# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

# 761136Orig1s000

# MULTI-DISCIPLINE REVIEW

**Summary Review**
**Office Director**
**Cross Discipline Team Leader Review**
**Clinical Review**
**Non-Clinical Review**
**Statistical Review**
**Clinical Pharmacology Review**

# BLA Multi-Disciplinary Review and Evaluation

| | |
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| **Application Type** | New Molecular Entity |
| **Application Number(s)** | BLA 761136, Original 1 |
| **Priority or Standard** | Priority |
| **Submit Date(s)** | April 4, 2019 |
| **Received Date(s)** | April 4, 2019 |
| **PDUFA Goal Date** | December 4, 2019 |
| **Division/Office** | Division of Hematology Products/Office of Hematology and Oncology Products |
| **Review Completion Date** | October 29, 2019 |
| **Proper Name** | REBLOZYL |
| **Nonproprietary Name** | luspatercept-aamt |
| **Pharmacologic Class** | Erythroid maturation agent |
| **Code name** | ACE-536 |
| **Applicant** | Celgene Corporation |
| **Dosage form** | Powder for solution for injection |
| **Applicant proposed Dosing Regimen** | Recommended starting dose is 1 mg/kg once every 3 weeks by subcutaneous injection |
| **Applicant Proposed Indication(s)/Population(s)** | Adult patients with beta thalassemia-associated anemia who require red blood cell (RBC) transfusions |
| **Recommendation on Regulatory Action** | Approval |
| **Recommended Indication(s)/Population(s)** (if applicable) | Treatment of anemia in adult patients with beta thalassemia who require regular red blood cell (RBC) transfusions |
| **Recommended Dosing Regimen** | Recommended starting dose is 1 mg/kg once every 3 weeks by subcutaneous injection. |

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OPQ=Office of Pharmaceutical Quality
OPDP=Office of Prescription Drug Promotion
OSI=Office of Scientific Investigations
OSE= Office of Surveillance and Epidemiology
DEPI= Division of Epidemiology
DMEPA=Division of Medication Error Prevention and Analysis
DRISK=Division of Risk Management

# Glossary

| | |
|---|---|
| AC | advisory committee |
| ADME | absorption, distribution, metabolism, excretion |
| AE | adverse event |
| AR | adverse reaction |
| BLA | biologics license application |
| BPCA | Best Pharmaceuticals for Children Act |
| BRF | Benefit Risk Framework |
| CBER | Center for Biologics Evaluation and Research |
| CDER | Center for Drug Evaluation and Research |
| CDRH | Center for Devices and Radiological Health |
| CDTL | Cross-Discipline Team Leader |
| CFR | Code of Federal Regulations |
| CMC | chemistry, manufacturing, and controls |
| COSTART | Coding Symbols for Thesaurus of Adverse Reaction Terms |
| CRF | case report form |
| CRO | contract research organization |
| CRT | clinical review template |
| CSR | clinical study report |
| CSS | Controlled Substance Staff |
| DHOT | Division of Hematology Oncology Toxicology |
| DMC | data monitoring committee |
| ECG | electrocardiogram |
| eCTD | electronic common technical document |
| ETASU | elements to assure safe use |
| FDA | Food and Drug Administration |
| FDAAA | Food and Drug Administration Amendments Act of 2007 |
| FDASIA | Food and Drug Administration Safety and Innovation Act |
| GCP | good clinical practice |
| GRMP | good review management practice |
| ICH | International Conference on Harmonization |
| IND | Investigational New Drug |
| ISE | integrated summary of effectiveness |
| ISS | integrated summary of safety |
| ITT | intent to treat |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mITT | modified intent to treat |
| NCI-CTCAE | National Cancer Institute-Common Terminology Criteria for Adverse Event |
| NDA | new drug application |
| NME | new molecular entity |

| | |
|---|---|
| OCS | Office of Computational Science |
| OPQ | Office of Pharmaceutical Quality |
| OSE | Office of Surveillance and Epidemiology |
| OSI | Office of Scientific Investigation |
| PBRER | Periodic Benefit-Risk Evaluation Report |
| PD | pharmacodynamics |
| PI | prescribing information |
| PK | pharmacokinetics |
| PMC | postmarketing commitment |
| PMR | postmarketing requirement |
| PP | per protocol |
| PPI | patient package insert (also known as Patient Information) |
| PREA | Pediatric Research Equity Act |
| PRO | patient reported outcome |
| PSUR | Periodic Safety Update report |
| REMS | risk evaluation and mitigation strategy |
| SAE | serious adverse event |
| SAP | statistical analysis plan |
| SGE | special government employee |
| SOC | standard of care |
| TEAE | treatment emergent adverse event |

# 1 Executive Summary

## 1.1. Product Introduction

Luspatercept-aamt (ACE-536) is a recombinant fusion protein that binds select endogenous transforming growth factor-beta superfamily ligands. By binding to specific endogenous ligands, luspatercept diminishes Smad 2/3 signaling. Luspatercept-aamt promoted erythroid maturation through differentiation of late-stage erythroid precursors (normoblasts) in mice. In a model of B-thalassemia, luspatercept-aamt decreased abnormally elevated Smad 2/3 signaling and improved hematology parameters associated with ineffective erythropoiesis in mice.

Luspatercept-aamt is indicated for the treatment of anemia in adult patients with beta thalassemia who require regular blood cell transfusions. The recommended starting dose of luspatercept is 1.0 mg/kg once every 3 weeks administered by subcutaneous (SC) injection. If a patient does not achieve a reduction in RBC transfusion burden after at least 2 consecutive doses (6 weeks) at the 1.0 mg/kg starting dose, the dose may be increased to 1.25 mg/kg (maximum dose). Treatment should be discontinued if a patient does not experience a decrease in transfusion burden after administration of 3 doses (9 weeks) at the maximum dose level.

## 1.2. Conclusions on the Substantial Evidence of Effectiveness

Study ACE-536-B-THAL-001, also referred to as the BELIEVE trial, is an ongoing Phase 3, double-blind, randomized, placebo-controlled study to compare the efficacy and safety of luspatercept-aamt versus placebo for the treatment of patients with documented β-thalassemia or HgbE/B-thalassemia who require regular RBC transfusions (6-20 RBC units in the 24 weeks prior to randomization). The patients 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. Three-hundred and thirty-six eligible patients were randomized to luspatercept-aamt treatment (n=224) or placebo (n=112) in a 2:1 ratio. Patients in each treatment arm also received best supportive care (BSC) for β-thalassemia including RBC transfusions, iron-chelating agents, as dictated by prespecified thresholds.

