 (3.0, 205.9)

[a] "Missing" category includes subjects in the population who had no result for the parameter listed.
[b] Transfusions that occurred on Study Day 1 (Dose 1 Day 1) were counted as part of the baseline RBC transfusion burden (refer to the statistical analysis plan, Appendix 16.1.9).
[c] The 24-week pretransfusion Hb threshold was defined as mean of a subject's all documented pretransfusion Hb values during the 24 weeks prior to Dose 1 Day 1. The 12-week pretransfusion Hb threshold was defined as mean of a subject's all documented pretransfusion Hb values during the 12 weeks prior to Dose 1 Day 1.
[d] The ECOG Grade: 0 = fully active, able to carry on all predisease performance without restriction; 1 = restricted in physically strenuous activity but ambulatory, and able to carry out work of a light or sedentary nature, eg, light house work or office work.
[e] The value of LIC was either the value collected from electronic case report form or the value derived from the T2*, R2*, or R2 parameter, depending on which techniques and software were used for MRI LIC acquisition.
[f] If myocardial iron content was missing, it was derived from nonmissing myocardial T2* value: $45/(T2^*)^{1.22}$.
Source: Table 14.1.7.1.

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Table 14.2.16.5
Analysis of Transfusion Window (days) by 12-week Interval and Overall
ITT Population

	Luspatercept + BSC (N=224)	Placebo + BSC (N=112)
Transfusion Window [a] at Baseline [b]		
n	224	112
Mean	22.2	22.9
SD	4.95	4.92
Median	21.3	22.0
Q1, Q3	19.0, 26.0	19.8, 28.0
Min, Max	9, 33	11, 32

Medical History

All subjects had at least one medical history condition or β-thalassemia comorbidity, and the proportion of subjects with specific medical history condition and/or comorbidities was generally similar (difference of $\leq$ 10% of subjects) between the 2 treatment groups. Overall, the medical history conditions or β-thalassemia comorbidities were typical of a population with TD β-thalassemia.

Nearly all patients (>93%) had (severe) iron overload (more than 1000 mcg/L serum ferritin or LIC >7mg Fe/g dw and received iron chelation therapy (ICT).

Numbers analysed

Table 41: Analysis populations

Analysis Population	Number of Subjects		
	Luspatercept + BSC	Placebo + BSC	Total
ITT Population[a]	224	112	336

BSC = best supportive care; HRQoL = health-related quality of life; ITT = intent to treat; NA = not applicable; PK = pharmacokinetic.
[a] Included all subjects who were randomized, regardless of whether they received treatment or not.

Outcomes and estimation

Primary Endpoint: Red Blood Cell Transfusion Burden Reduction (≥ 33% Reduction) from Baseline with a Reduction of at least 2 Units from Week 13 to Week 24

A greater proportion of subjects reached the primary efficacy endpoint (≥ 33% reduction in RBC transfusion burden during the fixed Week 13 to Week 24 interval) in the luspatercept + BSC treatment group (21.4% of subjects) than in the placebo + BSC arm (4.5% of subjects).

Table 42: RBC transfusion burden reduction (≥ 33% reduction) from baseline to the fixed week 13 to week 24 interval (ITT population)

	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)
Number of Responders, n (%)[a]	48 (21.4)	5 (4.5)
Difference in Proportions (%) (95% CI)[b] (Luspatercept – Placebo)	17.0 (10.4, 23.6)	
Common Risk Difference (%) (95% CI)[b] (Luspatercept – Placebo)	17.0 (10.4, 23.6)	
Odds Ratio (95% CI)[c]	5.79 (2.24, 14.97)	
p-value[c]	< 0.0001	

BSC = best supportive care; CI = confidence interval; CMH = Cochran-Mantel-Haenszel; ITT = intent to treat; RBC = red blood cell.

[a] Subjects with ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24 compared with the 12-week interval on or prior to Dose 1 Day 1.

[b] Difference in proportions (luspatercept – placebo) and 95% CIs were estimated from the unconditional test. Common risk difference (luspatercept – placebo) and 95% CIs were estimated from the CMH method stratified by the geographical regions defined at randomization.

[c] Odds ratio (luspatercept over placebo), 95% CIs, and p-value were estimated from the CMH test stratified by the geographical regions defined at randomization.

Note: Transfusion records collected up to a minimum of (death date, study discontinuation date, last dose date + 20, 11 May 2018) were used for the analysis.

Key secondary Endpoint 1: Red Blood Cell Transfusion Burden Reduction (≥ 33% Reduction) from Baseline with a Reduction of at least 2 Units from Week 37 to Week 48

Table 43: RBC transfusion burden reduction (≥ 33% reduction) from baseline to the fixed week 37 to week 48 interval (ITT population)

	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)
Number of Responders, n (%)[a]	44 (19.6)	4 (3.6)
Difference in Proportions (%) (95% CI)[b] (Luspatercept – Placebo)	16.1 (9.8, 22.3)	
Common Risk Difference (%) (95% CI)[b] (Luspatercept – Placebo)	16.1 (9.8, 22.4)	
Odds Ratio (95% CI)[c]	6.44 (2.27, 18.26)	
p-value[c]	< 0.0001	

BSC = best supportive care; CI = confidence interval; CMH = Cochran-Mantel-Haenszel; ITT = intent to treat; RBC = red blood cell.

[a] Subjects with ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48 compared with the 12-week interval on or prior to Dose 1 Day 1.

[b] Difference in proportions (luspatercept – placebo) and 95% CIs were estimated from the unconditional test. Common risk difference (luspatercept – placebo) and 95% CIs were estimated from the CMH method stratified by the geographical regions defined at randomization.

[c] Odds ratio (luspatercept over placebo), 95% CIs, and p-value were estimated from the CMH test stratified by the geographical regions defined at randomization.

Note: Transfusion records collected up to a minimum of (death date, study discontinuation date, last dose date + 20, 11 May 2018) were used for the analysis.

Source: Table 14.2.2.1.

Key secondary Endpoint 2: Red Blood Cell Transfusion Burden Reduction (≥ 50% Reduction) from Baseline with a Reduction of at least 2 Units from Week 13 to Week 24

Table 44: RBC transfusion burden reduction (≥ 50% reduction from baseline to the fixed week 13 to week 24 interval (ITT population)

	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)
Number of Responders, n (%)[a]	17 (7.6)	2 (1.8)
Difference in Proportions (%) (95% CI)[b] (Luspatercept – Placebo)	5.8 (1.6, 10.1)	
Common Risk Difference (%) (95% CI)[b] (Luspatercept – Placebo)	5.8 (1.6, 10.1)	
Odds Ratio (95% CI)[c]	4.55 (1.03, 20.11)	
p-value[c]	0.0303	

BSC = best supportive care; CI = confidence interval; CMH = Cochran-Mantel-Haenszel; ITT = intent to treat; RBC = red blood cell.

[a] Subjects with ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24 compared with the 12-week interval on or prior to Dose 1 Day 1.

[b] Difference in proportions (luspatercept – placebo) and 95% CIs were estimated from the unconditional test. Common risk difference (luspatercept – placebo) and 95% CIs were estimated from the CMH method stratified by the geographical regions defined at randomization.

[c] Odds ratio (luspatercept over placebo), 95% CIs, and p-value were estimated from the CMH test stratified by the geographical regions defined at randomization.

Note: Transfusion records collected up to a minimum of (death date, study discontinuation date, last dose date + 20, 11 May 2018) were used for the analysis.

Key secondary Endpoint 3: Red Blood Cell Transfusion Burden Reduction (≥ 50% Reduction) from Baseline with a Reduction of at least 2 Units from Week 37 to Week 48

Table 45: RBC transfusion burden reduction (≥ 50 % reduction) from baseline to the fixed week 37 to week 48 interval (ITT population)

	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)
Number of Responders, n (%)[a]	23 (10.3)	1 (0.9)
Difference in Proportions (%) (95% CI)[b] (Luspatercept – Placebo)	9.4 (5.0, 13.7)	
Common Risk Difference (%) (95% CI)[b] (Luspatercept – Placebo)	9.4 (5.0, 13.7)	
Odds Ratio (95% CI)[c]	11.92 (1.65, 86.29)	
p-value[c]	0.0017	

BSC = best supportive care; CI = confidence interval; CMH = Cochran-Mantel-Haenszel; ITT = intent to treat; RBC = red blood cell.

[a] Subjects with ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48 compared with the 12-week interval on or prior to Dose 1 Day 1.

[b] Difference in proportions (luspatercept – placebo) and 95% CIs were estimated from the unconditional test. Common risk difference (luspatercept – placebo) and 95% CIs were estimated from the CMH method stratified by the geographical regions defined at randomization.

[c] Odds ratio (luspatercept over placebo), 95% CIs, and p-value were estimated from the CMH test stratified by the geographical regions defined at randomization.

Note: Transfusion records collected up to a minimum of (death date, study discontinuation date, last dose date + 20, 11 May 2018) were used for the analysis.

Key Secondary Endpoint 4: Change from Baseline in Red Blood Cell Transfusion Burden to the Fixed Week 13 to Week 24 Interval

Table 46: mean change in transfusion burden (RBC units /12 weeks) from baseline to the fixed week 13 to week 24 interval (ITT population)

	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)
Baseline[a]		
n	224	112
Mean (SD)	6.86 (1.998)	6.88 (1.829)
Median (Min, Max)	6.12 (3.0, 14.0)	6.27 (3.0, 12.0)
Week 13 - 24		
n	210	102
Mean (SD)	6.15 (2.434)	7.55 (2.228)
Median (Min, Max)	6.00 (0.0, 15.0)	8.00 (1.0, 13.0)
Mean Change From Baseline (Week 13 to Week 24)		
n	210	102
Mean (SD)	-0.67 (1.795)	0.66 (1.774)
Median (Min, Max)	0.00 (-6.0, 5.0)	0.00 (-6.0, 4.4)
LS Mean (SE)[b]	-0.67 (0.123)	0.68 (0.176)
LS Mean of Difference (Luspatercept – Placebo) (95% CI)[b]	-1.35 (-1.77, -0.93)	
p-value[b]	< 0.0001	

ANCOVA = analysis of covariance; BSC = best supportive care; CI = confidence interval; ITT = intent to treat; LS = least squares; Max = maximum; Min = minimum; RBC = red blood cell; SD = standard deviation; SE = standard error.

[a] Baseline was defined as the total number of RBC units transfused during the 12-week interval on or prior to Dose 1 Day 1.

[b] Estimates were based on an ANCOVA model with geographical regions defined at randomization and baseline transfusion burden as covariates.

Other analysis, endpoints and subgroup analyses of interest

It is noted that no alpha control is applied for endpoints discussed below this point and that described results are mainly relevant for assessment of internal validity of data and description of study population, but these results need to be interpreted with caution in terms of the risk for false positive conclusions.

In addition to the fixed period of analysis for the primary and key secondary endpoints on reduction of transfusion burden, a rolling analysis, to reflect clinical practice, was also conducted per the pre-defined SAP, see Table 47.

Table 47: Rolling analysis for ≥ 33 and ≥ 50% reduction in RBC transfusion burden during any consecutive 12 or 24 weeks

Endpoint	Luspatercept (N=224)	Placebo (N=112)
≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units for 12 consecutive weeks compared to the 12-week interval prior to treatment		
Any consecutive 12 weeks*	158 (70.5)	33 (29.5)
Difference in proportions (95% CI)[a]	41.1 (30.7, 51.4)	
Any consecutive 24 weeks*	92 (41.1)	3 (2.7)
Difference in proportions (95% CI)[a]	38.4 (31.3, 45.5)	
≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units for 12 consecutive weeks compared to the 12-week interval prior to treatment		
Any consecutive 12 weeks*	90 (40.2)	7 (6.3)
Difference in proportions (95% CI)[a]	33.9 (26.1, 41.8)	
Any consecutive 24 weeks*	37 (16.5)	1 (0.9)
Difference in proportions (95% CI)[a]	15.6 (10.5, 20.8)	

CI: confidence interval.

[a] Difference in proportions (luspatercept + BSC – placebo + BSC) and 95% CIs estimated from the unconditional exact test.

[b] Estimates are based on ANCOVA model with geographical regions and baseline transfusion burden as covariates

For the overall ITT population, the mean reduction in RBC transfusion burden per subject in RBC units from baseline was 4.75 RBC units/48 weeks in the luspatercept + BSC treatment group. At baseline, the average patient had received approx. 7 units within 12 weeks or 14 units within 24 weeks, which translates to approx. 28 RBC units/48 weeks. A reduction of 4.75 RBC units/48 weeks translates to approx. -17% or 3-5 transfusion-visits less per year.

The transfusion window time for the overall ITT population is longer for every fixed 12-week interval during the study than at baseline. Data are available until week 84 for a small number of patients. The LS mean of difference between the luspatercept and placebo group however is rather small: 3.1 (0.9, 5.3 95% CI) and not statistically significant (p-value = 0.006).

Red blood cell transfusion independence: 9 patients (4%) in the luspatercept group vs 0 in the placebo group achieved 12 week transfusion independence at one point during the study. 24 patients (10.7%) (N=2 in the placebo group) were TI for 8 weeks, 38 patients (17%) (N=7 in the placebo group) for 6 weeks. The difference between the two arms is numerically notable, although not statistically significant.

The median (mean) time from first dose of study drug to first erythroid response was 12.0 (56.1) days in ≥ 33% reduction in RBC transfusion burden responders and 24.5 (80.5) days in ≥ 50% reduction in RBC transfusion burden responders.

For the overall ITT population, in the luspatercept + BSC treatment group, there was a modest improvement (increase) in the mean pretransfusion Hb levels from baseline during all fixed 12-week intervals (mean increases from 0.09 to 0.38 g/dL). In the placebo + BSC treatment group, the mean pretransfusion Hb levels during all fixed 12-week intervals remained relatively stable from baseline (mean changes between -0.04 and +0.03 g/dL). Overall, pretransfusion Hb levels were stable over the 48 weeks of treatment, indicating that subjects were transfused at the same Hb level as before coming into the study.

Changes in Hb concentrations from baseline were summarized by fixed 12-week intervals. For the overall ITT population, in the luspatercept + BSC treatment group, the mean Hb levels increased in the range of 0.20 to 0.54 g/dL from baseline across the individual fixed 12-week intervals.

- **Mean change in serum ferritin level (ITT):**

Table 48: mean change in mean serum ferritin level (ITT population)

	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)
Baseline[a]		
n	220	111
Mean (SD), µg/L	2096.91 (1756.649)	1845.05 (1669.133)
Median (Min, Max), µg/L	1441.25 (88.0, 6400.0)	1301.50 (136.0, 6400.0)
Postbaseline[b]		
n	214	104
Mean (SD), µg/L	1831.97 (1844.266)	1988.91 (1783.991)
Median (Min, Max), µg/L	1000.25 (63.3, 6400.0)	1224.67 (144.8, 6400.0)
Change From Baseline		
n	212	104
Mean (SD), µg/L	-248.02 (800.021)	106.62 (526.174)
Median (Min, Max), µg/L	-192.88 (-2971.1, 3066.5)	106.00 (-1334.3, 2055.0)
LS Mean (SE)[c]	-233.51 (50.471)	114.28 (71.049)
LS Mean of Difference (Luspatercept – Placebo) (95% CI)[c]	-347.80 (-516.95, -178.65)	
p-value[c]	< 0.0001	

ANCOVA = analysis of covariance; BSC = best supportive care; CI = confidence interval; ITT = intent to treat; LS = least squares; Max = maximum; Min = minimum; SD = standard deviation; SE = standard error.

[a] Baseline mean serum ferritin was calculated as mean of ferritin values during the 12 weeks on or prior to Dose 1 Day 1/randomization.

[b] Postbaseline mean serum ferritin was calculated as mean of ferritin values during the last 12 weeks on or prior to the end date of the first 48-week treatment if efficacy cutoff date was on or after 48 weeks; or mean of ferritin values within last 12 weeks on or prior to the efficacy cutoff date if efficacy cutoff was before 48 weeks, but on or after 12 weeks; otherwise missing. The efficacy cutoff date was defined as min (death date, study discontinuation date, last dose date + 20, 11MAY2018).

[c] Estimates were based on an ANCOVA model with geographical regions defined at randomization and baseline serum ferritin as covariates.

- **Mean Change in Liver Iron Concentration (ITT)**

Table 49: Mean change in derived liver iron concentration at week 48 (ITT population)

	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)
Baseline[a]		
n	211	110
Mean (SD), mg/g dw	9.62 (9.963)	9.36 (10.241)
Median (Min, Max), mg/g dw	5.47 (0.8, 42.0)	4.89 (0.2, 43.0)
Week 48		
n	202	103
Mean (SD), mg/g dw	9.93 (10.194)	9.27 (10.357)
Median (Min, Max), mg/g dw	5.81 (0.8, 41.6)	4.74 (0.8, 43.0)

Change From Baseline at Week 48[a]		
n	202	103
Mean (SD), mg/g dw	0.10 (5.760)	0.08 (5.229)
Median (Min, Max), mg/g dw	0.03 (-24.9, 19.9)	-0.02 (-19.5, 16.9)
LS Mean (SE)[b]	0.34 (0.384)	0.23 (0.531)
LS Mean of Difference (Luspatercept – Placebo) (95% CI)[b]	0.11 (-1.16, 1.38)	
p-value[b]	0.8685	

ANCOVA = analysis of covariance; BSC = best supportive care; CI = confidence interval; dw = dry weight; ITT = intent to treat; LIC = liver iron concentration; LS = least squares; Max = maximum; Min = minimum; SD = standard deviation; SE = standard error.
[a] Baseline was defined as the last value on or before the first dose of study drug was administered; if multiple values were present for the same date, the average of these values was used.
[b] Estimates were based on an ANCOVA model with geographical regions defined at randomization and baseline LIC as covariates.
Note: If a subject had 1 postbaseline assessment, it was used as "Week 48". If a subject had multiple postbaseline assessments, the last one was used as "Week 48"; the average of the rest was used as "Week 24." All LIC values were used for the analysis except the 2 LIC values that were collected too far away from Week 48.
The value of LIC was either the value collected from the electronic case report form or the value derived from the T2*, R2*, or R2 parameter, depending on which techniques and software were used for magnetic resonance imaging LIC acquisition. Subjects with an LIC value > 43 were not included in the analysis.

- **Mean Change in Myocardial T2***

Table 50: mean change in myocardial T2 * MRI at week 48 (ITT population)

	Luspatercept + BSC (N = 224)	Placebo + BSC (N = 112)
Baseline[a]		
n	224	112
Mean (SD), ms	33.52 (16.170)	34.76 (10.665)
Median (Min, Max), ms	34.65 (3.0, 205.9)	36.30 (6.4, 57.5)
Week 48		
n	201	100
Mean (SD), ms	31.99 (11.304)	34.78 (10.680)
Median (Min, Max), ms	33.50 (2.7, 79.8)	36.07 (5.9, 53.9)
Change From Baseline at Week 48[a]		
n	201	100
Mean (SD), ms	-1.83 (15.084)	0.02 (6.843)
Median (Min, Max), ms	-0.80 (-174.5, 45.4)	-0.82 (-18.0, 24.2)
LS Mean (SE)[b]	-2.20 (0.684)	0.20 (0.961)
LS Mean of Difference (Luspatercept – Placebo) (95% CI)[b]	-2.39 (-4.67, -0.12)	
p-value[b]	0.0391	

ANCOVA = analysis of covariance; BSC = best supportive care; CI = confidence interval; ITT = intent to treat; LS = least squares; Max = maximum; Min = minimum; MRI = magnetic resonance imaging; SD = standard deviation; SE = standard error.
[a] Baseline was defined as the last value on or before the first dose of study drug was administered; if multiple values were present for the same date, the average of these values was used.
[b] Estimates were based on an ANCOVA model with geographical regions defined at randomization and baseline myocardial T2* as covariates.
Note: If during the 48-week double-blind Treatment Period, a subject had only 1 assessment, it was counted as the "Week 48" Visit; if a subject had multiple assessments, the last one was used as the "Week 48" Visit.

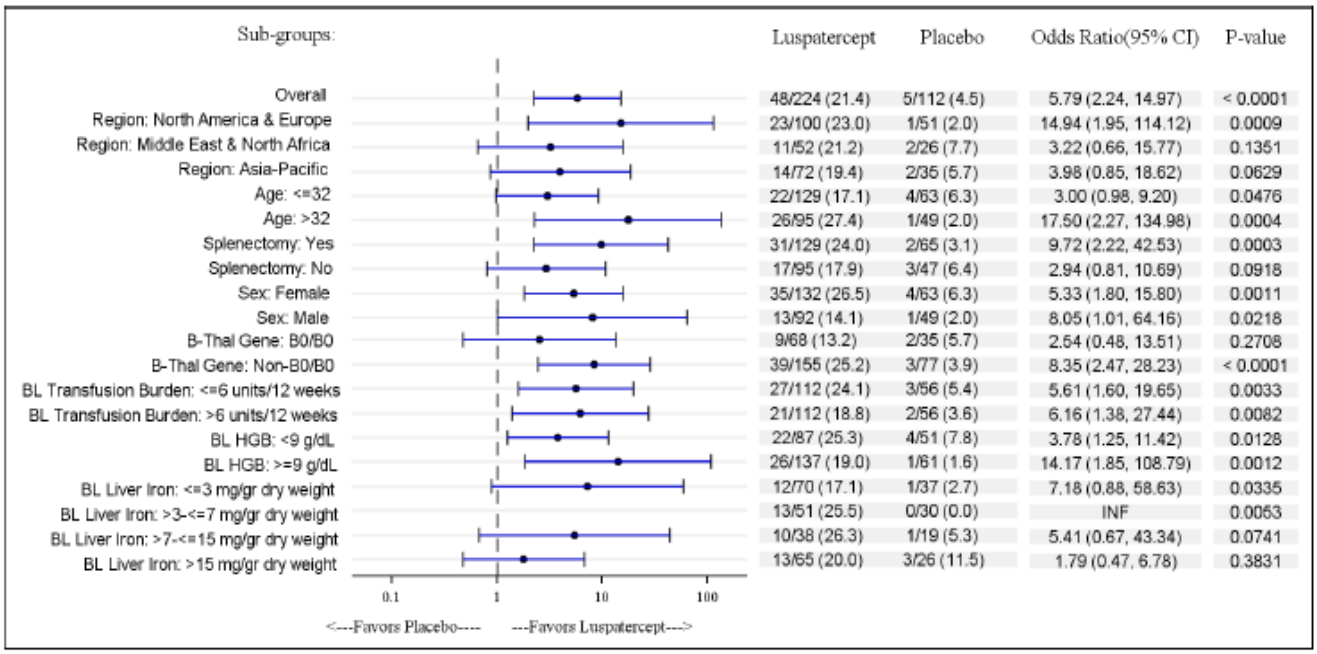
Most subjects in both treatment groups were on iron chelation monotherapy at baseline and postbaseline (60.7% in each treatment group). Use of iron chelation therapy (at baseline and postbaseline) was reported in 23.2% and 19.6% of subjects in the luspatercept and placebo treatment groups, respectively. There were no significant changes from baseline in the mean daily doses of deferasirox, deferiprone, or deferoxamine mesylate/deferoxamine between the 2 treatment groups.

There was no difference between the Luspatercept and the Placebo group in the QoL endpoints SF-36 and TranQoL based on descriptive statistics applied.

Ancillary analyses

Subgroup Analyses

Figure 16: Forest plot of RBC transfusion burden reduction (≥ 33 % reduction) from baseline from week 13 to week 24 (ITT population)



BL = baseline; B-Thal = β-thalassemia; CI = confidence interval; HGB = hemoglobin; INF = infinity; ITT = intent to treat; RBC = red blood cell.