The efficacy of luspatercept-aamt in adult patients with beta thalassemia was established based upon the proportion of patients achieving RBC transfusion burden reduction (≥ 33% reduction from baseline) with a reduction of at least 2 units from week 13 to week 24. The efficacy response rates for a ≥ 33% reduction from baseline in transfusion was 21.4% (48/224) in the luspatercept-aamt + BSC group and 4.5% (5/112) in the placebo + BSC group ($p < 0.0001$). When the response rate was assessed over weeks 37 to 48, the percentage of patients with a response demonstrated a sustained effect (19.6%, 44/224 subjects in the luspatercept-aamt + BSC group and 3.6%, 4/112 subjects in the placebo + BSC group; $p < 0.0001$).

## 1.3. Benefit-Risk Assessment

**Benefit-Risk Summary and Assessment**

Beta thalassemia's are inherited disorders characterized by absent or reduced production of the B-globin chains of hemoglobin and the reduced synthesis of the B-globin in patients with B-thalassemia leads to an imbalance in the a/B-globin chain ratio and excess of unpaired a-globin chains leading to premature death of RBCs or their precursors in the bone marrow. Beta thalassemia results in ineffective erythropoiesis leading to anemia and a number of subsequent pathophysiologic complications: hemolysis, hypercoagulability, transfusional iron overload secondary to frequent RBC transfusions and ineffective hematopoiesis, heart disease, and hepatic cirrhosis. There are no FDA-approved therapies for B-thalassemia. Patients with Beta Thalassemia who require regular transfusions remain dependent on periodic red blood cell transfusions to maintain an acceptable hemoglobin (Hb) range and also require iron chelation therapy due to transfusional iron overload. Blood transfusions are associated with transmission of blood-borne pathogens and viruses (including HIV and hepatitis), transfusion reactions, alloimmunization, and transfusional iron overload

Luspatercept-aamt is an erythroid maturation agent and is a recombinant fusion protein that binds select endogenous TGF-B superfamily ligands, thereby diminishing Smad2/3 signaling. In a model of B-thalassemia, luspatercept-aamt decreased abnormally elevated Smad2/3 signaling and improved hematology parameters associated with ineffective erythropoiesis in mice.

The efficacy of luspatercept-aamt in adult patients with B-thalassemia was evaluated in study ACE-536-B-THAL-001 (BELIEVE study), a Phase 3, double-blind, randomized, placebo-controlled study that compares the efficacy and safety of luspatercept-aamt versus placebo for the treatment of anemia in adult patients with β-thalassemia who require regular RBC transfusions (6-20 RBC units in the 24 weeks prior to randomization). Patients 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. The study included a double-blind treatment period (Weeks 1 to 48), long-term treatment period (after Week 48; patients continued to receive the study drug to which they were initially randomized) and a post-treatment follow-up period of 156 weeks after the last dose of study treatment. Eligible patients including those randomized to placebo treatment were given the option of open-label luspatercept-aamt treatment for up to 5 years after unblinding for the primary analysis.

Three-hundred and thirty-six eligible patients were randomized to luspatercept-aamt treatment (n=224) or placebo (n=112) in a 2:1 ratio. Patients in each treatment arm also received best supportive care (BSC) for β-thalassemia including RBC transfusions, iron-chelating agents, as

dictated by prespecified thresholds. Patients received a starting dose of luspatercept-aamt 1mg/kg subcutaneous injection every 3 weeks.

The overall median age was 30 years (range 18-66) with 42% male, 54.2% white, 34.8% Asian and 0.3 % Black or African-American. The majority of patients had beta thalassemia (76%), HbE/beta thalassemia (15%), or beta thalassemia combined with alpha-thalassemia (7.7%). The baseline median transfusion burden was 6.12 (3,14) in the luspatercept-aamt and 6.27 (3,12) in the placebo arm. In the luspatercept-aamt arm, 58% had prior splenectomy and 58% in the placebo arm had a prior splenectomy. The median age that patients started regular transfusion was balanced between the arms with the median of age 2 (range: 0,52) and 2 (range: 0,51) in the luspatercept-aamt and placebo arms, respectively.

The efficacy of luspatercept-aamt in adult patients with beta thalassemia was established based upon the proportion of patients achieving RBC transfusion burden reduction (≥ 33% reduction from baseline) with a reduction of at least 2 units from week 13 to week 24. The efficacy results demonstrated a response rate of 21.4% (48/224) in the luspatercept-aamt + BSC group and 4.5% (5/112) in the placebo + BSC group ($p < 0.0001$). When the response rate was assessed over Weeks 37 to 48, the percentage of patients with a response demonstrated a sustained effect, (19.6%, 44/224 subjects in the luspatercept-aamt + BSC group and 3.6%, 4/112 subjects in the placebo + BSC group; $p < 0.0001$).

Similarly, a greater proportion of subjects in the luspatercept + BSC group achieved ≥ 50% reduction in transfusion burden during the fixed Week 13 to Week 24 interval (7.6%; 17/224) compared with the placebo + BSC group (1.8%; 2/112; $p = 0.0303$). When the response rate was assessed over Weeks 37 to 48, the percentage of subjects with a ≥ 50% reduction in RBC transfusion burden improved slightly in the luspatercept + BSC group (10.3%, 23/224), again demonstrating a sustained effect; the response rate remained constant in the placebo + BSC groups (0.9%, 1/112, p-0.0017).

A total of 332 subjects randomized to the luspatercept-aamt (N = 223) and placebo (N = 109) treatment arms were included in the safety analysis. The median treatment duration was similar between the luspatercept treatment group (63.3 weeks) and the placebo treatment group (62.1 weeks).