Note: Transfusion records collected up to a minimum of (death date, study discontinuation date, last dose date + 20, 11 May 2018) were used for the analysis.

Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 51: Summary of efficacy for trial ACE-536-B-THAL-001

Title: A phase 3, double-blind, randomised, placebo-controlled, multicentre study to determine the efficacy and safety of Luspatercept (ACE-536) versus placebo in adults who require regular red blood cell transfusions due to β-thalassemia	
Study identifier	ClinicalTrials.gov identifier: NCT02604433 EudraCT number: 2015-003224-31 https://clinicaltrials.gov/ct2/show/NCT02604433 (last access 17.07.2019)
Design	Study ACE-536-B-THAL-001 is an ongoing Phase 3, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of luspatercept + BSC versus placebo + BSC in adults who require regular RBC transfusions due to β-thalassemia. The study consists of a Screening/Run-in Period, a double-blind Treatment Period, a double-blind Long-term Treatment Period, an Open-label Phase, and a Post-treatment Follow-up Period. Starting dose level was 1.0 mg/kg, up-titration to 1.25mg/kg and down-titration to 0.8 mg/kg, 0.6 mg/kg and 0.45 mg/kg was possible as well as delay of dose. The study was conducted at 65 study centers in 15 countries.

	Duration of main phase: Duration of Run-in phase: Duration of LTTP[1]: Duration of Extension phase[2] Duration of follow-up:		48 weeks 12 weeks (+12 weeks historical documentation) Up to 48 weeks Up to 5 years total treatment duration 156 weeks post last dose
Hypothesis	Superiority		
Treatments groups	Luspatercept + BSC		SC injection (q3w), N=224
	Placebo + BSC		SC injection (q3w), N=112
Endpoints and definitions	Primary endpoint	RBC Transf. Reduction ≥ 33% (min. 2 units) baseline to week 13-24	To determine the proportion of subjects treated with Luspatercept + BSC vs. placebo + BSC who achieved erythroid response, defined as ≥ 33% reduction from baseline in transfusion burden (units RBCs/time) with a reduction of at least 2 units, from week 13 to week 24. Baseline: Number of RBC units transfused in the 12 weeks prior to randomization
	Key Secondary Endpoint 1 (hierarchical)	RBC Transf. Reduction ≥ 33% (min. 2 units) baseline to week 37-48	See above. To evaluate effect after longer duration of treatment.
	Key Secondary Endpoint 2 (hierarchical)	RBC Transf. Reduction ≥ 50% (min. 2 units) baseline to week 13-24	See above. To investigate rate of responders who halve their transfusion burden.
	Key Secondary Endpoint 3 (hierarchical)	RBC Transf. Reduction ≥ 50% (min. 2 units) baseline to week 37-48	See above. To investigate rate of responders who halve their transfusion burden after longer duration of treatment.
	Key Secondary Endpoint 4 (hierarchical)	Mean change from baseline in transfusion burden (RBC units) from Week 13 to Week 24	Change from baseline expressed as continuous variable
Database lock	Data cut-off date for the study report was 11 May 2018. The study is ongoing.		

<u>Results and Analysis</u>

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat (ITT-) population after 24 and 48 weeks of Luspatercept treatment		
Effect estimate per comparison	Primary endpoint	*Comparison groups*	*Luspatercept + BSC vs. Placebo + BSC*
		Number of Responders, n (%)	48/224 (21.4) vs. 5/112 (4.5)

		Difference in proportions (%) (95% CI)	17.0 (10.4, 23.6)
		Odds Ratio (95% CI)	5.79 (2.24, 14.97)
		P-value	<0.0001
	Key Secondary Endpoint 1	Number of Responders, n (%)	44/224 (19.6) vs. 4/112 (3.6)
		Difference in proportions (%) (95% CI)	16.1 (9.8, 22.3)
		Odds Ratio (95% CI)	6.44 (2.27, 18.26)
		P-value	<0.0001
	Key Secondary Endpoint 2	Number of Responders, n (%)	17/224 (7.6) vs. 2/112 (1.8)
		Difference in proportions (%) (95% CI)	5.8 (1.6, 10.1)
		Odds Ratio (95% CI)	4.55 (1.03, 20.11)
		P-value	0.0303
	Key Secondary Endpoint 3	Number of Responders, n (%)	23/244 (10.3) vs. 1/112 (0.9)
		Difference in proportions (%) (95% CI)	9.4 (5.0, 13.7)
		Odds Ratio (95% CI)	11.92 (1.65, 86.29)
		P-value	0.0017
	Key Secondary Endpoint 4	mean (SD)	-0.67 (1.795) vs. +0.66 (1.774)
		LS mean of Difference (95% CI)	-1.35 (-1.77, -0.93)
		P-value	<0.0001

[1] Long term treatment period, no results provided (double-blind), ongoing

[2] no interim efficacy results provided (open label), ongoing

Updated efficacy data; data cut off 07 Jan 2019

- Subject disposition

As of the 07 Jan 2019 data cut-off date, in the pooled luspatercept treatment group of the β-thalassemia Data Pool, the proportion of subjects who discontinued treatment was 35.2% (101 of 287 subjects). The proportion of subjects who discontinued from treatment in the placebo treatment group was 26.6% (29 of 109 subjects). A total of 92 subjects who received placebo during the double-blind Treatment Period in Study ACE-536-B-THAL-001 crossed over to receive luspatercept during the Open-label Phase of the study.

As of the 07 Jan 2019 data cut-off date, in the pooled luspatercept treatment group of the β-thalassemia Data Pool, the proportion of subjects who discontinued the study was 14.3% (41 of 287 subjects). The proportion of subjects who discontinued from the study in the placebo treatment group was 5.5% (6 of 109 subjects). A total of 267 subjects in the pooled luspatercept treatment group (including cross-over subjects) were still receiving treatment as of the 07 Jan 2019 data cut-off date.

In both, the original MAA and this updated data report, the most frequently cited reason for treatment discontinuation and study discontinuation in the pooled luspatercept treatment group was withdrawal by subject.

- Efficacy

A postbaseline mean increase in ranging from +0.13 to +0.97 RBC units/24 weeks was reported in the placebo + BSC treatment group. At fixed 48-week intervals, the mean change from baseline in RBC transfusion burden in the luspatercept + BSC treatment group in Study ACE-536-B-THAL-001 was **-** 4.75 RBC units/48 weeks for Week 1 to Week 48 and -5.99 RBC units/48 weeks for Week 49 to Week 96. For the 48-week intervals, the postbaseline mean increase in RBC transfusion burden was +1.04 RBC units/48 weeks for Week 1 to Week 48 and +0.31 RBC units/48 weeks for Week 49 to Week 96 in the placebo + BSC treatment. In the "Phase 3-like Population" of the Phase 2 studies, a reduction in the mean RBC transfusion burden was observed for each time period measured, which ranged from -6.60 to -8.78 RBC units/24 weeks and -13.31 to -16.56 RBC units/48 weeks.

From the time of the data cut-off date for the original MAA to the 07 Jan 2019 data cut-off date for this updated report, the response rates for both, ≥ 33% and ≥50% RBC transfusion burden reduction in any 12-week interval in the Phase 3 study increased:

≥ 33% reduction: 76.3% subjects for 07 Jan 2019 data cutoff date versus 70.5% subjects for the original MAA;

≥ 50% reduction: 44.6% subjects for 07 Jan 2019 data cutoff date versus 40.2% subjects for the original MAA.

A similar result was observed for the time period in any 24-week interval in the Phase 3 study. The response rates for both ≥ 33% and ≥ 50% RBC transfusion burden reduction in any 24-week interval in the Phase 3 study increased:

≥ 33% reduction: 45.1% subjects for 07 Jan 2019 data cut-off date versus 41.1% subjects for the original MAA;

≥ 50% reduction: 20.5% subjects for 07 Jan 2019 data cut-off date versus 16.5% subjects for the original MAA).

For the "Phase 3-like population" of the Phase 2 studies, the response rates for both ≥ 33% and ≥ 50% RBC transfusion burden reduction in any 12-week interval remained the same in the 07 Jan 2019 data cut-off date versus the initial cut-off date. The response rates for both ≥ 33% and ≥ 50% RBC transfusion burden reduction for any 24-week interval also remained the same.

With updated data, a total of 83.0% (142/171) of responders experienced more than 1 response during the entire treatment period and 47.4% (81/171) of responders had > 5 episodes.

An increase in the median total duration of the transfusion burden reduction was observed with longer exposure. For those subjects in Study ACE-536-B-THAL-001 who achieved an erythroid response (≥ 33% transfusion burden reduction) during any rolling 12-week interval, the median total duration (cumulative) of RBC transfusion burden reduction was longer in the luspatercept + BSC treatment group (397.0 days) than in the placebo+ BSC treatment group (171.0 days). Among the subjects with ≥ 50% transfusion burden reduction during any rolling 12-week interval, the median total duration (cumulative) of RBC transfusion burden reduction was 276.0 days in the luspatercept + BSC treatment group and 169.0 days in the placebo + BSC treatment group. The median total duration of the transfusion burden reduction increased with longer exposure (median total duration of the transfusion burden reduction was 210.0 days in the original MAA versus 276.0 days as of the 07 Jan 2019 data cut-off date).

For the 48-week interval Week 49 to Week 96, a continued decrease of the transfusion burden reduction was observed with extended exposure compared to placebo.

The percentage of subjects who achieved red blood cell transfusion independence (RBC-TI) during any consecutive rolling 6-week, 8-week, or 12-week interval continued to be higher in the luspatercept + BSC group compared with the placebo + BSC group:

- RBC-TI during any 6-week interval: the response rate increased slightly compared with original MAA (response rate 17.0% for luspatercept and 6.3% for placebo for the original versus 21.0% for luspatercept and 8.0% for placebo for updated data
- RBC-TI during any 8-week interval: The response rate continued to be higher for the luspatercept group vs placebo group and the difference was statistically significant. For the luspatercept group, the response rate increase slightly compared with the original MAA (response rate 10.7% for luspatercept and 1.8% for placebo for the original versus 11.2% for luspatercept and 1.8% for placebo for the updated data.
- RBC-TI during any 12-week interval: The response rate for the luspatercept group in the 07 Jan 2019 data update (4.0%) was unchanged from the initial cut-off date

Among the subjects in the luspatercept + BSC treatment group who maintained RBC transfusion independence for ≥ 6, ≥ 8, and ≥ 12 weeks, the median longest duration of transfusion independence was 56.0, 74.0, and 270.5 days, respectively.

In Study ACE-536-B-THAL-001, subjects in the luspatercept + BSC treatment group showed a modest improvement (increase) in the mean pretransfusion Hgb levels from baseline during all fixed 12-week intervals (mean increase of 0.09 to 0.38 g/dL). In the placebo + BSC treatment group, the mean pretransfusion Hgb levels during all fixed 12-week intervals remained relatively stable from baseline (mean changes between -0.04 and 0.10 g/dL).

Iron Parameters

Serum ferritin

In Study ACE-536-B-THAL-001, a significantly greater decrease from baseline to Week 96 (last 12 weeks) in mean serum ferritin was observed in the luspatercept + BSC group compared with the placebo group (nominal $p < 0.0001$) as of the 07 Jan 2019 data cut-off date. The between-group difference in least squares (LS) mean change from baseline in serum ferritin level at Week 96 (last 12 weeks) was -549.50 µg/L, which favored luspatercept treatment versus placebo.

As of the 07 Jan 2019 data cut-off date, overall for the luspatercept group, 42.9% of subjects with mean serum ferritin ≥ 1000 µg/L at baseline shifted to < 1000 µg/L during the last 24 weeks on or prior to Week 96, versus 7.1% of subjects in the placebo group

45.5% of luspatercept responders (who had ≥ 33% transfusion burden reduction during any 12-week interval) experienced a shift in serum ferritin category from ≥ 1000 µg/L at baseline to < 1000 µg/L.

A higher decrease in serum ferritin was observed in responders versus the overall luspatercept group or placebo group.

Liver Iron Concentration (LIC)

The mean changes from baseline in LIC for responders in the luspatercept + BSC group at Week 96 were as follows:

- -1.33 mg/g dw for subjects in the luspatercept + BSC group who were ≥ 33% responders in any rolling 12 weeks (Table BEE.193a.9.1b.1.b)

- -1.82 mg/g dw for subjects in the luspatercept + BSC group who were ≥ 33% responders in any rolling 24 weeks (Table BEE.193a.9.1b.2.b)
- -1.61 mg/g dw for subjects in the luspatercept + BSC group who were ≥ 50% responders in any rolling 12 weeks (Table BEE.193a.9.1b.3.b)
- -1.54 mg/g dw for subjects in the luspatercept + BSC group who were ≥ 50% responders in any rolling 24 weeks (Table BEE.193a.9.1b.4.b)

Mean change from baseline in the ITT was -0.38mg/g dw at week 96.

Myocardial Iron by MRI

As of the 07 Jan 2019 data cut-off date, the overall mean myocardial iron T2* at Week 96 in Study ACE-536-B-THAL-001 remained in the normal range of cardiac iron (> 20 ms; Kirk, 2009). The mean change from baseline in myocardial iron T2* for the luspatercept group in Study ACE-536-B-THAL-001 was -0.39 ms at Week 96, which was not a clinically significant change. The LS mean difference for the luspatercept + BSC treatment group versus the placebo + BSC treatment group was -2.95 ms (95% CI: -41.38, 8.49) (p = 0.6101).

For shifts from baseline in myocardial iron T2* at Week 96, the majority of subjects in the overall luspatercept group remained in the same category as baseline.

Iron Chelation Therapy

No significant change from baseline in mean daily dose was observed but a trend in favour of IP (slightly higher decrease of daily dose compared to placebo) was noted.

Analysis performed across trials (pooled analyses and meta-analysis)

A document named 'integrated summary of clinical efficacy' was provided in the dossier for the beta-thalassemia dataset, including data from ACE-536-BTHAL-001 (phase III), A536-04 and A536-06 (phase II). The ITT population for the comparison of efficacy across studies included 336 subjects in Study ACE-536-B-THAL-001 (224 randomized to luspatercept + BSC and 112 randomized to placebo + BSC) and 24 subjects in the "Phase 3-like Population" of the Phase 2 studies.

Due to the differences in the subject populations and efficacy endpoints between the Phase 2 and Phase 3 studies, the Phase 2 and Phase 3 data sets were not pooled for integrated efficacy analyses. A subset of subjects from the Phase 2 studies that approximated the dosing of the Phase 3 study and matched key inclusion criteria was identified and efficacy results were presented next to one another for comparison.

Baseline characteristics of the "Phase 3-like Population" were consistent with those of the Phase 3 population, except for a lower mean LIC at baseline and a higher rate of splenectomy among subjects in the Phase 2 studies, and the more diverse, global subject population in the Phase 3 study compared with the limited number of sites in the Phase 2 studies.

Duration of treatment and follow-up has to be considered in comparisons between the Phase 3 and Phase 2 studies. The median duration of treatment for the data cut included in this submission was longer in Phase 2 (e.g., 715 days [approximately 102 weeks] in extension Study A536-06) than in Study ACE-536-B-THAL-001 (449 days [64.1 weeks] in the luspatercept group and 448 days [64.0 weeks] in the placebo group).

The "Phase 3-like Population" of the Phase 2 studies performed comparable in many endpoints and thus provided consistent supportive evidence for the efficacy of luspatercept.

Clinical studies in special populations

No studies in patients with renal or hepatic impairment, in elderly or paediatric patients (not included in the label) were conducted in the beta-thalassaemia indication. In the pivotal trial ACE-536-B-THAL-001 most patients were < 32 years of age and only 22 patients (6.5%) were > 50 years old, the oldest patient was 52 (see baseline characteristics in the description of the main study above). In A536-04 the oldest patient was 62 years old (A536-06 was the long-term extension of this study). Age was not restricted in terms of an upper age limit in the trials, but no elderly patients were recruited (the oldest patient was 66 years).

Data from the MDS development, where the population is significantly older, and also respective POP PK analyses showed no clinically significant difference in AUC or clearance across age groups (< 65, 65 74, and ≥ 75 years for MDS patients; 18 23, 24 31, 32 41, and 42 66 years for beta-thalassaemia patients).

Inclusion/exclusion criteria restricted the population in the pivotal trial to patients without significant hepatic or renal disease. Excluded were patients with liver disease with alanine aminotransferase (ALT) > 3 x the upper limit of normal (ULN) or history of evidence of cirrhosis and patients with creatinine clearance < 60 mL/min (per Cockcroft-Gault formula).

Supportive studies

Besides the pivotal phase III study ACE-536-BTHAL-001, two phase II studies were submitted to support Luspatercept for the treatment of beta-thalassaemia; A536-04 and A536-06. Two phase II studies and one phase III study submitted to support the 'MDS' indication also evaluated erythroid response.

2.6.1. Discussion on clinical efficacy in beta-thalassaemia

Design and conduct of clinical studies

In the dose escalating study A536-04, six dose levels were evaluated (0.2, 0.4, 0.6, 0.8, 1.0 and 1.25 mg/kg). Data indicates that there could be an increased response with increased dose. This was more clear based on the PD surrogate Hgb used in NTD subjects than for number of RBC units which was used as efficacy marker in TD subjects. Data from the NTD dataset seem informative for the TD population as well. The PD parameter Hgb likely is more sensitive to reflect dose response, directly related to the postulated mechanism of action of the drug and also more objective (while decision for transfusion could be influenced by several factors).

Study A536-06 (ongoing) is the long-term extension study of A536-04 and evaluates erythroid response in patients receiving 0.8-1.25mg/kg (N=10). The preliminary results support the conclusion from the parent study.

The dose finding was not extensive, the sample size in the phase II studies for beta thalassemia was small (N=33 in NTD patients and N=31 in TD patients in the parent trial) and thus, the description of the dose-exposure-response relationship is based on limited data.

As the phase II studies are still ongoing, they are of value for the assessment of long-term performance. Updated data provided after the clock-stop with a data cut-off 07 Jan 2019 supports the primary analyses and does not indicate a loss of response over time.

The **pivotal Phase 3 study (ACE-536-B-THAL-001)** is a double blind, randomised (2:1), placebo-controlled, multicentre (65 centres) study of luspatercept & BSC vs. placebo & BSC in adults who

require regular red blood cell transfusions. The study is still ongoing; the initial submission included the 48-week results. After the clock stop, updated results were provided (see below).

Submitting only one pivotal trial could be acceptable in this case, if results from the phase III in MDS (also investigating erythroid response) are supportive, also considering that beta-thalassaemia is an orphan disease with high unmet medical need.

The design of the study ACE-536-B-THAL-001, the choice of comparator and choice of the target patient population are largely in line with the EMA Scientific Advice (2015) and overall acceptable. The patient population included in the study is a rather severely affected one. At baseline, this patient population received about 2 units of RBCs every 3 weeks. The patients were severely iron overloaded with multiple thalassaemia-associated co-morbidities. There are some minor differences in baseline- and disease characteristics between the treatment arms, but they seem of little clinical relevance.

The primary endpoint is a responder analysis of the proportion of patients with a $\geq$ 33% reduction from baseline in transfusion burden (units RBCs/time) of at least 2 RBC units from week 13 to 24. Four secondary endpoints that were hierarchically tested evaluated also 50% reduction, response at later time intervals (week 37-48), and mean change from baseline (to week 13 – 24). A responder analysis bears the risk of loss of information compared to an analysis based on continuous outcomes. However, the Applicant gave a clinical justification for the responder definition, which was accepted also in the EMA SA: A reduction in RBC transfusion burden of 33% or greater is considered clinically meaningful for transfused patients based on the associated decrease in accumulation of transfusional iron and related complications. It was estimated that a patient who achieves reduction in transfusion need from 2 RBC units every 4 weeks to 2 RBC units every 6 weeks would thus reduce the transfusional iron intake by approximately 1700 mg/year, based on an estimated 200-mg iron/RBC unit (Cohen, 2008; Porter, 2001). In a patient weighing 50 kg, this would translate into a reduction of liver iron content of approximately 3 mg/g dw (Angelucci, 2000), which has been considered a clinically meaningful change in recent iron chelation studies in β-thalassemia populations (Taher, 2012; Cappellini, 2006). Reduced transfusional iron can also lead to a reduction in the dose of iron chelators (Cohen, 2008), thus reducing the risks and costs associated with ICT.

Overall, while the responder analysis has some weaknesses, in context with the other endpoints evaluated and the updated data on efficacy from the ongoing studies, the dataset seems sufficiently mature to base a benefit/risk assessment on.

In the study as well as for the set-up of the baseline data, the numbers of transfusion units given to a patient within a pre-defined time frame (12 or 24 weeks) was counted. The decision for transfusion (length of transfusion window, number of units transfused per visit…) and luspatercept dosing (dose, interval…) could have direct influence on the (primary) endpoint(s) of this study, both including subjective or variable elements. This makes the trial closer to a real world setting but stresses the necessity of impeccable blinding and randomisation techniques, which are considered robust.

Statistical methods - planned and performed – are in general considered adequate.

Efficacy data and additional analyses

The number of responders was 21.4% (N=48/224) in the luspatercept + BSC group and 4.5%, (N=5/112) in the placebo + BSC group; the treatment effect on the primary endpoint is statistically significant ($p< 0.001$). The calculated odds ratio was 5.79 (95% CI; 2.24, 14.97) and the difference in proportions was 17% (95% CI; 10.4, 23.6).

While there is a clear difference between the groups in responders, the percentage of responders is rather small for the fixed period of analysis. It is noticed that in the power calculation, the response rate was expected to be twice as high as observed in the results. The Applicant attributes this to the

differences between study designs and study population included in the phase 2 that served as basis for the power calculations and the patient population actually recruited in phase 3 (EU setting vs global setting and other subject specific variation). However, the clinical relevance of the observed luspatercept effect is difficult to determine based on the responder analysis alone, and needs to be assessed together with results from the other endpoints and analyses:

Statistical significance was reached also in all other hierarchically tested secondary endpoints, but key secondary endpoint 1-3 also evaluated binary outcomes and key secondary endpoint 4 evaluated the mean change from baseline to the fixed week 13 - 24 interval only (ITT), although 48 week data are available. A reduction of 0.67 RBC units in the luspatercept group within the 12 week period was found. A reduction of -4.75 RBC units/48 weeks (ITT), corresponding to approximately -17% or 3-5 transfusion visits less, was measured in another secondary endpoint (not statistically powered). Usually, patients receive (1-)2 units per transfusion visit and thus the reduction in terms of units and transfusion-associated hospital visits seems fairly modest.

Trends for favourable outcomes with luspatercept were also observed for most of the other secondary endpoints (but not for QoL endpoints and change in LIC, see below) further evaluating red blood cell transfusion burden reduction, iron parameters, etc., and for all associated subgroup analyses, which strengthens the internal validity of the data.

The rate of discontinuations was around 12% until week 48 and rather balanced between study arms.

Post-hoc, the Applicant recognised that measures of Hb change during the study were not reliable indicators of drug effect, as 14 days were not sufficient to exclude the influence of transfusion on Hb. This could have led to unjustified dose delays/reductions. The Applicant therefore proposed increase of the 'no transfusion' window to 3 weeks and amended the titration/dosing rules in the SmPC accordingly.

In the 12 weeks prior to randomization (the time period were 'baseline' was established), prospectively collected RBC transfusion counts were lower compared to the preceding 12 weeks (12-weeks of historical retrospective transfusion data that was transcribed from medical records) and also compared to transfusion data collected in the placebo group during the treatment phase. It seems that the baseline RBC transfusion counts were (slightly) underestimated for unknown reasons. However, baseline transfusion counts were very similar in the Placebo and Verum groups, and also very similar, if higher, in the Placebo and Verum groups in the 12-week historical dataset. Therefore this observation (the lower transfusion counts at baseline) seems unlikely to significantly have influenced the relevant efficacy study outcomes.