The most frequently common adverse reactions (in ≥ 10% of subjects) in patients with beta-thalassemia treated with luspatercept were headache (26%), bone pain (20%), arthralgia (19%), fatigue (14%), cough (14%), abdominal pain (14%), diarrhea (12%), and dizziness(11%). Grade 3 and 4 treatment-emergent adverse events in the phase 3 trial occurred in 29% of patients in the luspatercept-aamt arm and 14.7% of patients in the placebo arm with the most common being bone pain (1.3%), anemia (3.1%), hyperuricemia (2.7%), and ALT increase (0.9%). Serious TEAEs were higher in the luspatercept treatment arm (15.2% than in the placebo arm (5.5%). The most frequently reported (in more than 1 patient) serious TEAEs in the luspatercept treatment group were anemia, cellulitis, cerebrovascular accident, cholangitis, deep vein

thrombosis, and pyrexia. Serious adverse reactions were reported in 1% of patients receiving luspatercept-aamt and included cerebrovascular accident and deep vein thrombosis. There was 1 death reported in the 3-month safety follow up in a 26-year-old male treated with luspatercept-aamt who developed neutropenic sepsis, pancytopenia and renal failure resulting in death due to an unconfirmed report of development of AML (M6) erythroleukemia.

Permanent discontinuation due to an adverse reaction (Grades 1-4) occurred in 5.4% of patients who received luspatercept-aamt. The most frequent adverse reactions leading to permanent discontinuation in patients who received luspatercept-aamt included arthralgia (1%), bone pain (< 1%) and headache (<1%). Dosage reductions due to an adverse reaction occurred in 2.7% of patients who received luspatercept-aamt and the most frequent adverse reactions requiring dosage reduction in > 0.5% of patients who received luspatercept-aamt included hypertension and headache. Dosage interruptions due to an adverse reaction occurred in 15.2% of patients who received luspatercept-aamt and the most frequent adverse reactions requiring dosage interruption in > 1% of patients included upper respiratory tract infections, ALT increase and cough.

Clinically relevant adverse reactions in < 5% of patients include vertigo/vertigo positional, syncope/presyncope, injection site reactions and hypersensitivity. Safety analyses of liver function tests demonstrated aspartate aminotransferase values ≥ 3 x ULN were observed in 11.2% of patients in the luspatercept treatment group and 4.6% of patients in the placebo treatment group at any time postbaseline. There was 1 serious TEAE of drug-induced liver injury (DILI) reported in a luspatercept treated patient, which was considered by the investigator to be related to luspatercept and concomitant clarithromycin therapy. Inclusion of liver function laboratory abnormalities in section 6 of the USPI is warranted to describe these findings.

Important identified safety findings with the use of luspatercept included thrombosis/thromboembolism and hypertension. In adult patients with beta thalassemia, thromboembolic events were reported in 8/223 (3.6%) treated patients. Reported thromboembolic events included deep vein thromboses, pulmonary embolus, portal vein thrombosis, and ischemic strokes. Patients with known risk factors for thromboembolism may be at further increased risk of thromboembolic conditions. Hypertension was reported in 10.7% (61/571) of luspatercept-aamt treated patients. The incidence of grade 3-4 hypertension ranged from 1.8% to 8.6% and reflects exposure to luspatercept-aamt as a single agent administered across a range of doses (0.125mg/kg to 1.75mg/kg) in 571 patients. In adult patients with beta thalassemia with normal baseline blood pressure, 13 (6.2%) patients developed systolic blood pressure (SBP)> 130mm Hg and 33 (16.6%) patients developed diastolic blood pressure (DBP) > 80mmg Hg.

In non-clinical data there appears to be a safety signal for carcinogenicity and renal toxicity. At all dose levels lower F1 pup body weights and

adverse kidney findings such as membranoproliferative glomerulonephritis, tubular atrophy/hypoplasia and vessel ectasia) were observed. In addition, in repeat-dose toxicity studies, juvenile rats developed hematological malignancies at the 10mg/kg resulting in exposures (based on area under the curve) approximately 8 times the maximum recommended human dose of 1.25mg/kg. The mean urinary albumin/creatinine values at baseline and throughout the 48 week treatment period remained < 200mg/g which is in the lower microalbuminuria range albeit with periodic but transient fluctuations. There were decreases in renal function that were transient observed in the luspatercept-aamt group and generally occurred in patients with known risk factors for kidney injury. As luspatercept may be administered long-term to patients with beta-thalassemia the long-term safety and follow-up reports of ongoing studies will be important to assess the long-term safety of this product.

As with all therapeutic proteins, there is a potential for immunogenicity. Of the 284 patients with beta thalassemia treated with luspatercept-aamt and evaluable for the presence of anti-luspatercept aamt antibodies, 4 patients (1.4%) tested positive for treatment emergent anti-luspatercept-aamt antibodies including 2 (0.7%) who had neutralizing antibodies. There were no severe acute systemic hypersensitivity reactions reported for patients with anti-luspatercept-aamt antibodies in clinical trials and no association between hypersensitivity type reaction or injection site reaction and presence of anti-luspatercept-aamt antibodies.

In summary, patients with beta thalassemia who require regular transfusions have no available therapy and remains an area of high unmet medical need. Transfusions are a key component of the treatment of beta thalassemia however regular transfusions lead to iron overload in a disease with ineffective erythropoiesis. A reduction of RBC units that a patient receives is an important and clinically meaningful endpoint. The efficacy results from Study ACE-536-B-Thal-001 (BELIEVE Study) support the conclusion of effectiveness for luspatercept-aamt for the treatment of anemia in patients with beta thalassemia who require regular transfusions. The demonstration of a ≥33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units for 12 consecutive weeks (week 13- week 24) response rate of 21.4% (48/224) in the luspatercept + BSC group compared to 4.5% (5/112) in the placebo + BSC group ($p < 0.0001$) and response rate of 44% (19.6) for luspatercept-aamt + BSC compared to 4% (3.6) for the placebo arm during Weeks 37 to 48 provides meaningful clinical benefit for patients with beta-thalassemia who require regular transfusions. The subgroup analysis demonstrated a consistent treatment effect for luspatercept-aamt across all the subgroups, albeit this is a descriptive finding only.