Subgroup analyses and description of disease characteristics between responders and non-responders (primary endpoint) show some minor differences. Therefore the Applicant was asked to comment on subgroups with smaller response (e.g. patients with more severe disease, renal impairment, patients with $ß^0/ß^0$ mutation or patients <32 years). Patients with $ß^0/ß^0$ mutation in particular have very high unmet need as they are excluded from treatment with the recently approved gene-therapy product Zynteglo and a treatment option for them would be very desirable. Additional analyses revealed that while some patients (with ß0/ß0 mutation, with start of transfusions <2 years of age, with renal disease) might have a somewhat smaller benefit from treatment, the effect is still robust across all subgroups, and there is no indication to exclude specific subgroups from the label (see also Guideline on the Investigation of Subgroups in Confirmatory Clinical Trials (EMA/CHMP/539146/2013)). Respective results were included in section 5.1 of the SmPC.

Iron parameters are of major interest, as the iron burden in beta thalassaemia patients is associated with severe (long-term) risks and reduced life expectancy. Iron chelation therapy was not standardised

or stratified for in the study, but the groups were comparable in terms of chelator product used. Overall, 97% of patients were on iron chelator treatment.

Over the 48 weeks of luspatercept treatment ICT did not change significantly. Iron levels were reduced in some body compartments; (small) results in favour of luspatercept were seen for change in serum ferritin and myocardial T2*.

However, included patients were highly iron overloaded both at baseline and after 48 weeks, and an ICT dose decrease was therefore not indicated (from an efficacy perspective). Treatment with Luspatercept did not result in significant reduction in LIC over the 48-week double-blind treatment period and the Applicant states that this could be due to a delayed response of this parameter, requiring a longer observational period. It is noted that the primary responder threshold (33% reduction in RBC units) as initially justified by an estimated yearly decrease in LIC by ~ 3 mg/g dw (see above), could not be shown in ACE-536-B-THAL-001 at the cut-off presented for this application.

There was no difference between the Luspatercept and the placebo group in quality of life endpoints SF-36 and TranQoL. The Applicant claims that this is because of the good baseline values, which were better than in comparable populations from historical data sources (Sobota, 2011, Klaasen, 2014) and that this starting point makes it more challenging to demonstrate further improvement in HRQoL, despite responding to treatment. This rationale can be followed to a certain degree; however, it also questions the relevance of a reduction in transfusion units/visits for patients since the studied population had a good QoL despite the need for many transfusions and severe co-morbidities. The Applicant further claims that beneficial effects on QoL may not be observable in the initial years of treatment, but only manifest later. However, this cannot be verified based on the available data.

Initially, 48-week double-blind data was submitted in the dossier. As the study is still ongoing, further data became available and **updated efficacy (and safety) data with data cut-off 07 Jan 2019** was provided during the evaluation:

Data from an additional 92 subjects became available from study ACE-536-B-THAL-001 for patients who crossed over from the initial placebo group into the open-label phase, where they started to receive luspatercept. At this point in time, no more placebo data is collected, which has to be considered when comparing the groups. Updated long term data from patients initially randomised to luspatercept and then transferred into the open-label phase (still receiving luspatercept) seem also included in the updated dataset, yielding in a mix of controlled and uncontrolled data.

RBC transfusion burden expressed in the responder analyses ($\geq$ 33% or $\geq$ 50% reduction in RBC transfusion burden from baseline with a reduction of at least 2 units) and evaluated during rolling 12- or 24 week intervals yielded results consistent with the original MAA set, indicating superiority of luspatercept over placebo. Time to erythroid response did not differ relevantly either between the original and the updated dataset. Assessment of multiple response periods over the treatment duration indicates that many responders achieve several response periods (during any 12- or 24-week interval).

Results for the endpoint 'transfusion independence' (only a small proportion of subjects achieved transfusion independence during any 6-, 8-, or 12 week intervals) were in line with data from the initial cut-off date and indicate a beneficial effect of luspatercept as well. Pre-transfusion Hb values remained relatively stable (which would be a treatment goal in the management of ß-thalassemia), with a slight increase of pretransfusion Hb in the luspatercept group compared to baseline and to placebo.

The change from baseline in transfusion burden is considered a more sensitive and informative endpoint compared with the responder analyses. For the 48-week interval of Week 1 - 48, a decrease of -4.67 RBC transfusion units compared to baseline was observed in the luspatercept group. In contrast, transfused RBC units remained relatively stable (or increased slightly) in the placebo group

(+1.04 RBC units/48 weeks for Week 1 – 48). The difference in the change in RBC transfusion burden (Units/48 Weeks) is -5.83, with a corresponding nominal 95% confidence interval of (-7.01, -4.65).

For the 48-week interval for Week 49 - 96, a continued decrease of -5.66 units compared to baseline was seen in the luspatercept group. The corresponding result for the placebo group is +0.31 RBC units, but comparisons are not considered meaningful, because only 3 placebo patients remain.

Iron parameters

With a mean reduction in RBC transfusion burden of -4.75 RBC units/48 weeks in the overall ITT population on luspatercept, the average patient would be expected to be spared from approx. 1 g of transfusion iron per year (calculated result). The reduction of RBC units and thus, iron burden is higher in the (predefined) responder groups.

Serum ferritin levels continued to respond to luspatercept treatment and decreased over time in luspatercept patients (while slightly increasing in placebo patients).

Based on the original and updated numbers on LIC, it is difficult to assess the beneficial effect of luspatercept on liver iron over time and compared with placebo. Confidence intervals of mean changes are very broad and results are difficult to interpret as the patient population available for assessment decreases for each time point. However, it is acknowledged that the mean change from baseline in LIC in the second year of luspatercept treatment showed a small decrease (as compared with the first 48 weeks, where a small increase was observed). The Applicant claims that clinically relevant LIC results might only manifest after longer treatment duration and agrees to further collect relevant data in the long-term open-label follow-up Study ACE-536-LTFU-001, and to report respective results to the authorities annually for 5 years.

It is reassuring that when comparing mean changes over time between responders and non-responders in the luspatercept group, it seems that a reduction in transfusion units as defined for the responder thresholds correlates with LIC as the mean change in LIC was always better (i.e., higher decrease) in responders compared to non-responders.

While the recorded LIC reductions in the Reblozyl study are far below from what has been observed in registrational studies with iron chelators such as e.g. deferasirox (see SmPC Exjade), it is acknowledged that the pivotal luspatercept study was not primarily designed to investigate iron reduction. As no longer-term data are available, the potential for iron sparing in the liver with chronic luspatercept treatment remains not fully elucidated.

Mean change in myocardial iron over 48 weeks indicates a slight advantage of luspatercept over placebo, but no clinically meaningful improvement.

Regarding concomitant iron chelator treatment, updated data shows a trend for a larger decrease in ICT in the luspatercept group compared with placebo over time. However, these differences are very small and likely not clinically meaningful and the corresponding confidence intervals are wide.

Special Populations

No dedicated studies in patients with renal or hepatic impairment, in elderly or paediatric patients (not included in the label) were conducted in the beta-thalassemia indication.

In the pivotal trial ACE-536-B-THAL-001 most patients were < 32 years of age and only 22 patients (6.5%) were > 50 years old, the oldest patient was 52. In A536-04 the oldest patient was 62 years old. Age was not restricted in terms of an upper age limit in the trials, but no elderly patients were recruited. Transfusion dependent beta-thalassemia patients have a life expectancy of around 40-50 years, improvement of survival during recent years has been frequently reported by different authors. Based on the dataset provided, efficacy in elderly beta-thalassemia patients cannot be evaluated.

However, data from the MDS development, where the population is significantly older, are available and also respective POP PK analyses found no clinically significant difference in AUC or clearance across age groups (< 65, 65-74, and ≥ 75 years for MDS patients; 18-23, 24-31, 32-41, and 42-66 years for beta-thalassaemia patients). There is no indication for a different pathophysiological response in elderly beta-thalassemia patients.

Inclusion/exclusion criteria restricted the population in the pivotal trial to patients with no significant hepatic or renal disease therefore no dose recommendations can be made for these severe cases (please see SmPC).

Some patients with mild-moderate renal and hepatic impairment were included in the studies, however. In the POP-PK model mean luspatercept AUCss was higher in subjects with β-thalassemia who had mild renal impairment (+38%) than those with normal renal function, but the Applicant considers this aberration irrelevant. There are indications that renally impaired beta-thalassemia patients could have impaired efficacy and this might need to be reflected in the PI. The amount of patients with mild/moderate renal impairment in the beta thalassemia study was however small and the results should be interpreted with caution.

Another subgroup of interest are splenectomised patients, as they will likely be treated also later in clinical practice. More than half of the phase III population (57.7%) were splenectomised patients. Subgroup analyses of main efficacy results showed positive results for patients with and without spleen, with a trend for better response in splenectomised patients.

2.6.2. Conclusions on clinical efficacy in beta-thalassemia

In conclusion, available data from the pivotal study supports a rather small, if considering the fixed period of analysis alone , but robust effect of luspatercept on reduction in transfusion burden during 96 weeks of treatment in adult patients with transfusion-dependent anaemia associated with ß-thalassaemia.

2.7. Clinical safety

The safety of luspatercept has been examined in a clinical development programme comprising 571 subjects exposed to luspatercept (including 260 subjects with myelodysplastic syndromes [MDS], 287 subjects with β-thalassaemia, and 24 healthy, postmenopausal females) in 7 clinical studies, i.e. one phase I trial in healthy volunteers (A536-02), one open-label phase II ascending dose study followed by an extension study for each indication (MDS: A536-03 & A536-05; β-thal: A536-04 & A536-06) and one randomized, double-blind pivotal phase III study for each indication (MDS: ACE-536-MDS-001; β-thal: ACE-536-B-THAL-001). Safety data are presented in 3 separate data pools: the total luspatercept Data pool, the MDS Data Pool and the β-thalassemia Data Pool.

Patient exposure

The total luspatercept pool includes 571 subjects, the total placebo pool 193 subjects. The total luspatercept pool contains all subjects treated with luspatercept from the two MDS phase 2 studies (A536-03 & A536-05, n=107), the two β-thalassemia phase 2 studies (A536-04 & A536-06, n=64), the MDS phase 3 study (ACE-536-MDS-001, n=153), the β-thalassemia phase 3 study (ACE-536-B-THAL-001, n=223) and the phase 1 study with healthy subjects (A536-02, n=24). The total placebo pool contains all subjects treated with placebo from the MDS phase 3 study (ACE-536-MDS-001, n=76), the

β-thalassemia phase 3 study (ACE-536-B-THAL-001, n=109) and the phase 1 study with healthy subjects (A536-02, n=8).

Duration of exposure

Table 52

Table 14.3.1.1.3
Treatment Exposure
Overall Safety Population

	Phase 2 & 3 (MDS & BTHAL)				A536-02		Pooled	
	Luspatercept < 1.0 mg/kg (N=50)	Luspatercept >= 1.0 mg/kg (N=497)	Luspatercept All (N=547)	Placebo (N=185)	Luspatercept (N=24)	Placebo (N=8)	Luspatercept (N=571)	Placebo (N=193)
Total Person-Year [a]	59.36	540.47	599.82	167.55	1.84	0.69	601.66	168.24
Treatment Duration (weeks)								
n	50	497	547	185	24	8	571	193
Mean	61.9	56.7	57.2	47.3	4.0	4.5	55.0	45.5
SD	56.27	30.48	33.62	21.14	1.02	0.93	34.60	22.39
Median	32.4	58.1	57.6	50.7	4.0	5.0	55.1	49.7
Q1, Q3	15, 115	33, 71	29.4, 72	24, 64.7	3, 5	4, 5	24.1, 71	24, 64.4
Min, Max	3.0, 160.1	1.7, 169.3	1.7, 169.3	7.4, 89.4	3.0, 5.0	3.0, 5.0	1.7, 169.3	3.0, 89.4
Number of Dose Received								
n	50	497	547	185	24	8	571	193
Mean	20.1	18.3	18.5	15.7	1.5	1.8	17.8	15.1
SD	18.24	9.96	10.97	7.09	0.51	0.46	11.26	7.48
Median	11.0	18.0	18.0	17.0	1.5	2.0	18.0	16.0
Q1, Q3	5, 39	10, 23	9, 23	8, 22	1, 2	1.5, 2	8, 23	8, 22
Min, Max	1.0, 53.0	1.0, 53.0	1.0, 53.0	3.0, 30.0	1.0, 2.0	1.0, 2.0	1.0, 53.0	1.0, 30.0

[a] Total person-years is defined as the sum of treatment duration in years for all subjects in the treatment arm.
Treatment duration is defined as (Treatment end date - Date of first dose of IP + 1) / 7. Treatment end date is defined as min [(the last dose date + 20), death date, study discontinuation date, study cut-off date].

For MDS, the mean treatment duration of the entire MDS data pool was 49.0 weeks (median 45.6), in the phase 2 studies (A536-03/-05) 52.4 weeks (median 30.9), and in the phase 3 study (ACE-536-MDS-001) 46.6 weeks (median 49.0) for Luspatercept-treated and 30.6 weeks (median 24.0) for placebo-treated subjects. 171 (63.6%), 123 (45.7%), and 98 (36.4%) subjects received a minimum of 6, 12, and 18 months of luspatercept treatment at any dose, respectively.
For ß-thalassemia, the mean treatment duration of the entire ß-thalassemia data pool was 64.7 weeks (median 63.4), in the phase 2 studies (A536-04/-06) 78.2 weeks (median 78.5), and in the phase 3 study (ACE-536-ß-THAL-001) 60.8 weeks (median 63.3) for Luspatercept-treated and 58.9 weeks (median 62.1) for placebo-treated subjects. 256 (67.5%), 230 (60.7%), and 204 (53.8%) β-thalassemia subjects received a minimum of 6, 12, and 18 months of luspatercept treatment at any dose, respectively.

Dose

The studies included a variety of Luspatercept doses ranging from 0.0625 mg/kg in phase 1 (study A536-02) to 1.75 mg/kg in the phase 2 MDS study (study A536-03). In addition, the doses and thus exposure differs between the indication in the phase 2 studies: MDS phase 2 study A536-03: 0.125 mg/kg to 1.75 mg/kg Luspatercept versus β-thalassemia phase 2 study A536-04: 02 mg/kg to 1.25 mg/kg.

Adverse events

95.3% of subjects in the pooled luspatercept treatment group and 91.2% of subjects in the pooled placebo treatment group reported at least 1 TEAE. Incidence rates of serious TEAEs, TEAEs of Grade 3 or 4, TEAEs leading to dose interruption, and TEAEs leading to IP discontinuation, were higher in the pooled luspatercept treatment group than in the pooled placebo treatment group. It is noted that there was a shorter exposure duration in the MDS placebo treatment group than in the luspatercept treatment group. Fifteen subjects (10 subjects [1.8%], luspatercept; 5 subjects [2.6%], placebo) had Grade 5 (fatal) TEAEs during the study.

Table 53: Luspatercept Data Pool: Overview of TEAEs (Safety Population)

Category	Pooled Luspatercept N = 571 n (%)	Pooled Placebo N = 193 n (%)
Subjects with at least 1:		
TEAE	544 (95.3)	176 (91.2)
Serious TEAE	136 (23.8)	29 (15.0)
TEAE leading to death (CTCAE Grade 5)	10 (1.8)	5 (2.6)
CTCAE Grade 3 or 4	199 (34.9)	50 (25.9)
TEAE leading to dose interruption	83 (14.5)	15 (7.8)
TEAE leading to dose reduction	21 (3.7)	3 (1.6)
TEAE leading to permanent IP discontinuation	50 (8.8)	7 (3.6)

CTCAE = Common Terminology Criteria for Adverse Events; IP = investigational product; MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent adverse event.
Note: CTCAE version 4.03 was used for grading severity. Treatment-emergent adverse events include adverse events that started on or after the date of first dose and up to 63 days after the date of the last dose of study

Luspatercept data pool

In the Luspatercept Data Pool, the SOCs with the highest subject incidence of TEAEs in the pooled luspatercept treatment group were Infections and infestations, General disorders and administration site conditions, and Musculoskeletal and connective tissue disorders. The proportion of subjects with TEAEs in any SOC was generally numerically higher in the pooled luspatercept treatment group than in the pooled placebo treatment group. The most frequently reported TEAEs (at least 15% of subjects) in the pooled luspatercept treatment group were headache (Nervous system disorders SOC); back pain, bone pain, and arthralgia (Musculoskeletal and connective tissue disorders SOC); diarrhoea (Gastrointestinal disorders SOC); fatigue and pyrexia (General disorders and administration site conditions SOC); and cough (Respiratory, thoracic and mediastinal disorders SOC). This largely corresponds with the most frequently reported SOCs. With the exception of upper respiratory tract infection and pyrexia, TEAEs reported in at least 15% subjects in either treatment group were reported more frequently in the pooled luspatercept treatment group than in the pooled placebo treatment group. (For treatment-emergent AEs reported for at least 10% of subjects see clinical AR or Table 53 in the ISS).

MDS Data pool

Table 54: MDS Data Pool: Subject Incidence of TEAEs Reported for At Least 10% of Subjects in Either Treatment Group (Safety Population)

System Organ Class Preferred Term	Phase 2 (A536-03/-05)	Pivotal Phase 3 (Study ACE-536-MDS-001)		MDS Data Pool
	Luspatercept All N = 107 n (%) [EAIR][a]	Luspatercept 1.0 mg/kg N = 153 n (%) [EAIR][a]	Placebo N = 76 n (%) [EAIR][a]	Pooled Luspatercept N = 260 n (%) [EAIR][a]
Subjects with at least 1 TEAE	99 (92.5)	150 (98.0)	70 (92.1)	249 (95.8)

Vascular disorders	38 (35.5) [53.5]	34 (22.2) [29.3]	11 (14.5) [28.1]	72 (27.7) [38.5]
Hypertension	26 (24.3)	13 (8.5)	6 (7.9)	39 (15.0)
Nervous system disorders	40 (37.4) [51.6]	72 (47.1) [82.0]	21 (27.6) [59.8]	112 (43.1) [67.8]
Dizziness	9 (8.4)	30 (19.6)	4 (5.3)	39 (15.0)
Headache	14 (13.1)	24 (15.7)	5 (6.6)	38 (14.6)
Infections and infestations	63 (58.9) [107.8]	82 (53.6) [94.0]	31 (40.8) [92.2]	145 (55.8) [99.5]
Viral upper respiratory tract infection	22 (20.6)	12 (7.8)	4 (5.3)	34 (13.1)
Bronchitis	12 (11.2)	17 (11.1)	1 (1.3)	29 (11.2)
Urinary tract infection	9 (8.4)	17 (11.1)	4 (5.3)	26 (10.0)
Gastrointestinal disorders	40 (37.4) [51.8]	89 (58.2) [113.8]	27 (35.5) [81.9]	129 (49.6) [83.0]
Diarrhoea	18 (16.8)	34 (22.2)	7 (9.2)	52 (20.0)
Nausea	9 (8.4)	31 (20.3)	6 (7.9)	40 (15.4)
Constipation	2 (1.9)	17 (11.1)	7 (9.2)	19 (7.3)
General disorders and administration site conditions	52 (48.6) [84.1]	109 (71.2) [164.9]	39 (51.3) [141.2]	161 (61.9) [125.8]
Fatigue	25 (23.4)	41 (26.8)	10 (13.2)	66 (25.4)
Oedema peripheral	14 (13.1)	25 (16.3)	13 (17.1)	39 (15.0)
Asthenia	2 (1.9)	31 (20.3)	9 (11.8)	33 (12.7)
Injury, poisoning and procedural complications	22 (20.6) [24.1]	30 (19.6) [24.3]	18 (23.7) [45.1]	52 (20.0) [24.2]
Fall	9 (8.4)	15 (9.8)	9 (11.8)	24 (9.2)
Musculoskeletal and connective tissue disorders	44 (41.1) [61.9]	66 (43.1) [74.9]	29 (38.2) [91.5]	110 (42.3) [69.1]
Back pain	5 (4.7)	29 (19.0)	5 (6.6)	34 (13.1)
Myalgia	14 (13.1)	13 (8.5)	5 (6.6)	27 (10.4)
Arthralgia	10 (9.3)	8 (5.2)	9 (11.8)	18 (6.9)
Respiratory, thoracic and mediastinal disorders	35 (32.7) [44.7]	60 (39.2) [59.8]	28 (36.8) [82.6]	95 (36.5) [53.2]
Cough	12 (11.2)	27 (17.6)	10 (13.2)	39 (15.0)
Dyspnoea	14 (13.1)	23 (15.0)	5 (6.6)	37 (14.2)

EAIR = exposure-adjusted incidence rate; MDS = myelodysplastic syndromes; MedDRA = Medical Dictionary for Regulatory Activities; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.

[a] Exposure-adjusted incidence rates per 100 subject-years. The EAIR per 100 subject years is 100 times the number of subjects with the specific TEAE divided by the total exposure time (in years) to the event. Exposure time is the overall treatment exposure for subjects without the event and the time up to the first event start date for subjects with the event.

Note: Treatment-emergent adverse events include adverse events that started on or after the date of first dose and up to 63 days after the date of the last dose of study treatment. If a subject experienced multiple events under the

same SOC and PT, then the subject was counted only once for that SOC and PT level. MedDRA version 20.0 was for coding.

As of the initial data cut-off date, the most frequently reported (≥ 15.0%) TEAEs for the pooled luspatercept treatment group of the MDS Data Pool were fatigue, diarrhea, nausea, cough, dizziness, hypertension, and peripheral edema. As of the data cut-off date of 07 Jan 2019, in addition to the preferred terms mentioned above, headache, viral upper respiratory tract infection, and back pain were reported at an incidence of ≥ 15.0%. In general, compared with those reported in the original MAA, the nature and estimated incidence rates of TEAEs have not changed, and the EAIRs for the most frequently reported adverse event preferred terms (except peripheral edema) did not increase.