| Dimension | Evidence and Uncertainties | Conclusions and Reasons |
|---|---|---|
| **Analysis of Condition** | • B-thalassemia's are inherited disorders characterized by absent or reduced production of the B-globin chains of hemoglobin and the reduced synthesis of the b-globin in patients with B-thalassemia leads to an imbalance in the a/B-globin chain ratio and excess of unpaired a-globin chains leading to premature death of RBCs or their precursors in the bone marrow.<br>• Approximately 80 to 90 million people (approximately 1.5% of the global population) are carriers of a β-thalassemia mutation, with approximately 60,000 symptomatic individuals born annually.<br>• Beta thalassemia results in ineffective erythropoiesis leading to anemia and a number of subsequent pathophysiologic complications, including hemolysis, hypercoagulability, transfusional iron overload secondary to frequent RBC transfusions, heart disease, and hepatic cirrhosis | • Beta thalassemia impacts a large number of patients in the US and is associated with significant morbidity and mortality.<br>• Reduced survival in regularly transfused adult patients with thalassemia is largely due to the known iron overload complications in major organs, involving the heart, liver, and endocrine glands. |
| **Current Treatment Options** | • There are currently no approved FDA drugs to treat anemia secondary to beta thalassemia<br>• Patients with beta thalassemia who require regular transfusions are dependent on periodic red blood cell transfusions to maintain an acceptable hemoglobin (Hb) range and iron chelation therapy due to transfusional iron overload<br>• Blood transfusions are associated with transmission of blood-borne pathogens and viruses (including HIV and hepatitis), transfusion reactions, alloimmunization, and transfusional iron overload<br>• Hematopoietic stem cell transplantation (HSCT) from an identical family donor is a potential treatment option for pediatric patients but this therapeutic modality is often limited by lack of appropriate donors and potential risks of stem cell transplantation | • There is an unmet medical need for effective treatment options in patients with beta thalassemia who require regular transfusions. |

| Dimension | Evidence and Uncertainties | Conclusions and Reasons |
|---|---|---|
| **Benefit** | • Luspatercept treatment demonstrated clinically meaningful reductions in RBC transfusion burden in patients with beta thalassemia in the BELIEVE trial<br>• A greater proportion of luspatercept-treated patients achieved a ≥ 33% reduction from baseline in transfusion burden during weeks 13 to 24 (21.4% vs 4.5%)<br>• A greater proportion of luspatercept-treated patients achieved a ≥ 50% reduction from baseline in transfusion burden during any 12 week interval (40.2% vs 6.3%)<br>• These results were durable and robust as demonstrated by consistency across all subgroups, including $B^o/B^o$ genotypes | • Luspatercept treatment resulted in decreased RBC transfusion requirements in patients with beta thalassemia. |
| **Risk and Risk Management** | • The most common adverse reactions (in ≥ 10% of subjects) in patients with beta-thalassemia treated with luspatercept were headache (26%), bone pain (20%), arthralgia (19%), fatigue (14%), cough (14%), abdominal pain (14%), diarrhea (12%), and dizziness(11%).<br>• Grade 3 and 4 treatment-emergent adverse events in the phase 3 trial occurred in 29% of patients in the luspatercept-aamt arm and 14.7% of patients in the placebo arm with the most common being bone pain (1.3%), anemia (3.1%), hyperuricemia (2.7%), and ALT increase (0.9%).<br>• Serious adverse reactions were reported in 1% of patients receiving luspatercept-aamt and included cerebrovascular accident and deep vein thrombosis. There was 1 death reported in the 3-month safety follow up in a 26-year-old male treated with luspatercept-aamt who developed neutropenic sepsis, pancytopenia and renal failure resulting in death due to an unconfirmed report of development of AML (M6) erythroleukemia.<br>• Permanent discontinuation due to an adverse reaction (Grades 1-4) occurred in 5.4% of patients who received luspatercept-aamt. The most | • The overall risk for the proposed population appear acceptable. Long-term safety will be an important consideration and will be part of a post-marketing requirement/commitment.<br>• To minimize risks, labeling should include warnings and precautions for thrombosis and hypertension. Laboratory table for liver enzyme should also be included. Non-clinical data regarding renal finding should be included in the labeling. |

| Dimension | Evidence and Uncertainties | Conclusions and Reasons |
|---|---|---|
| | frequent adverse reactions leading to permanent discontinuation in patients who received luspatercept-aamt included arthralgia (1%), bone pain (< 1%) and headache (<1%)<br>• Important identified safety findings with the use of luspatercept included thrombosis/thromboembolism and hypertension. In adult patients with beta thalassemia, thromboembolic events were reported in 8/223 (3.6%) treated patients. Reported thromboembolic events included deep vein thromboses, pulmonary embolus, portal vein thrombosis, and ischemic strokes. Patients with known risk factors for thromboembolism may be at further increased risk of thromboembolic conditions.<br>• Hypertension was reported in 10.7% (61/571) of luspatercept-aamt treated patients. The incidence of grade 3-4 hypertension ranged from 1.8% to 8.6% and reflects exposure to luspatercept-aamt as a single agent administered across a range of doses (0.125mg/kg to 1.75mg/kg) in 571 patients. In adult patients with beta thalassemia with normal baseline blood pressure, 13 (6.2%) patients developed systolic blood pressure (SBP)> 130mm Hg and 33 (16.6%) patients developed diastolic blood pressure (DBP) > 80mmg Hg.<br>• In non-clinical data there appears to be a safety signal for carcinogenicity and renal toxicity. As luspatercept may be administered long-term to patients with beta-thalassemia the long-term safety and follow-up reports of ongoing studies will be important to assess the long-term safety of this product.<br>• As with all therapeutic proteins, there is a potential for immunogenicity. Of the 284 patients with beta thalassemia treated with luspatercept-aamt and evaluable for the presence of anti-luspatercept aamt antibodies, 4 patients (1.4%) tested positive for treatment emergent anti-luspatercept- | |

| Dimension | Evidence and Uncertainties | Conclusions and Reasons |
|---|---|---|
| | aamt antibodies including 2 (0.7%) who had neutralizing antibodies. There were no severe acute systemic hypersensitivity reactions reported for patients with anti-luspatercept-aamt antibodies in clinical trials and no association between hypersensitivity type reaction or injection site reaction and presence of anti-luspatercept-aamt antibodies. | |

Please note that this review address only the proposed indication of beta thalassemia. The Sponsor submitted the original BLA for treatment of myelodysplastic syndrome associated anemia and adult patients with beta thalassemia associated anemia. (b) (4)

There are a several considerations regarding the conclusion of efficacy and safety for luspatercept-aamt for the treatment of anemia in patients with beta thalassemia.

- The term transfusion dependent usually denotes patients with B-thalassemia major, severe hemoglobin E/B-thalassemia, and A-thalassemia major, was not used in the indication statement as there are patients with B-thalassemia intermedia, mild/moderate hemoglobin E/B-thalassemia or a-thalassemia intermediate (hemoglobin H) disease who require more frequent transfusions. In addition, the BELIEVE study enrolled patients with beta-thalassemia and HbE/beta thalassemia and beta-thalassemia with alpha thalassemia and all patients had to meet a transfusion entry criteria of 6-20 RBC units in prior 24 weeks). Therefore, there is evidence that luspatercept has efficacy in patients with beta thalassemia who require regular red blood cell transfusions.

- The impact of luspatercept-aamt on iron overload and reduction in iron by measuring serum ferritin, liver iron concentration and myocardial T2 was also assessed. There was no clinically meaningful differences in change from baseline in LIC and myocardial T2. There appears to be a trend toward reduction in serum ferritin with the mean serum ferritin concentration in the luspatercept-aamt group decreased by 248.02 ug/L from baseline with increase of 106.62ug/L in the placebo group from baseline. The mean baseline ferritin levels were 2096 and 1845 in the luspatercept-aamt and placebo groups, respectively. Although a meaningful reduction in liver iron concentration (LIC) was not observed at the time of the primary analysis, it may

be that the treatment duration is too short to demonstrate any change in visceral organ iron reductions. Longer follow-up will be needed to understand the impact of luspatercept-aamt on reduction of iron stores in organs.

- Lastly, for a disease in which there are no few to no available therapies and standard of care is chronic transfusion, decreasing or minimizing the number of transfusions a patient receives can be considered clinically meaningful. Although a reduction in liver iron concentrations was not demonstrated, the impact of this change may have been too early to assess at the time of data cut-off in this trial.

Overall, luspatercept-aamt was well tolerated and demonstrates an acceptable safety profile for the period of exposure provided in this application. The following important risks identified include thromboembolic events, hypertension, immunogenicity and non-clinical findings of hematological malignancies and kidney injury. The safety concerns of thromboembolic disease and hypertension will be addressed by including these in the warnings and precautions section of the label and requesting long-term follow-up data from ongoing trials. A description of the non-clinical findings for kidney injury and malignancies will be included in the prescribing information as well as requesting long-term follow-up data from ongoing clinical trials. Additionally, as this drug may be given life-long or intermittently life-long, characterization of the long-term safety of luspatercept will be important. To help mitigate this potential safety concern, post-marketing requirements for long-term follow-up of ongoing studies will be implemented. In conclusion, the benefit-risk assessment supports the recommendation for approval of luspatercept for the treatment of anemia in patients with Beta Thalassemia who require regular transfusions.

## 1.4. Patient Experience Data

**Patient Experience Data Relevant to this Application** (check all that apply)

| | | | | |
|---|---|---|---|---|
| X | **The patient experience data that were submitted as part of the application include:** | | | Section of review where discussed, if applicable [e.g., Section 6.1 Study endpoints] |
| | X | Clinical outcome assessment (COA) data, such as | | Discussed below and in section 6 of the review. |
| | | X | Patient reported outcome (PRO) | |
| | | ☐ | Observer reported outcome (ObsRO) | |
| | | ☐ | Clinician reported outcome (ClinRO) | |
| | | ☐ | Performance outcome (PerfO) | |
| | ☐ | Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.) | | |
| | ☐ | Patient-focused drug development or other stakeholder meeting summary reports | | |
| | ☐ | Observational survey studies designed to capture patient experience data | | |
| | ☐ | Natural history studies | | |
| | ☐ | Patient preference studies (e.g., submitted studies or scientific publications) | | |
| | ☐ | Other: (Please specify): | | |
| ☐ | **Patient experience data that were not submitted in the application, but were considered in this review:** | | | |
| | ☐ | Input informed from participation in meetings with patient stakeholders | | |
| | ☐ | Patient-focused drug development or other stakeholder meeting summary reports | | |
| | ☐ | Observational survey studies designed to capture patient experience data | | |
| | ☐ | Other: (Please specify): | | |
| ☐ | **Patient experience data was not submitted as part of this application.** | | | |

Patients in this study self-administered the SF-36 and the TranQoL. There were no prespecified statistical hypotheses in regard to comparison of the HRQoL endpoints and study was not powered to detect any differences in these assessments and thus all analyses are descriptive.

Tanya Wroblewski, MD
Cross Discipline Team Leader

# 2 Therapeutic Context

## 2.1.1. Analysis of Condition

Beta-thalassemia, one of the most common inherited hemoglobinopathies worldwide, is due to autosomal mutations in the gene encoding β-globin, which induce an absence or low-level synthesis of this protein in erythropoietic cells (Weatherall, 2001). About 80 to 90 million people (approximately 1.5% of the global population) are carriers of a β-thalassemia mutation, with approximately 60,000 symptomatic individuals born annually (Modell, 2007). Beta-thalassemia is characterized by a reduction of β-globin chains and subsequent imbalance in globin chains (α:non-α ratio) of the hemoglobin (Hb) molecule, which results in impaired or ineffective erythropoiesis. Complications resulting from ineffective erythropoiesis lead to anemia and a variety of subsequent pathophysiologic mechanisms, including hemolysis, iron overload, and hypercoagulability, which, in turn, are linked to clinical morbidities.

Nearly 200 different mutations that affect the β-globin gene have been described in patients with β-thalassemia, and patient genotypes may be either homozygous or compound heterozygous. Phenotypic findings, therefore, range widely in patients, from slight impairment to complete loss of β-globin chain synthesis (Thein, 2013). In addition to deficient β-globin chains, patients may also present with β-thalassemia combined with structural variants of Hb, such as hemoglobin E (HbE). This may lead to HbE/β-thalassemia, a highly heterogeneous disease, which is the most common form of thalassemia in India, Bangladesh, and Southeast Asia (Colah, 2010). The clinical symptoms vary widely; patients may be transfusion dependent or may require only occasional or intermittent transfusions.

Beta-thalassemia comprises a number of different phenotypes that vary in severity, including the following:

- Transfusion-dependent (TD) thalassemia: Includes patients with β-thalassemia major or severe forms of β-thalassemia intermedia or HbE/β-thalassemia who require regular red blood cell (RBC) transfusions. The term transfusion-dependent can also be stated as patients who require regular red blood cell transfusions.

- Non-transfusion-dependent (NTD) thalassemia: Includes patients with mild to moderate β-

thalassemia intermedia or HbE/β-thalassemia who may require infrequent transfusions to manage the disease and its complications.

- Beta-thalassemia trait (minor): Heterozygous patients with mild, usually asymptomatic anemia who generally do not require treatment. Such patients were excluded from the luspatercept target patient population.