ß-Thalassemia Data Pool

Table 55: β-thalassemia Data Pool: Subject Incidence of TEAEs Reported for At Least 10% of Subjects in Any Treatment Group (Safety Population)

System Organ Class Preferred Term	Phase 2 (A536-04/-06)	Pivotal Phase 3 (Study ACE-536-B-THAL-001)		β-thalassemia Data Pool
	Luspatercept All N = 64 n (%) [EAIR][a]	Luspatercept 1.0 mg/kg N = 223 n (%) [EAIR][a]	Placebo N = 109 n (%) [EAIR][a]	Pooled Luspatercept N = 287 n (%) [EAIR][a]
Subjects with at least 1 TEAE	64 (100.0)	214 (96.0)	101 (92.7)	278 (96.9)
Musculoskeletal and connective tissue disorders	55 (85.9) [264.2]	137 (61.4) [107.1]	61 (56.0) [84.0]	192 (66.9) [129.1]
Back pain	20 (31.3)	61 (27.4)	32 (29.4)	81 (28.2)
Bone pain	34 (53.1)	44 (19.7)	9 (8.3)	78 (27.2)
Arthralgia	26 (40.6)	43 (19.3)	13 (11.9)	69 (24.0)
Myalgia	29 (45.3)	22 (9.9)	11 (10.1)	51 (17.8)
Pain in extremity	9 (14.1)	21 (9.4)	9 (8.3)	30 (10.5)
Musculoskeletal pain	15 (23.4)	14 (6.3)	9 (8.3)	29 (10.1)
Neck pain	7 (10.9)	10 (4.5)	8 (7.3)	17 (5.9)
Nervous system disorders	46 (71.9) [118.3]	90 (40.4) [48.5]	32 (29.4) [33.1]	136 (47.4) [60.6]
Headache	39 (60.9)	58 (26.0)	26 (23.9)	97 (33.8)
Dizziness	11 (17.2)	25 (11.2)	5 (4.6)	36 (12.5)
General disorders and administration site conditions	47 (73.4) [190.2]	105 (47.1) [60.2]	45 (41.3) [52.4]	152 (53.0) [76.4]
Pyrexia	31 (48.4)	36 (16.1)	23 (21.1)	67 (23.3)
Asthenia	28 (43.8)	22 (9.9)	11 (10.1)	50 (17.4)
Fatigue	4 (6.3)	30 (13.5)	14 (12.8)	34 (11.8)
Injection site pain	8 (12.5)	8 (3.6)	3 (2.8)	16 (5.6)
Oedema peripheral	7 (10.9)	3 (1.3)	1 (0.9)	10 (3.5)
Infections and infestations	47 (73.4) [173.3]	141 (63.2) [93.0]	63 (57.8) [90.9]	188 (65.5) [105.2]
Upper respiratory tract infection	3 (4.7)	59 (26.5)	36 (33.0)	62 (21.6)

Influenza	16 (25.0)	19 (8.5)	6 (5.5)	35 (12.2)
Pharyngitis	11 (17.2)	20 (9.0)	13 (11.9)	31 (10.8)
Viral upper respiratory tract infection	9 (14.1)	14 (6.3)	2 (1.8)	23 (8.0)
Gastroenteritis	8 (12.5)	12 (5.4)	8 (7.3)	20 (7.0)
Rhinitis	16 (25.0)	3 (1.3)	4 (3.7)	19 (6.6)
Gastrointestinal disorders	40 (62.5) [97.0]	80 (35.9) [41.0]	36 (33.0) [36.8]	120 (41.8) [50.8]
Diarrhoea	25 (39.1)	27 (12.1)	11 (10.1)	52 (18.1)
Nausea	11 (17.2)	20 (9.0)	6 (5.5)	31 (10.8)
Abdominal pain	10 (15.6)	18 (8.1)	7 (6.4)	28 (9.8)
Vomiting	10 (15.6)	18 (8.1)	8 (7.3)	28 (9.8)
Abdominal pain upper	9 (14.1)	15 (6.7)	7 (6.4)	24 (8.4)
Dyspepsia	7 (10.9)	11 (4.9)	1 (0.9)	18 (6.3)
Respiratory, thoracic and mediastinal disorders	33 (51.6) [63.4]	71 (31.8) [33.6]	29 (26.6) [28.5]	104 (36.2) [39.5]
Oropharyngeal pain	21 (32.8)	28 (12.6)	12 (11.0)	49 (17.1)
Cough	16 (25.0)	32 (14.3)	12 (11.0)	48 (16.7)
Epistaxis	7 (10.9)	5 (2.2)	2 (1.8)	12 (4.2)

EAIR = exposure-adjusted incidence rates; MedDRA = Medical Dictionary for Regulatory Activities; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.

[a] Exposure-adjusted incidence rates per 100 subject-years. The EAIR per 100 subject years is 100 times the number of subjects with the specific TEAE divided by the total exposure time (in years) to the event. Exposure time is the overall treatment exposure for subjects without the event and the time up to the first event start date for subjects with the event.

Note: Treatment-emergent adverse events include adverse events that started on or after the date of first dose and up to 63 days after the date of the last dose of study treatment. If a subject experienced multiple events under the same SOC and PT, then the subject was counted only once for that SOC and PT level. MedDRA version 20.0 was for coding.

As of the initial data cut-off date, the most frequently reported (≥ 15.0%) TEAEs for the pooled luspatercept treatment group of the β-thalassemia data pool were headache, back pain, bone pain, arthralgia, pyrexia, upper respiratory tract infection, diarrhea, asthenia, oropharyngeal pain, and cough. As of the 07 Jan 2019 data cutoff date, no additional preferred terms were reported at an incidence of ≥ 15.0%. In general, compared with those reported in the original MAA, the nature and estimated incidence rates of TEAEs have not changed, and the EAIRs for individual adverse event preferred terms did not increase.

An analysis of adverse effects in relation to dose is discussed in the clinical AR and the ISS. No dose dependency of AEs could conclusively be determined.

Adverse events of special interest

Luspatercept data pool

Table 56: Summary of Adverse Events of Interest – Luspatercept Data Pool

Adverse Event of Interest Category	Luspatercept Data Pool	
	Pooled Luspatercept (N = 571) n (%)	Pooled Placebo (N = 193) n (%)
Malignancy EOI	16 (2.8)	1 (0.5)
Premalignant Disorder EOI	9 (1.6)	3 (1.6)
Embolic and Thrombotic Events EOI	23 (4.0)	4 (2.1)
Kidney Injury EOI	33 (5.8)	7 (3.6)
Hypertension EOI	64 (11.2)	11 (5.7)
Hypersensitivity Type Reactions EOI	48 (8.4)	8 (4.1)
Musculoskeletal Disorder-Bone Pain EOI	214 (37.5)	57 (29.5)

Myelodysplastic syndromes; EOI = event of interest.

Malignancy EOI

Malignancies were reported in the MDS data pool but not in the β-thalassaemia data pool as of the data cut-off date 11 May 2018. (Note: After database lock, a 27 year old β-thalassaemia patient developed unconfirmed AML M6 (acute erythroid leukaemia) after 27 treatment cycles and died from sepsis due to neutropenia. The histopathological diagnosis of AML M6 was confounded by the effects of erythroid hyperplasia due to the underlying beta-thalassaemia. Molecular screening found no mutations diagnostic for AML, which are present in almost 90% of AML cases, but could also not definitely rule out the possibility of erythroleukemia. In summary, the experts concluded that AML M6 cannot with absolute certainty be ruled out, however, available data points to a very unlikely probability of this diagnosis.

In the β-thalassemia clinical program, the only confirmed malignancy reported to up to the new cut-off data of July 2019 was a single event of hepatocellular carcinoma. An increased rate of hepatocellular carcinoma has been reported in patients with thalassemia (Borgna-Pignatti, 2014; Finianos, 2018; Zanella, 2016).

Table 57: Malignancy Events of Interest – MDS Data Pool

MDS Data Pool

SPM Category Preferred Term[a]	Phase 2 (A536-03/-05)	Pivotal Phase 3 (ACE-536-MDS-001)		MDS Data Pool
	Luspatercept All N = 107 n (%) [EAIR[b]]	Luspatercept 1.0 mg/kg N = 153 n (%) [EAIR[b]]	Placebo N = 76 n (%) [EAIR[b]]	Pooled Luspatercept N = 260 n (%) [EAIR[b]]
Malignancy EOI (95% confidence interval)	9 (8.4) [8.8] (4.6 – 16.9)	6 (3.9) [4.4] (2.0 – 9.9)	1 (1.3) [2.2] (0.3-15.9)	15 (5.8) [6.3] (3.8 – 10.5)
Hematologic Malignancies				

Transformation to AML[c] (95% confidence interval)	4 (3.7) [3.7] (1.4 – 9.9)	3 (2.0) [2.2] (0.7 – 6.8)	1 (1.3) [2.2] (0.3 – 15.9)	7 (2.7) [2.9] (1.4 – 6.0)
Solid Tumors				
Bronchial carcinoma	1 (0.9) [0.9]	0	0	1 (0.4) [0.4]
Non-small cell lung cancer	1 (0.9) [0.9]	0	0	1 (0.4) [0.4]
Renal cell carcinoma	1 (0.9) [1.0]	0	0	1 (0.4) [0.4]
Non-Melanoma Skin Cancer				
Basal cell carcinoma	1 (0.9) [0.9]	2 (1.3) [1.5]	0	3 (1.2) [1.2]
Squamous cell carcinoma	1 (0.9) [0.9]	0	0	1 (0.4) [0.4]
Squamous cell carcinoma of skin	0	1 (0.7) [0.7]	0	1 (0.4) [0.4]

AML = acute myeloid leukemia; EAIR = exposure-adjusted incidence rate; EOI = event of interest; MDS = myelodysplastic syndromes; MedDRA = Medical Dictionary for Regulatory Activities; SMQ = Standardized MedDRA Query; SPM = second primary malignancy; TEAE = treatment-emergent adverse event.

[a] Preferred terms are presented in descending order of subject incidence in the Pooled Luspatercept column for the MDS Data Pool and then the β-thalassemia Data Pool.

[b] Exposure-adjusted incidence rates per 100 subject-years. EAIR per 100 subject years is 100 times the number of subjects with the specific TEAE divided by the total exposure time (in years) to the event. Exposure time is the overall treatment exposure for subjects without the event and the time up to the first event start date for subjects with the event.

[c] Progression to AML.

In the MDS data pool, malignancy EOIs were reported for 15 subjects (5.8%; exposure-adjusted incidence rate (EAIR) 6.3/100 subject-years; 95% CI, 3.8 to 10.5) in the pooled luspatercept treatment group and 1 subject (1.3%; EAIR 2.2/100 subject-years; 95% CI, 0.3 to 15.9) in the placebo group. The 95% CIs were overlapping for the pooled luspatercept and placebo treatment groups for the EAIRs of malignancy EOIs.

The most frequently reported malignancy EOI in luspatercept-treated subjects in the MDS data pool was transformation to AML in 7 subjects (2.7%; EAIR 2.9/100 subject years) followed by basal cell carcinoma in 3 subjects (1.2%; EAIR 1.2/100 subject-years). All other malignancy EOIs were reported in 1 subject each (0.4%; EAIR 0.4/100 subject-years). The Applicant claims that the overall rates of malignancies were within the expected range for an elderly cohort with MDS (Balleari, 2015; Falantes, 2017).

Data cut-off date of 07 Jan 2019

As of the data cut-off date of 07 Jan 2019, malignancy EOIs were reported for 8.6% of subjects (EAIR 7.4 per 100 subject-years). There were no new malignancy events reported in the Phase 3 study, as of the data cutoff date of 07 Jan 2019. All new malignancies were reported from the Phase 2 studies, which included a subject population who had more follow-up time, compared to subjects in the Phase 3 study, and more indicators of severe disease, as described in the original MAA.

Data cut-off dates of 01 Jul 2019 (Phase 3 Study) and 13 Jul 2019 (Phase 2 Studies)

As of the data cut-off dates of 01 Jul 2019 (for the Phase 3 study) and 13 Jul 2019 (for the Phase 2 studies), 2 additional treatment-emergent malignancy events were reported since 07 Jan 2019. One subject in the luspatercept treatment group of the Phase 3 study reported basal cell carcinoma and one subject in the Phase 2 studies had transformation to AML.

Premalignant Disorder EOI

Premalignant disorder EOIs were reported in the MDS Data Pool but not in the β-thalassemia Data Pool.

Table 58: Progression to AML and High-risk MDS

MDS Data Pool

	Phase 2 (A536-03/-05)	Pivotal Phase 3 (ACE-536-MDS-001)		MDS Data Pool
	Luspatercept All N = 107 n (%) [EAIR[b]] (95% CI)	Luspatercept 1.0 mg/kg N = 153 n (%) [EAIR[b]] (95% CI)	Placebo N = 76 n (%) [EAIR[b]] (95% CI)	Pooled Luspatercept N = 260 n (%) [EAIR[b]] (95% CI)
Progression to AML				
Transformation to AML[c]	4 (3.7) [3.7] (1.4 - 9.9)	3 (2.0) [2.2] (0.7 – 6.8)	1 (1.3) [2.2] (0.3 – 15.9)	7 (2.7) [2.9] (1.4 – 6.0)
Progression to High-risk MDS				
Myelodysplastic syndromes[d]	6 (5.6) [5.6] (2.5 – 12.5)	0	1 (1.3) [2.2] (0.3 – 15.9)	6 (2.3) [2.5] (1.1 – 5.5)
Refractory anaemia with an excess of blasts[d]	0	1 (0.7) [0.7] (0.1 – 5.2)	0	1 (0.4) [0.4] (0.1 – 2.9)

AML = acute myeloid leukemia; CI = confidence interval; EAIR = exposure-adjusted incidence rate; MDS = myelodysplastic syndromes; MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent adverse event.

[a] Preferred terms are presented in descending order of subject incidence in the Pooled Luspatercept column for the MDS Data Pool.

[b] Exposure-adjusted incidence rates per 100 subject-years. EAIR per 100 subject years is 100 times the number of subjects with the specific TEAE divided by the total exposure time (in years) to the event. Exposure time is the overall treatment exposure for subjects without the event and the time up to the first event start date for subjects with the event.

[c] Progression to AML

[d] Progression to high-risk MDS

Juvenile animal toxicity studies with luspatercept have identified a potential signal for malignant transformation.

A total of 5 premalignant disorder EOIs were reported in the Phase 3 study in 2 subjects (1.3%; EAIR 1.5/100 subject-years) in the luspatercept group and in 3 subjects (3.9%; EAIR 7.0/100 subject-years) in the placebo group. Progression to high-risk MDS was reported for 1 subject in the luspatercept group (0.7%; EAIR 0.7/100 subject-years) with the PT "refractory anaemia with an excess of blasts" and for 1 subject (1.3%; EAIR 2.2/100 subject-years) in the placebo group. In addition, 1 subject (0.7%; EAIR 0.7/100 subject years) in the luspatercept group had intraductal papillary mucinous neoplasm and 2 subjects (2.6%; EAIR 4.7/100 subject-years) in the placebo group had actinic keratosis.

A proportion of patients with lower-risk MDS is expected to progress to higher-risk MDS or AML as part of the natural history of the disease. The rate of disease progression is influenced by a number of

prognostic factors. In the review of disease progression, it should be noted that the Phase 2 and Phase 3 populations were different as to prognostic factors. The Applicant claims that, as compared to the Phase 3 population, the Phase 2 population had more indicators of severe disease such as higher IPSS-R scores, lower rates of SF3B1 mutation (indicator of lower risk), and 35% of patients being ring sideroblast negative. Updated safety data shows that progression to AML occurred in 2 (2.6%) patients receiving placebo and 3 (2%) patients receiving luspatercept in the pivotal study. There was no signal for earlier transformation in luspatercept versus placebo treated subjects. Seven patients (6%) from the phase II studies showed disease progression at the updated cut-off July 2019.

An in-depth assessment was conducted to determine the background rate of clinical disease progression from other controlled clinical trials in a similar population of subjects with MDS and from an external MDS registry, and to further inform whether luspatercept treatment had any measurable impact on that rate. According to the Applicant, the outcome of that analysis indicates that luspatercept treatment does not pose an increased risk of progression to higher-risk MDS or AML as compared to placebo in the pivotal Phase 3 study and compared to background rates from the lenalidomide studies and external MDS registry data.

Kidney Injury EOI

Table 59: Kidney Injury Events of Interest – MDS Data Pool

	MDS Data Pool			
	Phase 2 (A536-03/-05)	Pivotal Phase 3 (ACE-536-MDS-001)		MDS Data Pool
	Luspatercept All N = 107 n (%) [EAIR[b]]	Luspatercept 1.0 mg/kg N = 153 n (%) [EAIR[b]]	Placebo N = 76 n (%) [EAIR[b]]	Pooled Luspatercept N = 260 n (%) [EAIR[b]]
Kidney Injury EOI[c]	6 (5.6) [5.7]	15 (9.8) [11.4]	4 (5.3) [9.2]	21 (8.1) [8.9]
Renal failure	2 (1.9) [1.9]	7 (4.6) [5.2]	2 (2.6) [4.5]	9 (3.5) [3.7]
Acute kidney injury	3 (2.8) [2.8]	4 (2.6) [3.0]	0	7 (2.7) [2.9]
Blood creatinine increased	2 (1.9) [1.9]	4 (2.6) [3.0]	2 (2.6) [4.6]	6 (2.3) [2.5]
Proteinuria	0	1 (0.7) [0.7]	0	1 (0.4) [0.4]
Renal impairment	0	1 (0.7) [0.7]	0	1 (0.4) [0.4]

EAIR = exposure-adjusted incidence rate; EOI = event of interest; MDS = myelodysplastic syndromes; MedDRA = Medical Dictionary for Regulatory Activities; SMQ = Standardized MedDRA Query.

[a] Preferred terms are presented in descending order of subject incidence in the Pooled Luspatercept column for the MDS Data Pool.

[b] Exposure-adjusted incidence rate in events per 100 subject-years. EAIR per 100 subject years is 100 times the number of subjects with the specific TEAE divided by the total exposure time (in years) to the event. Exposure time is the overall treatment exposure for subjects without the event and the time up to the first event start date for subjects with the event.

[c] The preferred terms used to evaluate this EOI category were based on the MedDRA broad scope of SMQ acute renal failure.

Table 60: Kidney Injury Events of Interest - β-thalassemia Data Pool

	β-thalassemia Data Pool			
	Phase 2 (A536-04/-06)	Pivotal Phase 3 (ACE-536-B-THAL-001)		β-thalassemia Data Pool
	Luspatercept All N = 64 n (%) [EAIR[b]]	Luspatercept 1.0 mg/kg N = 223 n (%) [EAIR[b]]	Placebo N = 109 n (%) [EAIR[b]]	Pooled Luspatercept N = 287 n (%) [EAIR[b]]
Kidney Injury EOI[c]	4 (6.3) [4.4]	8 (3.6) [3.1]	3 (2.8) [2.5]	12 (4.2) [3.5]
Proteinuria	4 (6.3) [4.4]	5 (2.2) [1.9]	2 (1.8) [1.7]	9 (3.1) [2.6]
Blood creatinine increased	1(1.6) [1.1]	2 (0.9) [0.8]	1 (0.9) [0.8]	3 (1.0) [0.8]
Blood urea increased	1 (1.6) [1.1]	0	0	1 (0.3) [0.3]
Creatinine renal clearance decreased	0	1 (0.4) [0.4]	0	1 (0.3) [0.3]
Glomerular filtration rate decreased	0	1 (0.4) [0.4]	0	1 (0.3) [0.3]

EAIR = exposure-adjusted incidence rate; EOI = event of interest; MedDRA = Medical Dictionary for Regulatory Activities; SMQ = Standardized MedDRA Query.

[a] Preferred terms are presented in descending order of subject incidence in the Pooled Luspatercept column for the β-thalassemia Data Pool.

[b] Exposure adjusted incidence rates in events per 100 subject years is 100 times the number of subjects with the specifc TEAE divided by the total exposure time (in years) to the event. Exposure

In the clinical phase 3 studies for MDS and ß-thalassemia, some events related to the kidney occurred more often in the luspatercept group. There were adverse kidney findings in non-clinical studies with luspatercept (see non-clinical part).

The impact on renal function (creatinine clearance) and on proteinuria was also investigated in the trials. Among luspatercept-treated subjects with renally associated AEs, renal function generally recovered substantially while the subject was still on treatment. Mean ACR (albumin/creatinine ratio) values remained clinically stable over time, with no prolonged elevations of mean ACR values in subjects in the Phase 2 and Phase 3 studies. According to the Applicant, administration of luspatercept was not associated with prolonged or irreversible worsening of clinically important indicators of kidney injury over the course of treatment.

The Applicant has provided a comprehensive discussion on kidney toxicity and a detailed overview of the kidney function parameters in the pivotal trial for beta-thalassaemia for subjects both on placebo and luspatercept. Comparative data of the safety profile for exposure below and above 48 weeks does not show an increase in renal AEs with longer treatment duration. Kidney parameters will further be monitored in the long-term extension study to gain additional information. Influencing factors for patients with beta-thalassaemia are chronic anaemia, hypoxia and iron overload as well as a possible role of iron chelators, which all contribute to the deterioration of renal function (Demosthenous et al, 2019, Hematology, 24:1, 426-438).

Embolic and Thrombotic EOI

For subjects with MDS, the assessment was unchanged from the original MAA with no difference in TEEs observed between luspatercept and placebo in the Phase 3 study.

An imbalance in thromboembolic events was observed in subjects treated with luspatercept in study ACE-536-B-THAL-001. Therefore, embolic and thromboembolic events (TEE) were further analysed.

The incidence of embolic and thrombotic events EOIs in the luspatercept data pool was 4.0% (23 subjects) in the pooled luspatercept treatment group and 2.1% (4 subjects) in the pooled placebo treatment group (similar if exposure adjusted). The higher incidence in the luspatercept pool was driven by splenectomised subjects from the β-thalassaemia data pool. The incidence of Grade 3 or 4 embolic and thrombotic events EOIs was similar between the pooled luspatercept treatment group (1.8%) and pooled placebo treatment group (1.0%). All of the Grade 3 or 4 embolic and thrombotic events EOIs were each experienced by at most 1 or 2 subjects per pooled treatment group.

Thromboembolic events are common complications of thalassaemia, especially thalassaemia intermedia (TI). The increased risk of thromboembolic events is likely due to abnormalities in platelet, red blood cell, endothelial cell, and thrombin activation which all contribute to hypercoagulable state (Thiersch, 2017; Taher, 2010 a,b). In addition to these haematological abnormalities, splenectomy has also been shown to be a major risk factor contributing to hypercoagulability among patients with thalassemia (Natesirinilkul, 2016). In the Phase 3 luspatercept treatment group in the ß-thalassemia study, 4.5% of subjects had a known history of thrombosis. The majority of subjects in the luspatercept and placebo treatment groups had a splenectomy prior to study participation (57.4% and 56.9%, respectively). All subjects who experienced thromboembolic events in this study were splenectomised. Occurrence of TEE was not correlated with elevated Hb levels.

As of the initial data cut-off date, in the pooled luspatercept treatment group of the β-thalassemia Data Pool, thromboembolic events (TEEs) were reported for 12 subjects (4.2%, EAIR 3.4 per 100 subject-years). All preferred terms in this category were reported by single subjects, with the exceptions of cerebrovascular accident, deep vein thrombosis, and thrombophlebitis superficial (3 subjects each, 1.0%, EAIR 0.8 per 100 subject-years).

As of the 07 Jan 2019 data cut-off date, TEEs were reported for 14 subjects (4.9%, EAIR 2.8 per 100 subject-years) in the pooled luspatercept treatment group of the β-thalassemia Data Pool excluding the cross-over subjects and 16 subjects (4.2%, EAIR 2.4 per 100 subject-years) including the cross-over subjects. Two subjects in the Phase 3 Study ACE-536-B-THAL-001 experienced transient ischemic attack since the original MAA, compared with no subject at the time of the original MAA. In the Phase 3 study, 1 event of ischemic stroke reported in the original MAA was updated to transient ischemic attack; this diagnosis was confirmed by imaging. There were 4 new subjects who reported TEEs, all of whom were splenectomized and had other recognised risk factors, including hypertension, increased platelets, history of thrombosis, and use of sex hormones.

As of the data cut-off dates of 01 Jul 2019 (for the Phase 3 study) and 13 Jul 2019 (for the Phase 2 studies), there was 1 additional TEAE under the Standardized MedDRA Query (SMQ) of TEE reported since the data cut-off date of 07 Jan 2019. The event, transient ischaemic attack, occurred in the Phase 3 Study ACE-536-B-THAL-001. However, this event was non-thrombotic/embolic in nature because there was no evidence of vessel occlusion. The event involved a 49-year-old female splenectomized subject with relevant medical history of hypertension.

Hypertension EOI

In the MDS Phase 3 study, the proportion of TEAEs was balanced; however, in the β-thalassemia Phase 3 study, there was a higher rate of hypertension TEAEs in the luspatercept treatment group, although proportions were generally less compared to the MDS population. None of these events were serious or led to treatment discontinuation. One (0.3%) subject in the beta-thalassaemia pool had an SAE of hypertension (Updated Safety 07Jan2019. Patients treated with Reblozyl had an average increase in systolic and diastolic blood pressure of 5 mmHg from baseline.

The incidence rate of hypertension TEAEs was similar in for the updated safety report compared to that in the initial data cut-off.