## 2.2. Analysis of Current Treatment Options

Current treatment options for β-thalassemia are extremely limited; packed red blood cell (RBC) transfusions and iron chelation therapy (ICT) remain the mainstay of treatment. As such, management of patients requires close monitoring and follow up from early childhood throughout adulthood with multidisciplinary teams from various medical specialties.

For patients with TD β-thalassemia, the standard of care involves regular blood transfusions, usually administered every 2 to 5 weeks, to maintain a pretransfusion Hb level of 9.0 to 10.5 g/dL, as a result of the malfunction of the bone marrow as early as 2 years of age (Cappellini, 2014). Lifelong dependence on transfusions is associated with transfusional iron overload as well as the risk of alloimmunization, transfusion reactions, and transmission of bloodborne pathogens and infections.

Many patients with β-thalassemia major require splenectomy due to hypersplenism occurring as a result of large numbers of cells being pooled and destroyed in the spleen's reticuloendothelial system (Cappellini, 2014b). This may compromise a patient's immune system and increase risk of infection secondary to encapsulated bacteria.

Transfusional iron load often leads to excessive iron deposition in the liver and cardiac tissue. Untreated iron overload can lead to hepatic dysfunction, cirrhosis, hepatic and/or cardiac failure. Three iron chelators are currently approved in the US for the treatment of iron overload in patients with TD β-thalassemia: deferoxamine in subcutaneous (SC) or intravenous injection, oral deferiprone in tablet or solution form, and oral deferasirox in dispersible tablet and, more recently, film-coated tablet forms (Taher, 2018b; Cappellini, 2014c; Musallam, 2013). Of note, these treatments address the iron overload that develops as a result of frequent RBC transfusion; they do not alter the underlying pathophysiology of β-thalassemia.

Hematopoietic stem cell transplantation (HSCT) from an identical family donor is a potential treatment option for children with thalassemia and is the only therapy with curative potential (SCCCAT, 2008). However, this option is limited by the availability of appropriate donors and by significant risks posed by the bone marrow transplant procedure itself.

# 3 Regulatory Background

## 3.1. U.S. Regulatory Actions and Marketing History

Luspatercept is not currently marketed in the United States (U.S.).

## 3.2. Summary of Presubmission/Submission Regulatory Activity

On June 14, 2011, IND 112562 was submitted as a multiple ascending dose study of ACE-536 in normal, healthy, post-menopausal women. The study was deemed safe to proceed on July 14, 2011.

On November 1, 2011, IND 112562 was placed on full clinical hold due to CMC issues. The Agency found that there were manufacturing violations of current good manufacturing practice (CGMP) regulations. Upon review of the supporting information, the full clinical hold was removed on February 15, 2012.

On March 11, 2013, luspatercept was granted orphan designation (#13-3898) for beta-thalassemia and on May 5, 2015, luspatercept was granted fast track designation for transfusion-dependent and non-transfusion-dependent beta-thalassemia.

On May 15, 2015, an End-of-Phase 2/Pre-Phase 3 meeting was held to seek agreement on their phase 3 study design for beta-thalassemia. The key points related to the proposed indication is the followings:

- Agency did not agree with the proposed clinical development program of conducting one trial per indication.
- The Agency agreed with the key enrollment criteria and the 2:1 randomization.
- The Agency recommended that the definition of clinical benefit in transfusion-dependent patients as a primary endpoint be modified to require 50% reduction in transfusion burden and a reduction of at least 2 units over a 12-week period.
- The Agency did not agree with the statistical analysis plan or the interim statistical analysis plan.

On May 25, 2018, Written Responses for a Guidance meeting request were issued regarding the content and format of a new marketing application.

The CMC Pre-BLA meeting was held on September 5, 2018, to discuss the data package within the Quality (Module 3) section of the anticipated Biologics License Application (BLA).

The Sponsor presented their top-line results for the clinical efficacy and safety data from the Phase 3 BELIEVE pivotal study (ACE-536-BTHAL-001) and two phase 2 studies (A536-04/06) to support their BLA submission at the pre-BLA meeting on October 4, 2018.

# 4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

## 4.1. Office of Scientific Investigations (OSI)

The two selected clinical sites were chosen with assistance from the OSI team utilizing the site selection tool. International site #481 (Dr. Maria Domenica Cappellini, Milan, Italy) was a high enroller site in the pivotal beta thalassemia study, ACE-536-B-THAL-001 and was noted to have a high number of protocol deviations and violations. In addition, site #481 had a relatively higher serious adverse event reporting profile compared to other high enrollee sites. Site #001 (Dr. Thomas M. Coates, Los Angeles, CA) was a high enrolling domestic site with several protocol violations and deviations reported.

Data from the two clinical sites are considered to be reliable in support of the requested indication. The inspection of Applicant's site found no significant deficiencies with oversight and monitoring of the trial. In general, the Applicant maintained adequate oversight of the clinical trial and appeared to be in compliance with Good Clinical Practices.

Please refer to the OSI review by Dr. Anthony Orencia M.D. and Min Lu M.D. M.P.H. for additional details.

## 4.2. Product Quality

The overall control strategy includes controlof raw materials, facilities and equipment, manufacturing process, and adventitious agents. The conditions used in manufacturing have been sufficienty validated, and the data submitted in this application suppor the conclusion that the manufacture of luspatercept is well controlled and yields a consistently high-quality product. Refer to the the product quality review by Sarah Johnson in the Office of Biotechnology Products(OBP) for more details.

## 4.3. Clinical Microbiology

From the Product Quality Microbiology Review and Evaluation by Aimee L. Cunningham Ph.D. M.P.H. and Reyes Candau-Chacon Ph.D, "the drug product portion of this BLA was reviewed from a sterility assurance and product quality microbiology standpoint and is recommended for approval." Please refer to the review written by the reviewers stated above for additional details.

## 4.4. Devices and Companion Diagnostic Issues

There are no devices or companion diagnostics associated with this drug product. Hence, this section is not applicable to BLA 761136.

# 5 Nonclinical Pharmacology/Toxicology

## 5.1. Executive Summary

Luspatercept-aamt (Reblozyl, ACE-536) is a recombinant fusion protein comprised of a modified form of the extracellular domain (ECD) of human activin receptor type IIB (ActRIIB) and a human IgG1 Fc domain. Luspatercept-aamt binds endogenous ligands of ActRIIB including the TGF-β superfamily ligands GDF8, GDF11, BMP6, and activin B; by binding and sequestering these ligands luspatercept-aamt acts as a ligand trap, inhibiting ActRIIB-mediated downstream signaling. Sequestration of GDF8, GDF11, and/or other ligands results in the inhibition of the Smad 2/3 pathway which is associated with impaired erythropoiesis, although the exact role of the Smad 2/3 signaling pathway in erythropoiesis is not fully understood.