Hypersensitivity Type Reactions EOI

The incidence of hypersensitivity type reactions EOIs in the luspatercept data pool, independent of the development of ADA, was higher in the pooled luspatercept treatment group (8.4%) than in the pooled placebo treatment group (4.1%), also for the EAIR. The imbalance was primarily driven by injection site reactions (eg, PTs of injection site erythema, injection site pruritus, injection site swelling, injection site reaction). None of the PTs that comprise the hypersensitivity type reactions EOI had an incidence of ≥ 2% in any treatment group. All of the hypersensitivity type reactions EOIs were Grade 1 or 2 in severity. No Grade 3 or higher hypersensitivity type reactions EOIs were reported in either treatment group. All hypersensitivity type reactions EOIs were nonserious. In the luspatercept clinical program, no anaphylactic reactions have been reported.

Musculoskeletal Disorder-Bone Pain EOI Category

The incidence of musculoskeletal disorder-bone pain EOIs in the Luspatercept Data Pool was higher in the pooled luspatercept treatment group (37.5%) than in the pooled placebo treatment group (29.5%) (similar if exposure adjusted). The most frequently reported musculoskeletal disorder-bone pain EOIs (≥ 10% of subjects in either treatment group) were back pain and bone pain. The incidence of back pain was similar between the pooled luspatercept treatment group (20.3%) and the pooled placebo treatment group (19.2%), whereas the incidence of bone pain was higher in the pooled luspatercept treatment group (15.9%) compared with the pooled placebo treatment group (6.2%), which accounted for the imbalance in the overall incidence. Bone pain is common in patients with β-thalassemia and the imbalance was primarily driven by subjects in the β-thalassemia studies.

The incidence of Grade 3 musculoskeletal disorder-bone pain EOIs was 2.6% in the pooled luspatercept treatment group and 1.0% in the pooled placebo treatment group. In the pooled luspatercept treatment group, the only Grade 3 events were bone pain and back pain (7 subjects each; 1.2%) and spinal pain (1 subject; 0.2%). In the pooled placebo treatment group, the only Grade 3 events were bone pain and back pain (1 subject each; 0.5%). No Grade 4 or higher musculoskeletal disorder-bone pain EOIs were reported. The median time to the first event was earlier in the pooled luspatercept treatment group compared with the pooled placebo treatment group (23.5 versus 61.0 days, respectively). However, the median total duration of musculoskeletal disorder-bone pain EOI was lower in the pooled luspatercept treatment group than in the pooled placebo treatment group (32.0 versus 53.0 days), respectively.

Serious adverse event/deaths/other significant events

Deaths

Considering the original data cut-off for MAA, a total of 27 subjects in the Luspatercept Data Pool died during the study (on treatment and off treatment): 17 subjects (3.0%) in the pooled luspatercept treatment group and 10 subjects (5.2%) in the pooled placebo treatment group.

While on treatment, 14 subjects died: 9 subjects (1.6%) in the pooled luspatercept treatment group and 5 subjects (2.6%) in the pooled placebo treatment group. The majority of these subjects died within 12 months after receiving the first dose of treatment.

While off treatment, 13 subjects died: 8 subjects (1.4%) in the pooled luspatercept treatment group and 5 subjects (2.6%) in the pooled placebo treatment group. The majority of these subjects died between 12 and 24 months after receiving the first dose of IP.

There were 10 subjects (1.8%) in the pooled luspatercept treatment group and 5 subjects (2.6%) in the pooled placebo treatment group who had Grade 5 (fatal) TEAEs. The majority of Grade 5 (fatal) TEAEs were experienced by 1 subject each, with the exception of sepsis (2 subjects [0.4%], pooled luspatercept) and general physical health deterioration (1 subject [0.2%], pooled luspatercept and 1 subject [0.5%], pooled placebo).

Similar to the underlying demographics of the population, the majority of subjects in the Luspatercept Data Pool who had a Grade 5 (fatal) TEAE were male (11 of 15 subjects), white (11 of 15 subjects), and from North America or Europe (14 of 15 subjects).

MDS

A total of 24 subjects in the MDS Data Pool died: 15 subjects (5.8%) in the pooled luspatercept treatment group and 9 subjects (11.8%) in the placebo treatment group. All deaths were assessed as not related or unlikely related to luspatercept by the investigator. There was no notable association between luspatercept treatment and type or frequency of events with fatal outcomes.

As of the data cut-off date of 07 Jan 2019, 19 additional deaths occurred in the pooled luspatercept treatment group bringing the total number of subjects in the pooled luspatercept treatment group who died to 34 (12.6%), and 5 additional deaths occurred in the placebo treatment group bringing the total number of subjects in the placebo treatment group who died to 14 (18.4%). Of the 19 additional deaths in the pooled luspatercept treatment group, 8 deaths occurred while the subject was on study and 11 deaths occurred while the subject was off study.

During the post-treatment phase of the study (off-study), 5 additional deaths occurred in the pooled luspatercept treatment group since the data cutoff date of 07 Jan 2019, bringing the total number of subjects in the pooled luspatercept treatment group who died to 39 (14.5%), and 4 additional deaths occurred in the placebo treatment group bringing the total number of subjects in the placebo treatment group who died to 18 (23.7%).

β-thalassaemia Studies

A total of 3 subjects in the β-thalassemia Data Pool died: 2 subjects (0.7%) in the pooled luspatercept treatment group and 1 subject (0.9%) in the placebo treatment group. None of the AEs leading to death were considered related to treatment.

After data cut-off, two further patients enrolled in study ACE-536-B-THAL-001 died:

- a young male adult died from neutropenia and sepsis following a diagnosis of AML M6 (Acute erythroid leukaemia).

The investigator assessed causal relationship between study therapy and fatal acute myeloid leukemia (M6) as suspected, and not suspected for neutropenia sepsis, neutropenic sepsis (second episode), thrombocytopenia, pancytopenia, multiple splenic abscess. Based on a study in Taiwan, which reported a 5.32-fold increased risk for haematological malignancy in thalassemia compared to the general population, and thalassemia patients given transfusions 9.31-fold more likely to develop haematological malignancy than non-transfused thalassemia patients (Chung WS et al, 2015), the investigator expressed underlying transfusion dependent thalassemia as possible risk factor to develop AML.

Additional information received including bone marrow biopsy showing erythroid hyperplasia with adequate granulopoiesis and megakaryopoiesis indicates that the pancytopenia is likely to be due to hypersplenism. The most recent bone marrow aspirate demonstrated mostly blastic cells belonging to erythropoiesis, with negative myeloid markers; the peripheral blood showed adequate granulocytosis

and no blasts. Based on the above picture it was considered difficult to discriminate between AML and thalassemia-related findings.

The sponsor considers the clinical course to be consistent with beta-thalassaemia major complicated by neutropenia possibly triggered by deferiprone therapy, splenomegaly/hypersplenism causing pancytopenia with subsequent sepsis, and does not attribute causality to study drug. A review of the biopsy samples by an independent pathologist concluded that the diagnosis of AML is not certain and further that it is still extremely unlikely that Luspatercept has played a causative role, or could have demonstrated changes in the bone marrow that can be compared to AML.

Independent expert opinion: all analyses after chemo no evidence of AML. At time of report, no confirmation of leukaemia. Independent molecular genetic analysis: unable to detect mutation in any of the typical predetermined breaking points of genes known in AML. Leukaemia mutations in at least one of the investigated genes are found in 90% of cases. Rare individual cases of malignant disease (MDS, AML) development known for thalassemia.

The Applicant has submitted full detailed documentation of this case. AML M6 cannot with absolute certainty be ruled out, however, available data point to a low probability of this diagnosis.

- A 57 year old male, died due to hepatic carcinoma and liver resection operation site infection.

The investigator assessed the causal relationship as follows: Well-differentiated hepatocellular carcinoma: blinded ACE-536 (not suspected). Per the investigator, the event was due to iron overload secondary to the underlying disease.

Updated safety data showed two additional on-study deaths in the luspatercept group, one was due to intracranial haemorrhage (post-neurosurgery), the other due to cardiac arrest in a subject with severe cardiac iron overload and suspected acute viral infection, leading to cardiac arrhythmia and death.

Serious adverse events

Table 61: SAE in Luspatercept Pool (Excerpt)

System Organ Class Preferred Term	Pooled Luspatercept N = 571 n (%)	Pooled Placebo N = 193 n (%)
Subjects with at least 1 SAE	136 (23.8)	29 (15.0)
Infections and infestations	50 (8.8)	13 (6.7)
Pneumonia	8 (1.4)	2 (1.0)
Urinary tract infection	4 (0.7)	2 (1.0)
Cellulitis	3 (0.5)	1 (0.5)
Sepsis	3 (0.5)	1 (0.5)
Septic shock	3 (0.5)	1 (0.5)
Device related infection	2 (0.4)	0
Erysipelas	2 (0.4)	0
Gastroenteritis	2 (0.4)	0
Lung infection	2 (0.4)	0
Vascular disorders	8 (1.4)	0
Deep vein thrombosis	2 (0.4)	0

Renal and urinary disorders	7 (1.2)	2 (1.0)
Acute kidney injury	2 (0.4)	0
Nephrolithiasis	2 (0.4)	0
Renal colic	2 (0.4)	0
Respiratory, thoracic and mediastinal disorders	7 (1.2)	2 (1.0)
Pulmonary embolism	2 (0.4)	0

Overall, the incidence of SAEs was higher in the pooled luspatercept treatment group (23.8%) than in the pooled placebo treatment group (15.0%). The SOC with the highest subject incidence of SAEs in the pooled luspatercept treatment group was Infections and infestations (incidence of 8.8%). Treatment-related SAEs were reported only in the pooled luspatercept treatment group (2.5% of subjects). Deep vein thrombosis was the only SAE assessed as related to treatment by the investigator experienced by > 1 subject (2 subjects [0.4%]).

In the MDS pool, the percentage of subjects with SAEs was higher in the pooled luspatercept treatment group (37.7%) than in the placebo treatment group (30.3%). The SOCs with the highest percentage of subjects with SAEs (at least 5% of subjects) in the pooled luspatercept treatment group were Infections and infestations; Neoplasms benign, malignant, and unspecified (including cysts and polyps); Cardiac disorders; Injury, poisoning and procedural complications; and Musculoskeletal and connective tissue disorders.

In the β-thalassemia data pool, the percentage of subjects with SAEs was higher in the pooled luspatercept treatment group (13.2%) than in the placebo treatment group (5.5%). The difference was driven by imbalances in the percentages of subjects with TEAEs in the Infections and infestations SOC (4.5% and 2.8%, respectively) and thromboembolic events, including cerebrovascular accident (1.0% and 0%) and deep vein thrombosis (0.7% and 0%).

For both indications, data provided with the newer cut-offs showed that the incidences and types of SAEs were similar to those in the original MAA.

Laboratory findings

In line with the intended PD effect of luspatercept, a treatment-dependent effect was observed for the incidence of haemoglobin > 11.5 g/dL: a higher proportion of subjects had haemoglobin > 11.5 g/dL in the pooled luspatercept treatment group (42.0%) than in the pooled placebo treatment group (28.0%).

A treatment-dependent effect was observed on the incidence of neutropenia (threshold < 0.5×10^9 cells/L; Table 61), with a nominally higher incidence in the pooled luspatercept treatment group than in the pooled placebo treatment group.

The incidence of thrombocytosis, based on a threshold of $\geq 500 \times 10^9$ cells/L, was treatment dependent, with a higher incidence in the pooled luspatercept treatment group than in the pooled placebo treatment group. For 8.1% of subjects (8.3/100 subject-years) in the pooled luspatercept treatment group and 5.7% of subjects (6.8/100 subject-years) in the pooled placebo treatment group, platelet counts were $\geq 1000 \times 10^9$ cells/L. 17 instances of TEEs occurred in 13 subjects (of whom 1 on placebo). Only three subjects had a baseline below 400 x10^9/L before the event, of whom 1 increased to 964 x109/L while the other two stayed below 400 x10^9/L. Reassuringly, the figures plotting platelet

count and occurrence of TEE over time for both populations do not show a spike in platelet numbers before the events of interest.

Table 62: Luspatercept Data Pool: Clinically Significant Abnormalities in Selected Hematology Parameters (Safety Population)

Threshold	Pooled Luspatercept N = 571 n (%)	Pooled Placebo N = 193 n (%)
Platelet count ≥ 1000×10^9 cells/L	46 (8.1)	11 (5.7)
Platelet count ≥ 500 to < 1000×10^9 cells/L	224 (39.2)	60 (31.1)
Hemoglobin > 11.5 g/dL	240 (42.0)	54 (28.0)

Neutrophil segmented was used for neutrophil count in the Phase 3 studies.

A trend towards elevated liver enzymes as well as bilirubin values can be observed in patients treated with luspatercept in both indications. Neither the mode of action nor the catabolic pathway of luspatercept are liver dependent. The underlying disease itself as well as concomitant medication/ infections etc. can influence liver parameters.

A slight decrease in creatinine clearance can be observed for patients in the luspatercept treatment groups versus patients who received placebo.

Safety in special populations

No clinically relevant differences were noted between age subgroups, genders, white- and non-white subjects or subjects with- and without splenectomy exposed to luspatercept.

No clinically relevant differences were noted across regions in the β-thalassemia data pool. There were too few subjects from the ROW (N = 3) compared to subjects from North America or Europe (N = 257) in the MDS Data Pool to draw any meaningful conclusions.

Analyses comparing incidences of TEAEs in patients with different degrees of hepatic impairment to that in patients with normal liver function found no specific trends towards a shift in AE profile in either the MDS, the beta-thalassaemia or the overall safety pool. Analyses according to renal function revealed no specific worrying trends for patients with mild or moderate impairment compared to patients with normal kidney function in any of the safety pools.

The safety of Luspatercept in children and adolescents under the age of 18 has not been established and this population is not included in the SmPC.

Pregnancy and lactation

Pregnant and lactating women were excluded from the study population and throughout the clinical development program. Female participants of childbearing potential and male participants in any luspatercept study were to use highly effective (Pearl index < 1% per year) birth control methods.

As of the safety data cut-off date, there are no data regarding the clinical effects of luspatercept in pregnancy. There have been no pregnancies reported in female subjects participating in luspatercept

clinical studies, and there was 1 pregnancy in the partner of a male subject in Study ACE-536-B-THAL-001. Given a lack of consent from the pregnant partner for collecting pregnancy data, no safety data for this pregnancy are available.

Updated safety data submitted during evaluation showed that an unintended pregnancy was reported in the Phase 3 study, requiring IP discontinuation. A 23-year-old subject experienced a spontaneous abortion on Study Day 517. The subject reported the last day of menses as Study Day 468, and had the last negative pregnancy test on Study Day 498. The subject had a positive pregnancy test on Study Day 505, with β-human chorionic gonadotropin of 1294.00 mIU/mL (0-3) and positive urine pregnancy test. After approximately 8 weeks of pregnancy, the subject complained of vaginal spotting and vaginal bleeding without abdominal pain. Based on the symptoms and ultrasound findings, the subject was diagnosed with threatened abortion. On Study Day 554, she had a follow-up abdominal ultrasound, which revealed a completed abortion. The event was not suspected to be related to study treatment (luspatercept) by the investigator.

Immunological events

Immunogenicity Assay Validation

The validation of the anti-drug antibody (ADA) methods and sample analysis were conducted in accordance with scientific guidance and in compliance with appropriate regulations.

Overall Incidence of Antidrug Antibodies in Clinical Studies

In the MDS population, in 8.85% of patients receiving luspatercept versus 3.95% receiving placebo developed treatment emergent ADAs. In β-thalassaemia, the incidence of TEADAs was lower, namely 1.41% of patients on luspatercept versus 1.87% of patients on placebo.

Preexisting ADAs could be determined in about 5% of MDS patients and ~ 1% of β-thalassaemia patients.

Correlation of Antidrug Antibody versus Safety

No obvious correlation of ADAs with specific TEAEs could be observed. The 4 instances of kidney injury observed in the MDS population, while positive for ADA, had significant underlying risk factors for kidney disease and were temporally associated with acute events that may lead to kidney injury.

Hypersensitivity

No systemic hypersensitivity reactions occurred in ADA positive subjects. In MDS, local reactions were observed in 4 patients who were ADA positive or had pre-existing ADA. However, similar reactions were also reported in ADA-negative patients in a comparable number. In β-thalassaemia, only ADA-negative subjects experienced hypersensitivity AEs or local reactions.

The observed influence of either pre-existing or treatment emergent anti-drug-antibodies on the safety profile of luspatercept appears limited, however, no long-term data are available.

Safety related to drug-drug interactions and other interactions

Drug-drug interaction studies with luspatercept have not been conducted.

Discontinuation due to adverse events

The incidence of TEAEs leading to treatment discontinuation was higher (8.8%) in the pooled luspatercept treatment group than in the pooled placebo treatment group (3.6%). AEs leading to study discontinuation were most often related to the mode of action of luspatercept (i.e. bone pain, back pain) or related to the disease (progression of MDS). In addition, in the β-thalassemia pool, nervous system disorders and headache were observed.

In the MDS Data Pool, the percentage of subjects with TEAEs leading to treatment discontinuation was similar in the pooled luspatercept (11.5%) and the placebo (7.9%) treatment groups. For the data cut-off date of 07 Jan 2019, the percentage of subjects with TEAEs leading to IP discontinuation was 16.4% (EAIR 13.7 per 100 subject-years) in the pooled luspatercept treatment group. The most frequently (≥ 1% of subjects) reported TEAEs leading to IP discontinuation in the pooled luspatercept treatment group were MDS (ie, progression to high-risk MDS; 7 subjects, 2.6%), transformation to AML (6 subjects, 2.2%), and general physical health deterioration and sepsis (3 subjects each, 1.1%).

In the β-thalassemia Data Pool, there were 19 luspatercept subjects in the pooled luspatercept treatment group (6.6%) and 1 (0.9%) subject in the placebo treatment group with TEAEs leading to discontinuation of IP. For the 07 Jan 2019 data cut-off date, the percentage of subjects with TEAEs leading to IP discontinuation was 8.7% (EAIR 5.0 per 100 subject-years) and 7.1% (EAIR 4.1 per 100 subject- years) in the pooled luspatercept treatment group of the β-thalassemia Data Pool excluding and including the cross-over subjects, respectively. As of the 07 Jan 2019 data cut-off date, arthralgia and bone pain remained the most frequently reported TEAEs leading to IP discontinuation, and the number of subjects reporting these events did not change.

Post marketing experience

Not post-marketing data were submitted in this application as luspatercept was not yet marketed in any country.

2.7.1. Discussion on clinical safety

Luspatercept is a novel first-in-class recombinant fusion protein that binds select transforming growth factor-beta (TGF-β) superfamily ligands and inhibits Smad2/3 signalling. In subjects suffering from MDS and β-thalassemia, Smad2/3 inhibition is expected to reduce the abnormal high Smad2/3 activity that hampers effective erythropoiesis.

While Luspatercept has shown promising effect on erythropoiesis during preclinical- and clinical trials, the mechanism of action and the implication of the interference in its totality ('off-target' effects, secondary pharmacodynamics) have not been extensively studied. The inhibition of TGF-ß superfamily ligands has many potential consequences for the body as TGF-ß signalling is involved in cellular development and differentiation of various tissues and cell types besides erythropoiesis. (Long-term) treatment with substances that regulate such pleiotropic pathways, especially pathways involved in cell growth, harbour an intrinsic potential of abnormal cell growth, among others.

The safety of luspatercept has been examined in a clinical development programme comprising 571 subjects exposed to luspatercept (including 260 subjects with myelodysplastic syndromes [MDS], 287 subjects with β-thalassemia, and 24 healthy, postmenopausal females) in 7 clinical studies, i.e. one phase I trial in healthy volunteers (A536-02), one open-label phase II ascending dose study followed by an extension study for each indication (MDS: A536-03 & A536-05; β-thal: A536-04 & A536-06) and one randomized, double-blind pivotal phase III study for each indication (MDS: ACE-536-MDS-001; β-

thal: ACE-536-B-THAL-001). Safety data are presented in 3 separate data pools: the total luspatercept Data pool, the MDS Data Pool and the β-thalassemia Data Pool. The total luspatercept pool includes 571 subjects, the total placebo pool 193 subjects.

Duration of exposure

For MDS, the mean treatment duration of the entire MDS data pool was 49.0 weeks (median 45.6), in the phase 2 studies (A536-03/-05) 52.4 weeks (median 30.9), and in the phase 3 study (ACE-536-MDS-001) 46.6 weeks (median 49.0) for Luspatercept-treated and 30.6 weeks (median 24.0) for placebo-treated subjects. As of the 07 Jan 2019 data cut-off date, in the pooled luspatercept treatment group of the MDS Data Pool, 269 subjects received at least 1 dose of luspatercept, increasing the overall exposure from 244.13 subject-years in the original MAA to 322.93 subject-years. The median total treatment duration increased from 45.6 weeks to 48.1 weeks, and the median number of doses received increased from 13.5 to 15.0.

For ß-thalassemia, the mean treatment duration of the entire ß-thalassemia data pool was 64.7 weeks (median 63.4), in the phase 2 studies (A536-04/-06) 78.2 weeks (median 78.5), and in the phase 3 study (ACE-536-ß-THAL-001) 60.8 weeks (median 63.3) for Luspatercept-treated and 58.9 weeks (median 62.1) for placebo-treated subjects. As of the 07 Jan 2019 data cut-off date, exposure for the pooled luspatercept treatment group in the β-thalassemia Data Pool increased from 355.70 subject-years in the original MAA to 503.14 subject-years for the 287 subjects (excluding the 92 subjects from Study ACE-536-B-THAL-001 who crossed over from placebo to luspatercept after the study was unblinded) and to 537.50 subject-years for the 379 subjects (including the cross-over subjects). In the pooled luspatercept treatment group of the β-thalassemia Data Pool, the median total treatment duration increased from 63.4 weeks to 95.6 weeks for the 287 subjects (excluding the cross-over subjects) and to 83.6 weeks for the 379 subjects (including the cross-over subjects). The median number of doses received increased from 21.0 to 31.0 and 27.0, respectively.

Dose

The studies included a variety of Luspatercept doses ranging from 0.0625 mg/kg to 1.75 mg/kg in MDS and 0.2 mg/kg to 1.25 mg/kg in ß-thalassemia studies.

Duration of follow-up

302 subjects completed 18 months of treatment with luspatercept, 98 patients with MDS and 204 patients with beta-thalassaemia. The study duration and duration of follow-up is of particular importance for the assessment of safety of this first-in-class agent, but follow-up duration in the MDS phase III trial was initially not fully clear. The Applicant has clarified the different protocol defined follow-up durations for the MDS and the beta-thalassaemia developments.

The Applicant has submitted updated safety results from the ongoing phase II studies, which include patients treated for considerably longer periods than in the phase III trials. The long term safety profile of >48 weeks of treatment was discussed in comparison to the safety profile <48 weeks of treatment, especially with regard to differences in detected signals as well as signals related to the kidney and malignancies. Overall, the safety profiles up to 48 weeks and after 48 weeks of exposure were comparable, with many AEs decreasing in frequency and no new safety signals emerging. A follow up of a minimum of three years will be provided for each indication, during which serious adverse events and specific adverse events of special interest agreed with PRAC will be collected in the long-term follow-up study ACE-536-LTFU-001 (please see Section 2.8 RMP below). Long-term safety is considered as missing information in the RMP.

Adverse Events

For MDS as well as ß-thalassaemia the overall TEAE rate is high, reflecting the severity of underlying diseases and disease burden in the populations. In the controlled studies, the proportion of TEAEs is mostly higher in the verum groups compared to placebo. There are some differences in the TEAE rates between the phase III and the phase II datasets that could be a consequence of the differences in exposure times (phase II studies were ongoing for longer and thus the exposer durations were longer compared to phase III). TEAEs reported in ≥ 15 and ≥ 5% of subjects with a > 2% higher frequency for luspatercept were overall comparable between the MDS and the ß-thalassemia dataset, apart from headache, diarrhoea, and pain (pain as a general disorder, to be distinguished from bone pain) which were no longer higher after adjustment for exposure in the ß-thalassemia phase III study.