The relative binding affinity of ACE-536 to the TGF-β superfamily ligands in vitro was BMP6 > activin B > GDF11 > GDF8, with little or no affinity to all other ligands tested. In an in vitro functional assay ACE-536 inhibited the activation of the Smad 2/3 pathway induced by GDF11 ($IC_{50}$=7.1 ng/mL), and to a lesser extent, GDF8 ($IC_{50}$=88 ng/mL). Despite binding BMP6 with greater affinity than GDF11 or GDF8, ACE-536 had no effect on BMP6-mediated Smad 1, 5, or 8 signaling in vitro at the concentrations tested.

Members of the TGF-β superfamily (ligands, receptors, and accessory molecules) are highly conserved across mammalian species, and luspatercept-aamt is pharmacologically active in healthy nonclinical species and in animal models of anemia and disease. In vivo pharmacology studies were conducted with ACE-536 and a murine ortholog (RAP-536) that is less immunogenic in mice. RAP-536 contains a murine IgG2a Fc region instead of a human IgG1 Fc region but is otherwise biochemically identical to ACE-536. In healthy cynomolgus monkeys ACE-536 treatment (10 mg/kg, SC) resulted in transient increase in reticulocytes (RETIC) and sustained increases in red blood cells (RBC), hemoglobin (HGB), and hematocrit (HCT). In healthy mice ACE-536 treatment (0.1-10 mg/kg, SC) resulted in dose-dependent increases in RBC, HGB, and HCT. Studies in healthy mice also suggested ACE-536 enhances the maturation of later stage erythroid precursors and acts independently of erythropoietin.

RAP-536 was evaluated in mouse models of MDS (NHD13 mice) and β-thalassemia (*Hbb*$^{th1/th1}$ mice). In the MDS model, RAP-536 treatment (10 mg/kg, SC, twice weekly (BIW) for 8 weeks) resulted in increases in measures of red cell mass (RBC, HCT, and HGB) and reduced erythroid hyperplasia. In a separate study chronic RAP-536 treatment (10 mg/kg, SC, BIW for 7 months) prevented worsening anemia in the MDS model but did not improve overall survival. In the mouse model of β-thalassemia RAP-536 treatment (1 mg/kg, SC, BIW for 2-3 months) resulted in improved hematology parameters (increased RBC, HGB, and HCT) and RBC quality (increased RBC life span, reduced hemolysis, and improved RBC morphology). Increased splenic Smad 2/3

phosphorylation was observed in both the mouse models of MDS and β-thalassemia, and Smad 2/3 phosphorylation was reduced after treatment with RAP-536.

The luspatercept-aamt toxicology program includes general toxicology studies (rats and cynomolgus monkeys), reproductive and developmental toxicology studies (rats and rabbits), and specialized toxicology studies (juvenile and renal toxicity studies in rats). Studies were conducted primarily by the SC route and in most studies ACE-536 was administered every 2 weeks (Q2W). All pivotal studies were conducted in GLP compliance. Toxicokinetic parameters were assessed in most of the toxicity studies.

The repeat-dose general toxicity studies were up to 3 and 6 months in duration in rats and cynomolgus monkeys, respectively. Pharmacologically-expected increases in measures of red cell mass were observed in all studies and partially or fully reversed following a recovery period; hematologic findings were accompanied by extramedullary hematopoiesis in the lymph nodes in the 6-month monkey study. Kidney findings were consistently noted in both species. Membranoproliferative glomerulonephritis was observed in the 3-month rat and 6-month monkey studies at exposures similar to or lower than the maximum recommended human dose and partially reversed following a recovery period. Glomerulonephritis was accompanied by microscopic evidence of immune complex deposition (IgG, IgM, and/or C3) in both species, but the incidence and titer of anti-drug antibodies (ADAs) in monkeys were low suggesting the kidney findings were directly related to ACE-536. Other findings indicating kidney toxicity included reversible increases in blood urea nitrogen (BUN, rats and monkeys) and creatinine (monkeys only). Adverse effects in the kidney were further evaluated in the investigative renal toxicity study in rats. Other organs/tissues identified in the general toxicity studies included the heart (decreased weight; rat), lung (decreased weight; rat), liver (decreased weight, vacuolation; rat), adrenal gland (increased weight, congestion, necrosis, mineralization; rat), stomach (mineralization; rat) choroid plexus (deposition, infiltration, degeneration; monkey), and thymus (congestion, involution; rat and monkey). One premature death at the high dose level in the 3-month rat study was attributed to disseminated pleomorphic lymphoma.

In a combined male and female fertility and early embryonic development study in rats, ACE-536 was administered SC and was associated with maternal toxicity and reductions in the average numbers of corpora lutea, implantations, and viable embryos; there were no adverse findings in male rats. In a subsequent study in females only, a 14-week recovery period was incorporated and there were no adverse effects on fertility parameters, demonstrating reversibility of these effects. Embryo-fetal developmental was evaluated in pregnant rats and rabbits using SC route of administration. ACE-536 was associated with maternal toxicity in rabbits and fetal toxicity in both species; fetal toxicity included increased resorptions and post-implantation loss, decreased litter size, increased incidence of skeletal variations, and reduced fetal weights. Pre- and postnatal development were evaluated in rats using SC route of administration. In the $F_1$ generation ACE-536-related observations included decreased body weights, adverse kidney findings, and delayed male sexual development (delayed preputial

separation). There were no adverse effects on fertility or reproductive parameters in the $F_1$ generation, and there were no findings in the $F_2$ generation ($F_1$ uterine examination).

Juvenile and renal toxicity studies were conducted in rats; these studies were not requested by the FDA. In the juvenile animal study ACE-536-related findings were generally similar to those observed in adult rats. Three malignancies were observed at the high dose level in the juvenile animals. This study was not designed to assess carcinogenicity and not all animals were evaluated for malignancies. The non-GLP renal toxicity study was conducted to better understand the effect of ACE-536 on the progression of renal injury. Increases in glomerular and tubulointerstitial findings and urinary kidney injury biomarkers were observed in nephrectomized animals treated with ACE-536 or RAP-536.