MDS: Focussing on the EAIR, the luspatercept group had significant higher rate of fatigue (35.4 vs 24.4), bronchitis (13.2 vs 2.3), influenza (7.6 vs 0), diarrhoea (29.1 vs 16.5), nausea (27.3 vs 14.2), dizziness (25.4 vs 9.3), headache (20.2 vs 12.0), dyspnoea (18.4 vs 11.8), back pain (24.0 vs 12.1) compared to placebo.

ß-thalassaemia: Focussing on the EAIR, the luspatercept group had significant higher rates of bone pain (20.6 vs 7.7), arthralgia (18.9 vs 11.6), dizziness (10.4 vs 4.2) and hyperuricaemia (6.5 vs 0.0) than placebo.

Exposure-safety analyses were conducted based on a population PK model for both indications, and an inverse E-R relationship was shown. However, in studies with dose titration for efficacy and various lengths of treatment duration among subjects, the dose- or exposure-TEAE relationship is confounded by effect of time and dose increase over time. Therefore additional dose- and exposure-response analyses were conducted by using the data during the first two treatment cycles when most subjects did not have any dose modifications. A dose- or exposure- dependent increase in the incidence of TEAEs ≥ Grade 3 was not found for either indication, although the dose-TEAE relationship was affected by the small sample size at most dose levels

Adverse Events of Interest

Adverse events of interest were selected based on non-clinical findings or clinical findings from the Phase 2 and/or Phase 3 studies and the proposed mode of action and included events of malignancy or premalignant disorders, embolic and thrombotic events, kidney injury, hypertension, hypersensitivity reactions and musculoskeletal disorder-bone pain.

Malignancy, Premalignant Disorders, Progression to AML or High-risk MDS

In the beta-thalassaemia clinical trial, one case of hepatocellular carcinoma was found in a subject with a medical history of liver fibrosis and liver iron overload prior to study entry. Hepatocellular carcinoma is a known complication of chronic iron overload and is not considered related to luspatercept treatment. For MDS, the number of subjects as well as the number of events is rather small in the MDS pool, hampering interpretation and reliability of conclusions. It is noted that a between-group comparison (luspatercept -placebo) has to be done with caution also because the placebo group in the phase III MDS study included significantly fewer data due to discontinuation of subjects (mostly due to lack of efficacy) in this treatment arm.

In the MDS Data Pool, malignancy EOIs were reported for 15 subjects (5.8%; EAIR 6.3/100 subject-years; 95% CI, 3.8 to 10.5) in the pooled luspatercept treatment group and 1 subject (1.3%; EAIR 2.2/100 subject-years; 95% CI, 0.3 to 15.9) in the placebo group. There were no meaningful differences in the rate of patients transforming to AML between the groups. Incidence rates of progression to AML also indicate no increased risk with luspatercept compared to external data sources. Solid tumours occurred only in the phase II MDS trial (in three patients), which was longer in

duration but not controlled. Updated safety data shows that progression to AML occurred in two (2.6%) patients receiving placebo and three (2%) patients receiving luspatercept in the pivotal study. There was no signal for earlier transformation in luspatercept versus placebo treated subjects. Seven patients (6%) from the phase II studies showed disease progression at the updated cut-off July 2019.

Carcinogenicity

No malignancies were reported in the β-thalassemia data pool until the original data cut-off. However, one possible event of AML M6 (erythroid leukaemia) in a 27 year old β-thalassemia patients was reported as late breaking information. This finding together with the occurrence of haematological malignancies in the juvenile toxicity study has led to concerns about the potential carcinogenicity of luspatercept treatment. During the evaluation of the MAA; the following data were submitted:

The Applicant has provided detailed narratives and clinical reports including results of bone marrow aspirates, molecular and cytogenetic evaluations as well as external experts' review of these data. The external haematology experts concluded that the histopathological diagnosis of AML M6 was confounded by erythroid hyperplasia due to the underlying beta-thalassaemia. Molecular screening found no mutations diagnostic for AML, which are present in almost 90% of AML cases, but could also not definitely rule out the possibility of erythroleukemia. In summary, the experts concluded that AML M6 cannot with absolute certainty be ruled out, however, available data points to a low probability of this diagnosis. Based on the submitted documentation this view can be supported.

Furthermore, the Applicant has identified one study from Taiwan (Chung, 2015) investigating the incidence of cancer in 2655 patients diagnosed with thalassaemia between 1998 and 2010 compared to a cohort comprised of 10 620 age and gender matched persons from the general population without thalassaemia. An increased risk for haematological malignancies could be identified for subjects with thalassaemia, most pronounced in those who had received blood transfusions. These data point to the possibility of a higher incidence rate of haematologic malignancies in patients with thalassaemia at baseline.

The Applicant discussed the non-clinical toxicity findings in the context of the mechanism of action of luspatercept and included a literature review and a discussion, highlighting the current state of knowledge and the remaining gaps. Apart from the experiments performed for this MA and presented in the dossier, literature offers a perspective on the potential carcinogenic risk associated with the inhibition of the targets of luspatercept, which are members of the superfamily of transforming growth factors (TGF)-β. Considering the complex ligand binding profile of luspatercept and the mixed results/conclusions on the role of these different ligands in tumour promotion or suppression, it is indeed very difficult to draw a conclusion about its possible influence on tumorigenesis. The risk of AML has been added as an important potential risk and will be monitored in study ACE-536-LTFU-001 (please see RMP).

The Applicant hypothesised that the mechanism of carcinogenicity observed in the juvenile toxicity study is specific to the developing hematopoietic system in immature rats and that this notion is supported by the data from the three-month repeat dose toxicity study in young and mature rats (Report 20017484), where no test article-related increased incidence of malignancies was noted. In consequence, the potential risk of a carcinogenic effect on the developing haematopoietic system might not be relevant to the adult populations sought to be included into the SmPC for MDS and beta-thalassaemia. However, clinical studies with infants/toddlers from the age of 6 months onwards are planned within the agreed PIP. Taking into account that the increased incidence of haematopoietic malignancies in the juvenile toxicity study might be related to a mechanism of carcinogenicity specific to the developing hematopoietic system, and that the early haematopoietic system in young children/infants exhibits specific risks towards malignant degeneration (Copley and Eaves 2013, DOI: 10.1038/emm.2013.98;), the CHMP was of the opinion that inclusion of young children and the

eventually proposed lower age range for paediatric trials should be reconsidered and off-label use in children was reflected as an important potential risk in the RMP.

Comparing phase 3 MDS subjects on placebo to those receiving luspatercept, no substantial differences were identified for progression from IPSS-R very low or low-risk to intermediate-risk. Specifically, there is no indication that subjects on luspatercept treatment worsen more often or earlier. For progression to high-risk MDS, the rates of subjects experiencing this AE are comparable for luspatercept and placebo, with 3.3% (5/153 subjects) and 2.6% (2/76 subjects), respectively.

Data from the complete clinical study programme in MDS, beta-thalassaemia and myelofibrosis does not point to an increase in the incidence of malignancies with luspatercept treatment. The provided updated safety data is appreciated and does not raise concern. But exposure is still limited to a median treatment duration of 48 (max 221) weeks for MDS and about 84 (max 231) weeks for beta-thalassaemia, which is not sufficient to exclude an increase in malignant transformation in the longer term. Continuing surveillance of this potential risk for long-term exposure is thus warranted.

Therefore, this major issue was considered adequately addressed by the Applicant through the presentation of updated data from the complete clinical development programme, evaluation of relevant published data and the initiation of pharmacovigilance measures as agreed in the RMP.

Thromboembolic Events

The incidence of embolic and thrombotic events EOIs in the Luspatercept Data Pool was 4.0% (23 subjects) in the pooled luspatercept treatment group and 2.1% (4 subjects) in the pooled placebo treatment group. The higher incidence of thromboembolic events in the luspatercept pool was driven by splenectomised subjects from the β-thalassemia Data Pool.

Thromboembolic events are common complications of thalassaemia, however, event rates in the placebo arm were small and smaller also compared to the phase III MDS placebo group. Event incidence rates in the phase II and phase III Luspatercept arms were comparable, also regarding the EAIRs, but higher compared to the placebo group of phase III.

All subjects with at least one embolic or thrombotic event in phase III were splenectomised, and splenectomy is indeed a known risk factor for thrombosis among patients with thalassaemia. A respective warning is included in section 4.4 of the SmPC. In the total ß-thalassaemia data pool, more than half of the subjects were splenectomised prior to the study.

Comparing Luspatercept-treated splenectomised subjects to Luspatercept-treated non-splenectomised subjects, the former had a higher incidence of infections and infestation (69.0% vs 56.5%), respiratory, thoracic and mediastinal disorders (40.9% vs 25.8%) and investigations (19.9% vs 6.5%). However, efficacy subgroup analyses showed that patients with splenectomy had a trend for better response (primary efficacy analysis) compared to non-splenectomised patients, thus rendering the B/R sufficiently positive for these patients.

In MDS, there are no clear indications of a higher incidence in thromboembolic event rates with luspatercept. However, the comparison is hampered by the fact that in phase III nearly all placebo patients discontinued and fewer data are available from this group. Thromboembolic events has been added as an important identified risk in the RMP and additional data will be provided in study ACE-536-LTFU-001 (please see pharmacovigilance plan in the RMP).

Kidney Injury

In the clinical phase III studies for MDS and ß-thalassemia, while some events related to the kidney occurred more often in the luspatercept group, the differences are small and no relation to treatment can be determined with certainty at this stage of the assessment. It is questioned whether the

performed clinical phase II and III were indeed sensitive to detect respective signals in humans. The extent of exposure might have been too limited (in terms of number of patients exposed, duration of treatment and observational periods) to detect changes in kidney function. However, the number of kidney injury events and proteinuria was higher in Studies A536-04/06, compared to Study ACE-536-THAL-001 (6,3% vs 3,6 for kidney injury and 6,3% vs 2,2% for proteinuria). The Applicant was asked to discuss whether these differences could be related to type of luspatercept formulation administered (liquid vs lyophilised). The difference in kidney injury events and proteinuria between Studies A536-04/A536-06 and ACE-536-B-THAL-001 was unlikely related to type of luspatercept formulation as almost all subjects with kidney injury EOI were on lyophilized powder formulation at the time of their first kidney injury event (3 out of 4 subjects in A536-04/A536-06 and all subjects in ACE-536-B-THAL-001.

With regards to laboratory findings, slight decrease in creatinine clearance can be observed for patients in the luspatercept treatment groups versus patients who received placebo. Furthermore, albumin/creatinine ratios appeared to be changed from the baseline in the luspatercept treatment group compared to placebo. The Applicant has provided a comprehensive discussion on kidney toxicity and a detailed overview over the kidney function parameters in the pivotal trial for beta-thalassaemia for subjects both on placebo and luspatercept. In addition, comparative data of the safety profile for exposure below and more than 48 weeks provided for both the MDS and the beta-thalassaemia trials do not show an increase in renal AEs with longer treatment duration. Kidney parameters will further be monitored in the long-term extension study to gain additional data. The SmPC recommends the monitoring of RFP in patients with kidney impairment in section 4.2. This measures are considered adequate. Influencing factors for patients with beta-thalassaemia are chronic anemia, hypoxia and iron overload as well as a possible role of iron chelators, which all contribute to the deterioration of renal function (Demosthenous et al, 2019, Hematology, 24:1, 426-438).

Hypertension

When looking at the comparison of the pooled luspatercept group with placebo, hypertension EOIs seem to occur more frequently with luspatercept treatment than with placebo. The incidence of hypertension EOIs is rather high in the MDS studies (note: significantly older patient population compared to ß-thalassemia) and highest in the phase II patient population (longest exposure), which seems to be the driver for the overall observed difference. In the controlled MDS trial, rates were balanced between luspatercept and placebo arms, however. In ß-thalassaemia, a slightly higher hypertension EOIs rate was observed in the luspatercept groups (phase III and II) compared to the placebo group in phase III, however, the differences were very small. Blood pressure monitoring is recommended in section 4.4 of the SmPC to alert the treating physician to this risk.

Hypersensitivity

Overall, the proportion of patients experiencing hypersensitive like events was higher in the pooled Luspatercept group compared to placebo (8.4 % vs. 4.1%), mostly driven by phase II results where the exposure and observational periods are longer. No anaphylactic reactions have been reported.

Musculoskeletal Disorder – Bone Pain

The incidence of musculoskeletal disorder-bone pain EOIs was higher in the pooled luspatercept treatment group (37.5%) than in the pooled placebo treatment group (29.5%), also, if the incidence was adjusted for exposure (EAIR). Back pain and bone pain are the predominant drivers and a relation to the MoA appears likely.

Deaths

27 deaths were reported from the clinical investigation programme in MDS and β-thalassaemia until data cut-off. 17 subjects (3.0%) died in the pooled luspatercept treatment group and 10 subjects (5.2%) in the placebo group. None of the deaths were assessed as related by the investigator. The provided narratives allow secondary assessment of the events leading to the death of each subjects and the investigators' assessment is conclusive and can be followed. The new cut-off July 2019 provided the following data: In the MDS programme, 39 (14.4%) subjects died in the luspatercept treatment group versus 18 (23.7%) in the placebo group on-study. In the beta-thalassaemia programme, 6 (2.1% excluding cross-over patients or 1.6% including cross-over) patients receiving luspatercept versus 1 (0.9%) patient receiving placebo died-on study.

SAE

Overall, the incidence of SAEs was higher in the pooled luspatercept treatment group (23.8%) than in the pooled placebo treatment group (15.0%). The SOC with the highest subject incidence of SAEs in the pooled luspatercept treatment group was Infections and infestations (incidence of 8.8%). Treatment-related SAEs were reported only in the pooled luspatercept treatment group (2.5% of subjects). Deep vein thrombosis was the only SAE assessed as related to treatment by the investigator experienced by >1 subject (2 subjects [0.4%]).

In the MDS pool, the percentage of subjects with SAEs was higher in the pooled luspatercept treatment group (37.7%) than in the placebo treatment group (30.3%). The SOCs with the highest percentage of subjects with SAEs (at least 5% of subjects) in the pooled luspatercept treatment group were Infections and Infestations; Neoplasms benign, malignant, and unspecified (including cysts and polyps); Cardiac disorders; Injury, poisoning and procedural complications; and Musculoskeletal and connective tissue disorders.

In the β-thalassaemia Data Pool, the percentage of subjects with SAEs was higher in the pooled luspatercept treatment group (13.2%) than in the placebo treatment group (5.5%). The difference was driven by imbalances in the percentages of subjects with TEAEs in the Infections and infestations SOC (4.5% and 2.8%, respectively) and thromboembolic events, including cerebrovascular accident (1.0% and 0%) and deep vein thrombosis (0.7% and 0%).

The nature of the observed SAEs reflects the different patient populations, with the MDS population significantly older and with more cardiac comorbidities than the β-thalassaemia subjects. Disease progression and cardiac disorders are clearly evident in the MDS data pool. Consistently reflected across the two different populations is the increased incidence of thromboembolic events and of infections in luspatercept treated subjects versus placebo.

Safety in special populations

Pregnant and lactating women were excluded from the study population and throughout the clinical development program. Given the findings in the nonclinical reproductive and developmental toxicity studies together with the high probability of a similar effect in humans due to the mode of action, a contraindication for pregnancy was included in section 4.3 of the SmPC. Additional measures (educational materials have also been put in place to assess HCP awareness of key messages included in the HCP Checklist for luspatercept including recommendations for counselling of WCBP and instructions for providing WCBP with the Patient Card, please see RMP). In addition, a study to evaluate the effectiveness of the additional risk Minimisation Measures in Europe has been requested (please see RMP).

Immunological Events

The observed influence of either pre-existing or treatment emergent anti-drug-antibodies on the safety profile of luspatercept appears limited and does not cause concern at the present time. The incidence

of TEADA was higher in subjects with MDS than in those with β-thalassemia (8.85% versus 1.41%). One potential explanation for such a difference might be the difference in MDS and beta-thalassaemia patients´ age (median age: 71 years vs. 30 years) and the disease-associated immune status, however, no firm conclusions can be drawn.

2.7.2. Conclusions on the clinical safety

The Applicant addressed the concerns raised during the evaluation of the MAA with the provision of updated safety data and additional analyses. The original data set with a cut-off of May 2018 was supplemented by comprehensive safety data until January 2019 and updated information for deaths, SAEs and AESIs up to July 2019. The new analyses did not reveal new safety signals, but rather alleviated most of the concerns highlighted earlier. Additional information pertaining to unfavourable effects and recommendations for patient management was introduced into the PI. The remaining uncertainties concerning the effect of luspatercept on renal function, malignancies and other adverse events of interest will be addressed by post-marketing study (please see RMP).

In conclusion, the safety database is considered sufficient to support a marketing authorisation of Reblozyl.

The CHMP considers the following measures necessary to address issues related to safety:

- ACE-536-LTFU-001: To evaluate the long-term safety, including TEEs (only in the β-thalassaemia population with splenectomy) and progression to AML and/or other malignancies/ pre-malignancies, of luspatercept in patients who have participated in Acceleron or Celgene-sponsored luspatercept clinical trials.

- Study to Evaluate the Effectiveness of the Additional Risk Minimisation Measures in Europe: To assess HCP awareness of key messages included in the HCP Checklist for luspatercept including recommendations for counselling of WCBP and instructions for providing WCBP with the Patient Card.

2.8. Risk Management Plan

Safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table 63: Summary of safety concerns

Summary of safety concerns	
Important identified risks	• TEEs (only in the β-thalassaemia population with splenectomy)
Important potential risks	• Haematologic malignancies (including AML) • Off-label use in paediatric patients (developmental toxicity of luspatercept) • Use during pregnancy and lactation
Missing information	• Long-term safety

Pharmacovigilance plan

Table 64: Ongoing and planned studies/activities in the post-authorisation pharmacovigilance development plan

Study/Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
ACE-536-LTFU-001/ Ongoing	To evaluate the long-term safety, including TEEs (only in the β-thalassaemia population with splenectomy) and progression to AML and/or other malignancies/ pre-malignancies, of luspatercept in patients who have participated in Acceleron or Celgene-sponsored luspatercept clinical trials.	TEEs (only in the β-thalassaemia population with splenectomy) Haematologic malignancies (including AML). Long-term safety.	Final report Interim safety updates	Q2 2029 Annually for the first 5 years.
Study to Evaluate the Effectiveness of the Additional Risk Minimisation Measures in Europe/ Planned	To assess HCP awareness of key messages included in the HCP Checklist for luspatercept including recommendations for counselling of WCBP and instructions for providing WCBP with the Patient Card.	Use during pregnancy and lactation.	Protocol submission	Within 3 months of marketing authorisation approval.

The MAH shall submit a protocol amendment for study ACE-536-LTFU-001 to the EMA within 2 months of marketing authorisation.

Risk minimisation measures

Table 65: Summary Table of Risk Minimisation Activities by Safety Concern

Safety Concern	Risk Minimisation Measures
Important Identified Risk	

Safety Concern	Risk Minimisation Measures
TEEs (only in the β-thalassaemia population with splenectomy)	**Routine risk minimisation measures:** SmPC Section 4.8 – TEEs (including DVT, portal vein thrombosis, ischaemic stroke and pulmonary embolism) are included as undesirable effects. PL Section 4 – Stroke symptoms and blood clots in the veins are included as possible side effects. SmPC Section 4.4 – Incidence of TEEs, risk factors and advice to consider thromboprophylaxis in higher risk patients. PL Section 2 – Advice regarding preventative measures and medications. SmPC Section 4.4 and PL Section 2 – Warning regarding luspatercept treatment in β-thalassaemia patients with a splenectomy and other TEE risk factors. SmPC Section 4.2 - Advice to interrupt luspatercept for persistent treatment-related Grade 3 or higher adverse reactions until the toxicity has improved or returned to baseline. **Additional risk minimisation measures:** None proposed. **Legal status:** Luspatercept is subject to restricted medical prescription.
Important Potential Risks	
Haematologic malignancies (including AML)	**Routine risk minimisation measures:** SmPC Section 5.3 – Haematologic malignancies were observed in juvenile rats. SmPC Section 4.2 - Advice to interrupt luspatercept for persistent treatment-related Grade 3 or higher adverse reactions until the toxicity has improved or returned to baseline. **Additional risk minimisation measures:** None proposed. **Legal status:** Luspatercept is subject to restricted medical prescription.
Off-label use in paediatric patients (developmental toxicity of luspatercept)	**Routine risk minimisation measures:** SmPC Section 4.1 – Target population is adults. SmPC Section 4.2 – Statement that the safety and efficacy of luspatercept in paediatric patients aged from 6 months to less than 18 years have not yet been established in β-thalassaemia and that luspatercept should be initiated by a physician experienced in treatment of haematological diseases. SmPC Section 5.3 – Nonclinical findings regarding pre- and post-natal development and juvenile toxicity. PL Section 2 – Statement that luspatercept is not recommended for use in children and adolescents under 18 years. **Additional risk minimisation measures:** None proposed. **Legal status:** Luspatercept is subject to restricted medical prescription.

Safety Concern	Risk Minimisation Measures
Use during pregnancy and lactation	**Routine risk minimisation measures:** SmPC Section 4.2 – Statement that luspatercept should be initiated by a physician experienced in treatment of haematological diseases. SmPC Section 4.3 – Contraindication in pregnancy. SmPC Section 4.6 – Instruction not to start luspatercept if the patient is pregnant, and to discontinue luspatercept if a patient becomes pregnant. SmPC Section 4.6 – Instructions to use effective contraception during and for at least 3 months after the last dose of luspatercept, and to have a pregnancy test prior to therapy. SmPC Section 4.6 – Advice whether to discontinue breast-feeding or luspatercept for 3 months after the last dose. SmPC Section 4.6 (cross-referencing to Section 5.3) – Nonclinical findings regarding reproductive toxicity, lactation and fertility. PL Section 2 – Contraindication regarding luspatercept treatment during pregnancy, pregnancy test prior to therapy, warnings and precautions regarding luspatercept therapy during breast-feeding, and advice regarding contraception usage. **Additional risk minimisation measures:** - Patient Card (for WCBP only). - HCP Checklist. **Legal status:** Luspatercept is subject to restricted medical prescription.
Missing information	
Long-term safety	**Routine risk minimisation measures:** None proposed. **Additional risk minimisation measures:** None proposed. **Legal status:** Luspatercept is subject to restricted medical prescription.

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.5 is acceptable.

2.9. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.10. New Active Substance

The applicant declared that luspatercept has not been previously authorised in a medicinal product in the European Union.

2.11. Product information

2.11.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.11.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Reblozyl (luspatercept) is included in the additional monitoring list as It contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU. Therefore, the SmPC and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

MDS-associated anaemia

The initially claimed indication was "Reblozyl is indicated for the treatment of adult patients with anaemia requiring transfusions due to very low- to intermediate-risk myelodysplastic syndromes (MDS), who have ring sideroblasts and loss or lack of response to, are ineligible for, or intolerant to erythropoiesis-stimulating agent (ESA) therapy".

The indication was later slightly amended to "Reblozyl is indicated for the treatment of adult patients with transfusion-dependent anaemia due to very low, low and intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts, who had an unsatisfactory response to or are ineligible for erythropoietin-based therapy (see section 5.1).

Myelodysplastic syndromes are acquired bone marrow disorders occurring predominantly in the elderly population characterised by ineffective haematopoiesis, development of peripheral cytopenias, and risk of progression to acute myeloid leukemia. In MDS patients classified as lower risk based on the IPSS criteria, progression to AML is delayed and survival is longer compared to patients with higher risk disease. Therefore, treatment in lower risk patients is mainly focussed on controlling anaemia (and other cytopenias) and improving quality of life. Anaemia is the most common cytopenia among patients with lower-risk MDS. The target population for Reblozyl are MDS patients with ring sideroblasts, most of which carry an SF3B1 mutation; the latter being associated with a more favourable disease course.

Patients requiring RBC transfusions of at least 2 units/8 weeks were included into the pivotal trial. This corresponds to a transfusion-dependent population as defined by the IWG 2006 criteria. The recruited patient population is also in line with the proposals for revised MDS IWG response criteria (Platzecker et al., 2019).

Beta-thalassaemia associated anaemia

The initially claimed indication was 'Reblozyl is indicated for treatment of adult patients with anaemia requiring transfusions due to beta thalassaemia (β thalassaemia).'

This was changed during assessment to "Reblozyl is indicated for the treatment of adult patients with transfusion-dependent anaemia associated with beta-thalassaemia (see section 5.1)" to more adequately reflect the target population.

The β-thalassaemias are a group of inherited disorders characterised by absent or reduced production of the β-globin chains of haemoglobin, the oxygen-carrying molecule in human RBCs.

Transfusion-dependent (TD) beta thalassaemia refers to the most severe form of beta thalassemia in which there is minimal to no beta globin chain production and consequently, little to no HbA and HbG, which then has to be externally substituted (by regular transfusions).

Patients with TD β-thalassaemia (which includes conventional β-thalassaemia major and severe forms of HgbE/β-thalassaemia) commonly come to clinical attention in early childhood (before 2 years of age) with severe anaemia (< 7 g/dL); they require life-long, regular blood transfusion therapy.

Patients with NTD β-thalassemia present later in childhood or adolescence with mild to moderate anaemia and require no or only occasional transfusions in instances of blood loss or worsening anaemia due to periods of physiological stress (e.g., during infections, surgery, or pregnancy).

3.1.2. Available therapies and unmet medical need

MDS-associated anaemia

Treatment with erythropoiesis-stimulating agents (ESAs) is the first line option in patients with lower risk MDS-associated symptomatic anaemia without del(5q) mutation and with serum EPO levels ≤500 mU/mL. Despite an initial response to ESA treatment, approximately 70% of patients eventually become unresponsive to ESAs. If no response occurs with ESAs alone, addition of G-CSF can be considered, especially in patients with ≥15% RS in the marrow (Greenberg 2017).

Second line treatment options include immunomodulatory agents such as lenalidomide (approved for lower risk MDS patients with a del(5q) mutation) and azacitidine (a hypomethylating agent approved in higher risk MDS patients). Immunosuppressive therapy with anti-thymocyte globulin (±cyclosporine) may be considered in selected younger patients with lower risk MDS.

Chronic RBC transfusions often remain the mainstay of therapy in insufficiently responding MDS patients, but RBC transfusions carry the risk of iron overload resulting in cardiac, hepatic or endocrine dysfunction and a higher infection risk, as well as immunogenicity. Whether iron overload indeed contributes to negative clinical outcomes in transfusion-dependent patients with MDS remains disputed, however (Fenaux 2009, Ades 2014).

In summary, an unmet medical need exists in transfusion-dependent MDS patients with symptomatic anaemia, who are ineligible for ESA or become unresponsive to ESA treatment.

Beta-thalassaemia associated anaemia

Transfusion dependent beta-thalassemia is a condition of high unmet medical need. The main available management options are RBC transfusions (leading to iron overload disorders), iron chelation therapy, (splenectomy) and hematopoietic stem cell transplantation.

Zynteglo, a gene therapy medicinal product, was recently authorised in the EU for the treatment of β-thalassaemia in patients with a non-β0/β0 genotype. No approved treatment addressing the underlying ineffective erythropoiesis and anaemia is available for the β0/β0 genotype.

3.1.3. Main clinical studies

MDS-associated anaemia

The pivotal Study ACE-536-MDS-001 is an ongoing Phase 3, double-blind, randomized, placebo-controlled study to determine the efficacy and safety of luspatercept versus placebo in subjects with anaemia due to IPSS-R very low-, low-, or intermediate-risk MDS with ring sideroblasts, who require RBC transfusions. In addition, subjects were refractory to (nonresponse or response that is no longer maintained), intolerant of, or ineligible for (serum EPO > 200 U/L) ESA treatment.

Eligible subjects were randomized to luspatercept (at a starting dose level of 1.0 mg/kg SC injection every 3 weeks [Q3W]) or placebo in a 2:1 ratio and entered the 24-week double-blind Primary Phase of the Treatment Period. If subjects did not achieve RBC transfusion independence after at least 2 consecutive doses at the 1.0 mg/kg starting dose level, luspatercept dose could be increased to 1.33 mg/kg; after at least 2 consecutive doses at the 1.33 mg/kg dose level, the luspatercept dose level could be increased to a maximum of 1.75 mg/kg.

Subjects who experienced clinical benefit and absence of disease progression per IWG-MDS criteria per protocol at the Week 25 Visit MDS Disease Assessment continued double-blind treatment in the Extension Phase of the Treatment Period until they met any discontinuation criteria as per protocol.

The primary objective of the study was to evaluate the effect of luspatercept as compared with placebo on RBC transfusion independence (RBC-TI) over > 8 weeks in these MDS patients. As of the clinical data cut-off date of 08 May 2018, enrolment in the study is complete; however, long-term treatment and follow-up are ongoing. The initial submission includes data for all subjects who either completed the Week 48 visit or discontinued treatment before Week 48 and entered Post-treatment Follow-up.

The ITT population for the evaluation of efficacy included 229 subjects (153 randomized to luspatercept and 76 randomized to placebo). The median duration of treatment was 49.0 weeks in the luspatercept treatment group and 24.0 weeks in the placebo group.

Beta-thalassaemia associated anaemia

Study ACE-536-B-THAL-001 is an ongoing Phase 3, double-blind, randomized, placebo-controlled study to compare the efficacy and safety of luspatercept versus placebo for the treatment of subjects with β-thalassaemia who require regular RBC transfusions.

Consenting subjects entered a 12-week screening/run-in period during which eligibility was assessed and 12 weeks of transfusion history was collected prospectively, in addition to 12-weeks of historical retrospective transfusion history (i.e., total of 24 weeks of RBC transfusion history for determination of eligibility). Eligible subjects were randomized to luspatercept or placebo in a 2:1 ratio. The study includes a double-blind treatment period [weeks 1 to 48] and long-term treatment period [after week 48; subjects continued to receive the study drug to which they were initially randomized]. A post-treatment follow-up period of 156 weeks after the last dose of study treatment is planned.

After unblinding for the primary analysis, eligible subjects (including those randomized to placebo) were given the option of open-label luspatercept treatment for up to 5 years (until all subjects initially assigned to luspatercept in the double-blind Treatment Period completed a total treatment duration of 5 years from Dose 1 of the double-blinded phase).

The primary objective of the study is to determine the proportion of subjects treated with luspatercept plus best supportive care (BSC) versus placebo plus BSC who achieve erythroid response. The primary endpoint was the percentage of "responders", defined as subjects achieving RBC transfusion reduction of ≥ 33% (min. 2 units) from baseline to week 13-24. Enrolment in the study is complete; however, long-term treatment and follow-up are ongoing. The initial submission includes data for all subjects, who were either treated for at least 48 weeks (and were continuing treatment after the cut-off date) or had discontinued treatment before the cut-off date and had entered post-treatment follow-up.

The ITT population of efficacy included 336 subjects (224 randomized to luspatercept plus BSC and 112 randomized to placebo plus BSC).

3.2. Favourable effects

MDS associated anaemia

The pivotal phase III study ACE-536-MDS-001 showed statistically significant results in favour of luspatercept in the primary and all hierarchically tested (key) secondary endpoints:

Primary analysis (pre-specified): RBC-TI ≥ 8 weeks from Week 1 to 24 was achieved in 58/153 (37.91%) and 10/76 (13.16%) patients in the luspatercept and Placebo arm, respectively.

Key secondary endpoints: RBC-TI ≥ 12 weeks from Week 1 to 24 and from Week 1 to 48 showed slightly lower effect sizes. A post hoc analysis on the effect of TI during 16 weeks was supportive of a sustained treatment effect (19% and 3.9% achieved 16 weeks of TI during weeks 1 to 24 in the luspatercept and placebo arm, respectively).

Additional endpoints evaluating the change from baseline in transfusions, modified haematologic improvement, duration of response, mean haemoglobin over time, changes in serum ferritin all showed results in favour of luspatercept.

The change from baseline in RBC transfusions in weeks 9 to 24 was -4.0 and 0 for luspatercept and Placebo, respectively; in weeks 33 to 48 -5.0 and -2.5 for luspatercept and Placebo, respectively.

The results of a reanalysis of the transfusion data to estimate the ratio in transfusion rates between treatment arms are in support of a reduction of transfusion frequency. Estimates for the rate ratio (i.e. the number of transfusion events with luspatercept treatment divided by the number of transfusion events with placebo during Weeks 1 to 24) range from 0.58 to 0.80 (95% CI) in favour of luspatercept. Results from the analysis of the mean difference in transfusion units using an ANCOVA model are consistent to those presented for the number of transfusion events. These indicate a reduction of the transfused RBC units in the luspatercept group (compared to placebo) by about 3.6 to 7.1 units (95% CI) during Weeks 1 to 24.

Subgroup analyses for the primary, key secondary endpoints and 'modified Hematological improvement – erythroid' (mHI-E) support the overall results for all investigated subgroups.

All additionally provided analyses investigating effects according to low and high transfusion burden indicate a slightly smaller effect in the higher baseline transfusion burden groups, although still in favour of luspatercept.

The data that is available in patients with very high transfusion burden (>8 units/8 weeks) is limited, which is reflected in the SmPC.

Phase 2 results, although derived from a broader patient population, provided proof of concept; in a 'phase 3 like' population, results were consistent with the Phase 3 results.

Beta-thalassaemia associated anaemia

The pivotal phase III study ACE-536-B-THAL-001 showed statistically compelling results in favour of luspatercept in the primary and all hierarchically tested (key) secondary endpoints:

Primary analysis (pre-specified): RBC transfusion reduction of ≥ 33% (min. 2 units) from baseline to week 13-24 was achieved in 21% (48/224) in the luspatercept & BSC arm versus in 4.5% (5/112) in the Placebo & BSC arm.

Key secondary endpoints evaluating 50% response and response at later time intervals (week 37-48) as well as mean change from baseline (to week 13 – 24) showed comparable effect sizes.

Additional endpoints evaluating response with rolling week analyses, time to erythroid response, duration of response, transfusion independence, change from baseline in serum ferritin and cardiac T2* showed results in favour of luspatercept.

The mean RBC transfusion burden reduction per subject in RBC units from baseline to Week 1 - 48 interval was 4.75 RBC units/48 weeks with luspatercept (ITT). The difference between Luspatercept and Placebo in the change in RBC transfusion burden is -5.83 (Units/48 Weeks, 95% CI; -7.01, -4.65).

For the 48-week interval Week 49 to Week 96, a continued decrease of -5.66 Units compared to baseline was observed in the Luspatercept group.

In the last 12 weeks of 96 weeks of treatment, a statistically significant mean decrease of -464.96 ug/L of serum Ferritin (+73.20 ug/L in the Placebo group) in the Luspatercept group compared to baseline was recorded.

By and large, the above stated results apply irrespective of baseline demographics and disease characteristics.

Results from the phase II dose escalating study A536-04 showed erythroid response defined as ≥ 20% reduction in RBC transfusion burden in TD subjects during any rolling 12-week window on treatment compared to pretreatment in 25 (80.6%) subjects, all of whom received luspatercept at dose levels of 0.6 to 1.25 mg/kg transfusion. Erythroid response could also be shown in the extension study A536-06 over 48 weeks. Results support choice of doses used in phase III.

3.3. Uncertainties and limitations about favourable effects

MDS-associated anaemia

A large proportion of placebo patients was discontinued from treatment after Week 24 for fulfilling the criteria of "progression of disease" or "lack of clinical benefit", hampering the statistical evaluation of potential long-term treatment effects beyond 24 weeks. Additional analyses using adequate imputation methods were provided that are supportive of the primary analysis. Nevertheless, the discontinuation of a large number of patients, especially in the placebo arm, limits the interpretation of the study. This appears especially relevant in view of the intended chronic treatment with luspatercept. To prevent futile treatment in case no clinical benefit is observed over several weeks at the highest recommended dose, a stopping rule has been introduced in the SmPC.

The provided sensitivity analyses on change of Hb over time indicate a small treatment effect in favour of luspatercept. However, all analyses on the effect of luspatercept on haemoglobin need to be interpreted with caution, as a large number of Hb values was likely influenced by previous transfusions. Importantly, neither the 14/3 rule nor the exclusion of all hb values within 14 days of transfusion that was applied in the requested sensitivity analyses were sufficient to rule out any influence of previous transfusions on Hb.

Luspatercept failed to show an effect on quality of life. The HRQoL results appear of limited value to support the primary analysis and the clinical relevance of the results due to imbalances between treatment arms in some subdomains.

The dose response is only weakly characterised, with dose-exposure-response analyses relying on clinical endpoints rather than PD parameters.

Beta-thalassaemia associated anaemia

The effect, though statistically compelling, seems small in absolute numbers. It is noticed that for the power calculation, the response rate was expected to be approximately twice as high (40% vs 20%) as actually seen in the trial (21.4% and 4.5%). The primary endpoint was a binary outcome, and thus less informative than the analysis of mean changes over time. The relevance of the achieved reduction in transfusion units/hospital visits for the quality of life of individual patients is not easy to interpret since the studied population indicated a good quality of life at baseline (which was not improved by luspatercept treatment) despite the need for frequent transfusions. To prevent futile treatment in case no clinical benefit is observed over several weeks at the highest recommended dose, a stopping rule has been introduced in the SmPC.

Inter-group comparisons after 48 weeks become less robust as the number of patients per group decreases. At week 96 only 3 placebo patients remain.

Luspatercept treatment also could not reduce liver iron content (LIC) after 48 weeks of treatment and no convincing evidence of a relevant reduction was seen after 96 weeks. It should be pointed out that the primary responder threshold (33% reduction in RBC units) was initially justified by an estimated yearly decrease in LIC by ~ 3 mg/g dw.

The dose response is only weakly characterised as the phase II dose response study was very small. Support from pharmacological investigations and models is also limited.

3.4. Unfavourable effects

In the juvenile toxicity study WIL-961003, 3 cases of haematologic malignancies (1 case each of lymphoma, myeloid- and lymphoid leukaemia), which developed within 6 months of treatment initiation, were observed in juvenile rats at a dose of 10 mg/kg. The Applicant hypothesises that the mechanism of carcinogenicity is specific to the developing hematopoietic system in immature rats and that this notion is supported by the data from the three-month repeat dose toxicity study in young and mature rats (Report 20017484), where no similar test article-related increased incidence of malignancies was noted.

In a mouse model of tumour progression (study PPR113), slight increases in tumour weight were observed after 6 administrations of RAP-536 (murine analogue of ACE-536) of 1 and 10 mg/kg. However, after further discussion it was concluded that the data obtained in this model were not adequate to judge on a potential tumorigenic effect of luspatercept.

In the repeat-dose toxicity studies in rats and *Cynomolgus* monkeys, membrano-proliferative glomerulonephritis in the kidney was observed at doses of 1 mg/kg and above. These histopathologic findings were accompanied by increases in BUN and creatinine at higher dose levels. Based on the nature and incidence of these kidney findings, a LOAEL in rats was determined at 1 mg/kg and a NOAEL in monkeys was established at 0.3 mg/kg.

<u>TEAEs</u>

95.3% of subjects in the pooled luspatercept treatment group and 91.2% of subjects in the pooled placebo treatment group reported at least one TEAE. Incidence rates of serious TEAEs, TEAEs of Grade 3 or 4, TEAEs leading to dose interruption, and TEAEs leading to treatment discontinuation were higher in the pooled luspatercept treatment group than in the pooled placebo treatment group.

The most frequently reported ($\geq$ 15.0%) TEAEs for the pooled luspatercept treatment group of the MDS Data Pool were fatigue, diarrhoea, nausea, cough, dizziness, hypertension, and peripheral edema in the original MAA. With the cut-off 07 Jan 2019, additionally headache, viral upper respiratory tract infection and back pain were reported at an incidence of $\geq$ 15.0%. For the pooled luspatercept treatment group of the β-Thalassaemia Data Pool, the most frequently reported ($\geq$ 15.0%) TEAEs were headache, back pain, bone pain, arthralgia, pyrexia, upper respiratory tract infection, diarrhoea, asthenia, oropharyngeal pain, and cough for the original MAA. No additional preferred terms at an incidence of $\geq$15.0% were reported at the later cut-off.

<u>Adverse events of special interest</u>

Malignancy, Premalignant Disorders, Progression to AML or High-risk MDS

MDS: Progression to AML occurred in two (2.6%) patients receiving placebo and three (2%) patients receiving luspatercept in the pivotal study. There was no signal for earlier transformation in luspatercept versus placebo treated subjects. Seven patients (6%) from the phase 2 studies showed disease progression at the updated cut-off July 2019. However, as compared to the phase 3

population, the phase 2 population had more indicators of severe disease. The incidence rates of solid tumors do not give rise to concern in comparison with published data on patients with or without MDS.

Beta-thalassaemia: In the beta-thalassamia clinical trials supporting the current MAA, in addition to the one unconfirmed AML case, a case of hepatocellular carcinoma occurred.

In other ongoing clinical trials in either MDS or beta-thalassaemia, not supporting the currently sought indications, no malignancies were reported. In a single arm phase II trial in myelofibrosis, transformation to AML was observed in one patient; transformation to AML is however also a characteristic of the underlying disease.

The risk of haematologic malignancies (including AML) has been added as an important risk in the RMP, monitoring of the risk will take place in study ACE-536-LTFU-001.

TEEs:

The incidence of embolic and thrombotic events in the luspatercept data pool was 4.0% (23 subjects) in the pooled luspatercept treatment group and 2.1% (4 subjects) in the pooled placebo treatment group. The higher incidence in the luspatercept group was driven by the splenectomised subjects (more than 50% of subjects) from the β-thalassaemia data pool. It is noted that all subjects with at least 1 embolic or thrombotic event in phase 3 were splenectomised, and splenectomy is indeed a known risk factor for thrombosis among patients with thalassaemia. A respective warning is included in section 4.4 of the SmPC. Thromboembolic events in splenectomised patients has been added as an important identified risk and monitoring of this risk will take place as part of study ACE-536-LTFU-001 (please see RMP).

The incidence of hypertension EOIs in the Luspatercept Data Pool was higher in the pooled luspatercept treatment group (11.2%) than in the pooled placebo treatment group (5.7%). The most frequently reported hypertension EOI (≥ 10% of subjects in either treatment group) was the preferred term of hypertension (10.7% and 4.7% of subjects in the pooled luspatercept and placebo treatment groups, respectively), driven by the (elderly) patient population in the MDS phase II study. Luspatercept was associated with mean increases in SBP and DBP of approximately 5 mm Hg from baseline. The proportion of patients experiencing hypersensitivity-like events was higher in the pooled luspatercept group compared to placebo (8.4 % vs. 4.1%), mostly driven by phase II results.

The incidence of musculoskeletal disorder-bone pain EOIs was higher in the pooled luspatercept treatment group (37.5%) than in the pooled placebo treatment group (29.5%). Back pain and bone pain are the predominant drivers and could be related to the MoA.

27 deaths were reported for the clinical investigation programme in MDS and β-thalassaemia until initial data cut-off. 17 subjects (3.0%) died in the pooled luspatercept treatment group and 10 subjects (5.2%) in the placebo group. None of the deaths were assessed as related by the investigator. The new cut-off July 2019 provided the following data: In the MDS programme, 39 (14.4%) subjects died in the luspatercept treatment group versus 18 (23.7%) in the placebo group on-study. In the beta-thalassaemia programme, 6 (2.1% excluding cross-over patients or 1.6% including cross-over) patients receiving luspatercept versus 1 (0.9%) patient receiving placebo died-on study.

The incidence of serious AEs was higher in the pooled luspatercept treatment group (23.8%) than in the pooled placebo treatment group (15.0%). The SOC with the highest subject incidence of SAEs in the pooled luspatercept treatment group was Infections and infestations (incidence of 8.8%). Deep vein thrombosis was the only SAE assessed as related to treatment by the investigator experienced by >1 subject (2 subjects [0.4%]). For both indications, data provided with the newer cut-offs showed incidences and types of SAEs similar to those in the original MAA.

The incidence of TEAEs leading to treatment discontinuation was higher (8.8%) in the pooled luspatercept treatment group than in the pooled placebo treatment group (3.6%).

Immunogenicity

Of 544 evaluable subjects treated with luspatercept, 27 subjects (4.96%) tested positive for TEADA against luspatercept, with neutralizing TEADAs detected in 11 subjects (2.02%). The incidence of TEADAs was 8.85% in subjects with MDS and 1.41% in subjects with beta-thalassemia.

3.5. Uncertainties and limitations about unfavourable effects

Non-clinical data indicates a potential for renal toxicity in multiple species even at subclinical doses; no NOAEL could be established for renal safety in rats and a NOAEL of only 0.3 mg/kg in the *Cynomolgus* monkey.

In the clinical studies, a slight decrease in creatinine clearance was observed for patients in the luspatercept treatment groups. However, other markers of renal injury were not appreciably increased and a comparison of the safety profile of exposures below and above 48 weeks yielded no worrying signals. Median exposure in the luspatercept treated group is approximately 48 weeks for MDS and 84 weeks for β-thalassaemia, while unlimited treatment duration is envisaged. Continuing surveillance of renal function is planned in the still ongoing clinical trials as well as in the long-term follow-up trial (please see RMP).

The interpretation of comparative safety data from the phase 3 trials is hampered by the fact that significantly less data from placebo patients is available. Most subjects in the placebo group of the phase 3 MDS study discontinued treatment early (mostly due to lack of efficacy), while in the β-thalassaemia trial such a differential drop-out was less noticeable.

No malignancies were reported in the β-thalassemia data pool until the original data cut-off. However, one possible event of AML M6 (erythroid leukaemia) in a 27 year old β-thalassemia patients was reported as late breaking information. Data from the complete clinical study programme in MDS, beta-thalassaemia and MF does not point to an increase in the incidence of malignancies with luspatercept treatment. The updated safety data does not raise concern, but exposure is still limited to a median treatment duration of 48 (max 221) weeks for MDS and 84 (max 231) weeks for beta-thalassaemia, which is not sufficient to exclude an increase in malignant transformation in the long term. Continuing surveillance of this potential risk for long-term exposure is thus warranted (please see RMP).

3.6. Effects Table

3.7. Benefit-risk assessment and discussion

Table 66: Effects Table for Reblozyl in MDS associated anaemia (data cut-off: 08 May 2018, updated data cut-off: 07 Jan 2019)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
	Favourable Effects					

Effect	Short Description		Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
				Luspatercept + BSC N=153	Placebo + BSC N=76	RCT, multicentre Pivotal trial	ACE-536-MDS-001
RBC-TI ⩾ 8 weeks (week 1-24)[$]	Proportions of subjects with RBC-TI (absence of RBC transfusion during any 8-week period (week 1-24)	Response rate 95% CI	%	37.91 (n=58) 30.20- 46.10	13.16 (n=10) 6.49- 22.87	p< 0.0001a	Prim. endpoint
RBC-TI ⩾ 12 weeks (week 1-48)[$]	Proportions of subjects with RBC-TI (absence of RBC transfusion during any 12-week period) (week 1-48)	Response rate 95% CI	%	33.33 (n=51) 25.93- 41.40	11.84 (n=9) 5.56- 21.29	P=0.0003[a]	Key secondary
RBC-TI ⩾ 12 weeks (week 1-24)[$]	Proportions of subjects with RBC-TI (absence of RBC transfusion during any 12-week period (week 1-24)	Response rate 95% CI	%	28.10 n=43 21.14- 35.93	7.89 n=6 2.95- 16.40	P=0.0002[a]	Key secondary
Transfusion Event Frequency (Week 1-24)		Interval Transfusion Rate (95% CI) Rate ratio (95% CI)	RBC events	6.26 (5.56, 7.05) n=153 0.68 (0.58, 0.80)	9.20 (7.98, 10.60) n=76	Re-analysis based on negative binomial regression with imputation of missing data based on baseline transfusion frequencies p< 0.0001[b]	Post hoc analysis
Transfusion Event Frequency (Week 25-48)		Interval Transfusion Rate (95% CI) Rate ratio (95% CI)	RBC events	6.27 (5.47, 7.19) n=153 0.72 (0.60, 0.86)	8.72 (7.40, 10.28) n=76	See above p=0.0004[b]	Post hoc analysis

Effect	Short Description		Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Serum Ferritin	Mean Change From Baseline in Mean Serum Ferritin Averaged Over Weeks 9 Through 24[d]	LS Mean (SE) 95% CI LS Mean Difference (SE) 95% CI	µg/L	9.9 (47.09) (-82.9, 102.7) -180.1 (65.81) -309.8, -50.4	190.0 (60.30) (71.2, 308.8)	0.0067[c]	Post hoc analysis
Serum Ferritin	Mean Change From Baseline in Mean Serum Ferritin Averaged Over Weeks 33 Through 48[d]	LS Mean (SE) 95% CI LS Mean Difference (SE) 95% CI	µg/L	0.2 (18.57) (-36.4, 36.8) -46.0 (25.95) (-97.2, 5.1)	46.2 (23.78) (-0.7, 93.0)	0.0775[c]	Post hoc analysis
Overall survival *Original MA*	Subjects alive (censored) Subjects died		%	92.2 n=141 7.8 n=12	88.2 n=67 11.8 n=9	Median Follow-up of 13.9 and 14.3 months, only (luspatercept vs. Placebo)	Secondary endpoint
Overall survival *Data cut off 07 Jan 2019*	Subjects alive (censored) Subjects died		%	85.6 n=131 14.4 n=22	81.6 n=62 18.4 n=14	Median Follow-up of 21.3 and 21.2 months (luspatercept vs. Placebo)	Data cut off 07 Jan 2019
Progression to AML			%	2.0 n=3	1.3 n=1	No additional subject from the phase 3 study progressed to AML as of 07 Jan 2019	Secondary endpoint
	Unfavourable Effects						
				Updated July 2019 Data N = 269	Updated July 2019 Data N = 76		Updated safety data, response to Q 184

Effect	Short Description		Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Death (on- and off-study)			n (%)	39 (14.5%) 16 (5.9) On-study 23 (8.6) Off-study	18 (23.7%) 4 (5.3) On-study 14 (18.4) Off-study	Pivotal phase III study (RCT) & uncontrolled phase II studies vs. Phase III placebo control group	
Malignancies EOI	Proportion of subjects with malignancy event		n (%) [EAIR]	25 (9.3) [7.3]	1 (1.3) [2.2]	See above; driven by transformation to AML in phase II with higher risk population	
Kidney injury EOI	Proportion of subjects with kidney injury event		n (%) [EAIR]	27 (10.0) [8.0]	5 (6.6) [11.1]	See above	

Abbreviations: CI: Confidence interval; RBC-TI: Red Blood Cell transfusion independence, ISS: integrated summary of clinical safety

Notes: [$]Rank in multiple testing: The primary efficacy endpoint was tested first at the 1-sided 0.025 significance level. In order to preserve the overall alpha level at 0.025 across the RBC-TI endpoints, formal statistical inference for the RBC-TI ≥ 12 weeks analysis (first tested for Week 1 to Week 48 and then Week 1 to Week 24) was to be made only if superiority of luspatercept was demonstrated for the primary efficacy endpoint (RBC-TI of ≥ 8 weeks), at the 1-sided 0.025 significance level.

[a] 2-sided p-value from Cochran-Mantel-Haenszel (CMH) test stratified for average baseline RBC transfusion requirement (≥ 6 units versus < 6 units of RBC per 8 weeks), and baseline IPSS-R score (very low or low versus intermediate).

[b]Nominal p-value and 95% CI are from negative binomial regression model with post-baseline transfusion frequency as dependent variable, and with treatment arm, baseline RBC transfusion burden (≥ 6 units versus < 6 RBC units/8 weeks), and baseline IPSS-R (very low/low versus intermediate), and baseline RBC transfusion event frequency per 24 weeks as independent variables

[c] If a subject did not have a serum ferritin value within the designated postbaseline interval, the serum ferritin is imputed from the baseline value. Analysis of covariance was used to compare the treatment difference between groups (including nominal p-value), with the change in serum ferritin as the dependent variable, treatment group (2 levels) as a factor, and baseline serum ferritin value as covariates, stratified by average baseline RBC transfusion requirement (≥ 6 units versus < 6 units of RBC per 8 weeks), and baseline IPSS-R (very low or low versus intermediate). Imputation of missing values by baseline.

Table 67: Effects Table for Reblozyl in beta-thalassemia associated anaemia (data cut-off: 11 May 2018 up to the week 48 data and 7 Jan 2019 for the week 96 data)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
			Luspatercept + BSC N=224	Placebo + BSC N=112	RCT, multicentre Pivotal trial	ACE-536-B-THAL-001
RBC Transfusion reduction [1]	Proportion of patients with ⩾ 33% (min. 2 units) RBC unit reduction, baseline to week 13-24	% 95% CI	21.4% (48/224) 16.2-27.4	4.5% (5/112)	*	
RBC Transfusion reduction [2]	Proportion of patients with ⩾ 33% (min. 2 units) RBC unit reduction, baseline to week 37-48	% 95% CI	19.6% (44/224) 14.7-25.5	3.6% (4/112)	*	
RBC Transfusion reduction [2]	Proportion of patients with ⩾ 50% (min. 2 units) RBC unit reduction, baseline to week 13-24	% 95% CI	7.6% (17/244) 4.5-11.9	1.8% (2/112)	*	
RBC Transfusion reduction [2]	Proportion of patients with ⩾ 50% (min. 2 units) RBC unit reduction, baseline to week 37-48	% 95% CI	10.3% (23/244) 6.6-15.0	0.9% (1/112)	*	
RBC Transfusion reduction [2]	Mean change from baseline in transfusion burden (RBC units) from Week 13 to Week 24	RBC units	-0.67	+0.66		
RBC Transfusion reduction [2]	Mean change from baseline in transfusion burden (RBC units) from Week 1 to Week 48 (ITT)	RBC units	-4.75	+1.04		
RBC Transfusion reduction [2]	Mean change from baseline in transfusion burden (RBC units) from Week 49 to Week 96 (ITT)	RBC units	-5.99	+0.31		
Mean Change in Serum Ferritin Level [3]	Change from baseline at week 48, mean (SD) (ITT)	µg/L (SD)	-248.02 (800.021)	+106.62 (526.174)	*	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Mean Change in Serum Ferritin Level during any 12-week interval until week 96 [3]	Change from baseline during any 12-week interval until week 96, mean (SD) (ITT)	µg/L (SD)	-412.16 891.697	+94.92 655.698	*	
Mean Change in LIC [3]	Change from baseline at week 48, mean (SD)	Mg/g dw (SD)	0.10 (5.760)	0.08 (5.229)		
Mean Change in LIC [3]	Change from Baseline for Week 96, mean (SD) (ITT)	Mg/g dw (SD)	-0.38 8.404	+1.86 2.067	Number of Placebo Patients at week 96; N=3	
Mean Change in Myocardial T2* [3]	Change from baseline at week 48, mean (SD)	ms	-1.83 (15.084)	+0.02 (6.843)	*	
Unfavourable Effects						
			Updated July 2019 Data Including Cross-over N = 379	Updated July 2019 Data N = 109		Updated safety data, response to Q 193
Death (on- and off-study)		n (%)	6 (1.6%) 3 (0.8) On-study 3 (0.8) Off-study	1 (0.9%) 1 (0.9) On-study 0 Off-study	Pivotal phase III study (RCT) & uncontrolled phase II studies vs. Phase III placebo control group	
Malignancies EOI	Proportion of subjects with malignancy event	n (%) [EAIR]	4 (1.1) [0.5]	0	See above; includes unlikely AML M6 and confounded by one event of splenectomy and one event of HCG increased	
Kidney injury EOI	Proportion of subjects with kidney injury event	n (%) [EAIR]	21 (5.5) [2.8]	3 (2.8) [1.4]	See above	
Thrombo-embolic events	Proportion of subjects with thromboembolic events	n (%) number /total	3.6% (8/223)	0.9% 2/223)		

Abbreviations:

RBC: red blood cell

Min.: minimum

BSC: Best Standard of Care
RCT: randomised controlled trial
LIC: Liver Iron Concentration
dw: dry weight
ms: milliseconds
EOI: event of interest
ISS: integrated summary of clinical safety

Notes:
*p< 0.05
[1]=primary endpoint
[2]=key secondary endpoint, hierarchically tested
[3]=secondary endpoint, not hierarchically tested (no alpha control)
[4]= including late breaking information on the death of a 27 year old β-thalassaemia patients (possible event of AML)

3.7.1. Importance of favourable and unfavourable effects

MDS

In patients classified as lower risk MDS based on the IPSS criteria, progression to AML occurs later and survival is longer compared with patients with higher risk MDS. Therefore, treatment is mainly focussed on controlling anaemia (and other cytopenias) and improving quality of life.

ESAs are an established first line treatment in MDS-associated anaemia, but the majority of patients will eventually become unresponsive to ESAs; others might not respond/not tolerate them at all. Especially patients with high endogenous EPO levels and high transfusion needs seem to respond poorly to ESA treatment, and are in need of other treatment options. These patients are proposed to be targeted with luspatercept treatment.

Transfusion independence and reduction of transfusion requirement are important objectives of treatment, and this was evaluated in the pivotal phase 3 trial. The primary and key secondary efficacy endpoints show a statistically significant result in favour of luspatercept. However, the clinical relevance of these responder endpoints (i.e. proportion of patients with any 8- or 12 weeks of RBC-TI during 24 or 48 weeks) is difficult to interpret. Understanding the effect in terms of transfusion burden reduction is important, as e.g. patients with high transfusion burden hardly achieve periods of transfusion independence. In addition, sustainability of the effect over time is a relevant consideration, as a single period of 8 or 12 weeks of transfusion independence may not justify taking the risk of experiencing unfavourable effects. An important limitation to the interpretation of long-term efficacy is however the high attrition rate in placebo patients, who were allowed to discontinue the study after week 24 in case of no clinical benefit.

Reassuringly, the presented secondary analyses mostly support the primary and key secondary results. The post hoc analyses most relevant for interpretation of the clinical relevance of the effect are those on the change in transfusion rates/units as well as serum ferritin levels over time, using adequate imputation methods for missing data.

Estimates for the rate ratio (i.e. the number of transfusion events with luspatercept treatment divided by the number of transfusion events with placebo during Weeks 1 to 24) range from 0.57 to 0.79 (95% CI) in favour of luspatercept. This result is consistent with a mean reduction of 3.6 to 7.1 units (95% CI) compared to placebo during the same period. The effect is largely maintained during weeks 25 to 48, albeit associated with a larger uncertainty due to the large number of imputed patients.

Of note, there is an indication of a smaller effect in patients with higher baseline transfusion burden. Efficacy and safety data in patients with a baseline transfusion burden of more than 12 units/8 weeks are scarce; this has been reflected in the SmPC.

Taken together, the treatment effect as observed in this study, albeit small, is regarded clinically relevant in patients experiencing a sustained response and is further supported by the results of serum ferritin levels that are in favour of luspatercept.

To prevent patients from receiving futile treatment, a stopping rule (i.e. stop after 9 weeks of treatment if no clinical benefit at highest dose) was introduced in the SmPC.

The focus of treatment with luspatercept is improvement of MDS associated anaemia, without any claims regarding delaying progression to higher MDS risk groups or AML and prolonging overall survival. Nonetheless, any detrimental effect in terms of progression of disease or shortened OS compared to patients treated with best standard of care would not be acceptable. Reassuringly, there is no indication of a detrimental treatment effect with the currently provided data. While the study is still ongoing and all patients will be further followed, the data are considered mature enough to draw meaningful conclusions on the benefit risk.

Beta-thalassaemia

Transfusion dependent beta-thalassemia is a condition of high unmet medical need. The main available management options are very limited and do not include all affected patients. HSCT is a potentially curative option, but only for young patients with an HLA matched HSC donor. The recently approved gene-therapy product Zynteglo is not indicated for patients with a ß0/ß0 genotype.

Transfusions are the key management tool in TD ß-thalassaemia. However, regular transfusions lead to an iron overload, which is associated with severe long-term risks and reduced life expectancy. The primary objective of reducing RBC transfusions has thus acknowledged relevance for patients. Compared with the current symptomatic treatment of anaemia with transfusions and iron chelators a treatment option addressing the underlying ineffective erythropoiesis is highly desirable.

The pivotal study showed statistically compelling results in favour of luspatercept in the primary and all hierarchically tested (key) secondary endpoints. Some patients (the so called "responders") experienced a significant erythroid response with luspatercept treatment, achieving a $\geq$ 33% reduction and some even 50% reduction in RBC units at week 13-24 compared to baseline. However, the fraction of patients with such clinical improvement is rather small (about 20%).

While the iron parameter serum ferritin improved from baseline with luspatercept treatment, other indicators, such as liver iron content, cardiac T2* values or iron chelator requirements, did not change significantly. Notably, the responder threshold for the primary endpoint was initially justified by an expected clinically relevant reduction in liver iron content (LIC). It is plausible that a reduction of transfused iron reduces the iron overload of ß-thalassemia patients; however, no such reduction was demonstrated in the phase 3 trial after 48 or 96 weeks of treatment. LIC measured by MRI appears to be superior to measurement of serum ferritin for estimating total body iron burden (Taher A. et al 2008) and the effect of long-term luspatercept treatment on LIC will be further monitored post marketing.

Many patients had no benefit from treatment and remained stable (or slightly deteriorated) in their transfusion need. To prevent futile treatment, a respective stopping rule (stop after 9 weeks of treatment if no clinical benefit at highest dose) was introduced in the SmPC .

Luspatercept did not demonstrate an improved effect on quality of life, but it was maintained in the ITT population. This is due to the fact that patients had a good quality of life at baseline making

demonstration of further improvement more challenging, or because effects on QoL may not be observable in the initial phase of treatment, but manifest only later.

Safety MDS/Beta-thalassaemia

There are uncertainties pertaining to long-term safety in both indications. Non-clinical findings suggest a carcinogenic potential with luspatercept in juvenile animals in whom the haematopoietic system is not yet fully developed. The Applicant has provided updated safety data from the complete clinical study programme in MDS, beta-thalassaemia and myelofibrosis. While this data does not raise concern, exposure is still limited to a median treatment duration of 48 (max 221) weeks for MDS and about 84 (max 231) weeks for beta-thalassaemia. This is not sufficient to exclude an increase in malignant transformation in the long term, and continuing surveillance of this potential risk is thus warranted (please see RMP).

Other safety findings probably associated with the use of luspatercept, such as e.g. TEEs and hypertension, are highlighted in the SmPC to alert the treating physician to these potential risks.

In addition, signals from non-clinical data indicate a detrimental effect of luspatercept on renal function. A slight decrease in creatinine clearance was observed in patients in the luspatercept treatment groups. Other markers of renal injury were not appreciably increased and a comparison of the safety profile for an exposure below and above 48 weeks also yielded no worrying signals. Nevertheless, continuing surveillance of renal function is planned in the still ongoing clinical trials as well as in the long-term follow-up trial.

3.7.2. Balance of benefits and risks

MDS

In the pivotal trial, luspatercept showed superiority over placebo in all primary and key secondary analyses. The mean reduction in RBC units was 3.6 to 7.1 units (95% CI) compared to placebo during weeks 1 to 24 (mean, -5.3 units), and the effect was largely maintained in the second period of the study (weeks 25 to 48). A small proportion of patients even achieved a sustained response period of transfusion independence. There is some uncertainty on the long-term treatment effects as a large proportion of patients mainly from the placebo arm discontinued the study after Week 24. Secondary analyses of change in transfusion rates and RBC units as well as in serum ferritin using appropriate imputation methods are in support of the primary/key secondary analyses. Although haemoglobin levels did not increase significantly over time, secondary analyses indicate that the reduction in transfused RBC units does not come at the expense of a decrease in haemoglobin.

For the elderly population of MDS patients (with a mean age above 70 years), chronic anaemia represents a significant burden associated with multiple complications, but frequent RBC transfusions are also burdensome. In transfusion-dependent MDS patients, for whom regular transfusions are a substantial burden due to adverse events or cumbersome scheduling of clinical appointments and affect their quality of life, the achieved reduction in transfusion frequency is considered beneficial, in line with the main goals of treatment in lower risk MDS (treatment of cytopenia(s) and improvement of QoL; Fenaux, 2014). To prevent futile treatment in case no clinical benefit is observed over several weeks at the highest recommended dose, a stopping rule has been introduced in the SmPC.

The updated safety profile of luspatercept (cut-off Jan 2019 for all AEs and July 2019 for deaths, SAEs and AESIs) shows a manageable adverse event profile, which is comparable to the safety outcomes with the initial cut-off (May 2018). No signal for an increase in progression to/ time to progression to high risk MDS or AML was identified. However, due to the still limited duration of exposure, uncertainty

remains with regard to long-term safety, which has to be addressed by continuing surveillance, both in the still ongoing clinical trials as well as in the long-term follow-up study ACE-536-LTFU-001.

For patients with a lower transfusion burden, the B/R balance is less favourable in view of the uncertainty on long-term safety and the potential risk for haematologic malignancies.

Beta-thalassaemia

Despite some minor uncertainties regarding interpretation of efficacy results, superiority of luspatercept over placebo in all primary and key secondary analyses has been robustly established. Some patients, the so called 'responders' achieved a remarkable reduction in RBC transfusion needs; the number of responders was only around 20%, however. The effect on RBC transfusions is considerably smaller in the average patient, but also robust and clinically relevant. The effect appeared durable over the 96 week treatment period.

Responses favouring luspatercept over placebo were fairly consistent across subgroups. In particular, patients with a ß0/ß0 mutation have a very high unmet medical need as they are excluded from treatment with the recently approved gene-therapy product (Zynteglo). Additional analyses revealed that while some patients (i.e. with ß0/ß0 mutation, start of transfusions <2 years of age, renal disease) might have a somewhat smaller benefit from treatment, the effect is still robust across all subgroups, and thus, there is no need to exclude specific subgroups from the SmPC.

There is some uncertainty on the effect on iron overload, as provided LIC results were below expectations, and LIC did not considerably drop during 96 weeks of luspatercept treatment. Longer observation might be needed to quantify the effect.

The updated safety data shows a manageable adverse event profile of luspatercept, which is comparable to the safety outcomes with the initial cut-off (May 2018). However, duration of exposure is still limited to a median treatment duration of about 84 (max 231) weeks, while long-term treatment over many years or even decades might be foreseen in a patient population requiring transfusions from a very young age onwards.

The remaining uncertainties with regards to long-term safety and durability of the beneficial effects are especially relevant for this indication considering the rather young population and chronic use, and will be addressed by continuing surveillance in the still ongoing clinical trials as well as in the long-term follow-up study ACE-536-LTFU-001.

3.7.3. Additional considerations on the benefit-risk balance

Clinical studies in infants/toddlers from the age of 6 months onwards are planned within the agreed PIP. Since the increased incidence of haematopoietic malignancies in the juvenile toxicity study might be related to a mechanism of carcinogenicity specific to the developing hematopoietic system, and as the early haematopoietic system in young children/infants exhibits specific risks towards malignant degeneration (Copley and Eaves 2013, DOI: 10.1038/emm.2013.98), the CHMP is of the opinion that inclusion of young children and the eventually proposed lower age range for paediatric trials should be reconsidered. Furthermore, off-label use in children has been added as an important potential risk in the RMP. Finally, having development of luspatercept in the paediatric population in mind, further mechanistic (*in vitro*) studies might be considered to help in further elucidating the risk for haematologic malignancies seen in juvenile animals.

This recommendation is further supported by the nephrotoxicity that was commonly found in most non-clinical studies and frequently persisted throughout recovery phases (also in the juvenile toxicity study). Finally, the other adverse effects observed in young rats even at the lowest dose administered

(adverse adrenal gland findings, problems in bone growth, lower heart weights etc.), should also be considered, when deciding to include young infants in the planned clinical trials.

3.8. Conclusions

The overall B/R of Reblozyl (luspatercept) in adult patients with transfusion-dependent MDS and beta-thalassaemia is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Reblozyl is not similar to Revlimid and Zynteglo within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Reblozyl is favourable in the following indication:

Reblozyl is indicated for the treatment of adult patients with transfusion-dependent anaemia due to very low, low and intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts, who had an unsatisfactory response to or are ineligible for erythropoietin-based therapy (see section 5.1).

Reblozyl is indicated for the treatment of adult patients with transfusion-dependent anaemia associated with beta thalassaemia (see section 5.1).

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regards to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

Prior to launch of Reblozyl in each Member State the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The MAH shall ensure that in each member state where Reblozyl is marketed, all HCPs who intend to prescribe Reblozyl are provided with an HCP Information Pack, containing the following:

1. Information on where to find latest SmPC;
2. HCP Checklist;
3. Patient Card (for WCBP only).

Healthcare Professional Checklist

The HCP Checklist is to be used before initiating treatment, at each administration, and then at regular intervals when performing follow-up. The HCP Checklist shall contain the following key messages:

- Information on studies in animals showing luspatercept reproductive and embryo-foetal toxicity and is therefore contraindicated during pregnancy.
- Reminder that luspatercept is contraindicated during pregnancy and in WCBP not using effective contraception.
- Need to provide counselling before treatment initiation and regularly thereafter regarding the potential teratogenic risk of luspatercept and required actions to minimise this risk.
- A pregnancy test must be carried out and negative results verified by the prescriber before starting treatment. It must be repeated at suitable intervals.
- Patients must use highly effective contraception during the treatment with luspatercept.
- While on treatment, women must not become pregnant. If a woman becomes pregnant or wants to become pregnant, luspatercept should be discontinued. Women of childbearing potential must use highly effective contraception during treatment with luspatercept and for at least 3 months following discontinuation of treatment with luspatercept.

- Need to provide counselling in the event of pregnancy and evaluation of the outcome of any pregnancy.
- Should a pregnancy occur during treatment or within 3 months following discontinuation of treatment with luspatercept, remind the patient that it should be reported to the HCP, NCA, and/or to Celgene by contacting the local e-mail address or visiting the URL provided in the material, irrespective of adverse outcomes observed.

Patient Card (for WCBP only)

The Patient Card is to be handed to WCBP by the HCP at the time of treatment initiation. The HCP is to request that the WCBP confirm whether they have the Patient Card prior to each subsequent administration and provide them with additional cards as needed. The Patient Card shall contain the following key messages:

- The need for a negative pregnancy test result prior to starting treatment with luspatercept in WCBP.
- The need for WCBP to use at least one highly effective method of contraception during treatment with luspatercept and for at least 3 months following discontinuation.
- The need to report to the doctor any suspected or confirmed pregnancy occurring during and for 3 months following discontinuation of treatment.

Conditions or restrictions with regards to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that luspatercept is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.