No genotoxicity studies were conducted or are required to support a marketing application for biotechnology-derived products such as luspatercept-aamt. No formal carcinogenicity studies were conducted. Given the malignancies observed in the general and juvenile toxicity studies in rats a relationship between luspatercept-aamt and carcinogenesis cannot be excluded.

Luspatercept-aamt enhances the maturation of existing erythroid precursors by inhibiting the Smad 2/3 pathway and acts independently of the erythropoietin receptor; a new Established Pharmacologic Class "erythroid maturation agent" was created for luspatercept-aamt. The luspatercept-aamt product label recommends the use of contraception during treatment with luspatercept-aamt and for at least 3 months after the last dose. The mean serum half-life of luspatercept-aamt was approximately 11 days in patients with beta thalassemia and 13 days in patients with MDS. The 3-month duration is based on 5 half-lives[1] of luspatercept-aamt in patients with MDS.

The nonclinical pharmacology and toxicology data submitted to this BLA are adequate to support the approval of luspatercept-aamt for the proposed indication.

## 5.2. Referenced NDAs, BLAs, DMFs

None

## 5.3. Pharmacology

Primary pharmacology

In surface plasmon resonance (SPR) analyses ACE-536 stably bound TGF-β superfamily ligands including human BMP6, activin B, GDF11, and GDF8 with subnanomolar to low-nanomolar

[1] FDA, 2019. Guidance for Industry, Oncology Pharmaceuticals: Reproductive Toxicity Testing and Labeling Recommendations. (https://www.fda.gov/media/124829/download (accessed September 24, 2019)

equilibrium dissociation constants ($K_D$=0.18-3.00 nM) at 37˚C. The binding profiles of ACE-536 and the murine orthologue, RAP-536, were similar. The affinity of ACE-536 or RAP-536 to TGF-β superfamily ligands of other species was not evaluated by SPR.

The functional activity of ACE-536 to inhibit various TGF-β superfamily ligands was evaluated in a reporter gene assay. ACE-536 inhibited the activation of the Smad 2/3 pathway induced by GDF11 and GDF8 with $IC_{50}$ values of 7.1 ng/mL and 88 ng/mL, respectively. ACE-536 was less potent in inhibiting activin B and activin A signaling ($IC_{50}$ values of 14,400 ng/mL and ~33,300 ng/mL, respectively), and did not affect Smad 1, 5, and 8 signaling mediated by BMP6, BMP9, and BMP10 at the concentrations tested ($IC_{50}$ values >3,000 ng/mL).

The ActRIIB protein is highly conserved across mammalian species. The human ActRIIB protein sequence is >99% similar to the mouse, rat, bovine, and rabbit sequences, and 100% identical to the cynomolgus monkey sequence; given the high protein sequence identity among mammalian species, ACE-536 and RAP-536 are expected to be pharmacologically active in healthy nonclinical species and in animal models of anemia and disease.

The in vivo pharmacologic activity of ACE-536 was evaluated in healthy cynomolgus monkeys. ACE-036 was administered (10 mg/kg, SC) on Days 1 and 8, and animals were sacrificed on Day 45. Increased RBC (1.0-1.2x baseline), HGB (1.0-1.3x baseline), and HCT (1.0-1.3x baseline) were observed by Day 3 and persisted until sacrifice; transient increase in RETIC (0.9-4.6x baseline) was also observed. Examination of bone marrow smears revealed an increase in erythroid counts.

The in vivo pharmacologic activity of ACE-536 and RAP-536 were evaluated in normal mice and in mouse models of MDS and β-thalassemia intermedia. Normal C57BL/6 mice were administered ACE-536 (0.1-10 mg/kg, SC, BIW) or vehicle for 8 weeks. Dose-dependent increases in RBC, HGB, and HCT were observed, and the differences from control were statistically significant ($p<0.001$) at ≥3 mg/kg. In separate studies in normal mice ACE-536 (10 mg/kg, IP, single dose) was administered alone, in combination with erythropoietin (1800 units/kg, IP, single dose), or in combination with an anti-erythropoietin monoclonal antibody. Coadministration of ACE-536 and erythropoietin resulted in greater increases in RBC (+20.3%), HGB (+23.1%), and HCT (+22.5%) than either agent alone (+5.6-9.2%). Coadministration of ACE-536 and anti-erythropoietin monoclonal antibody reversed the decline in RBC, HGB, and HCT observed after treatment with anti-erythropoietin monoclonal antibody alone. Following treatment with RAP-536 fewer splenic and bone marrow early erythroblasts and more later stage erythroid precursors were observed.

RAP-536 was evaluated in a transgenic mouse model of MDS. NHD13 mice were administered RAP-536 (10 mg/kg, SC, BIW) or vehicle. After 8 weeks of treatment increased RBC, HGB, and HCT (+16.9%, +12.5%, and +11.6%, respectively) were observed in RAP-536-treated mice compared to vehicle-treated mice, and levels were slightly below or similar to wild type mice.

The bone marrow of RAP-536-treated mice contained fewer erythroid precursors (31.7% vs 52.0% in vehicle-treated mice) and had normalized myeloid:erythroid ratios, indicating reduced erythroid hyperplasia. Smad 2/3 phosphorylation was decreased in the spleen sections of RAP-536-treated mice compared to vehicle-treated mice. RAP-536 demonstrated pharmacologic activity in models of early-, mid-, and late-stage MDS.

Another study was conducted in NHD13 mice to assess the ability of RAP-536 to prevent anemia in the MDS model. NHD13 mice were administered RAP-536 (10 mg/kg, SC, BIW) or vehicle beginning at 4 months of age. The effect of RAP-536 on hematological indices was apparent after 1 month of treatment. After 7 months of treatment improvement in hematological indices was observed in males (RBC [+13.8%], HGB [+19.8%], HCT [+14.8%]) and females (RBC [+27.2%], HGB [+21.5%], HCT [+22.9%]) compared to vehicle-treated mice; however, treatment with RAP-536 did not improve overall survival.

RAP-536 was evaluated in a mouse model of β-thalassemia intermedia. *Hbb*$^{th1/th1}$ mice were administered RAP-536 (1 mg/kg, SC, BIW) or vehicle. After 2-3 months of treatment increased RBC, HGB, and HCT (+28.6%, +15.6%, and +18.6%, respectively), and decreased RETIC and RDW (-33.4% and -19.4%, respectively) were observed in RAP-536-treated mice compared to vehicle-treated mice. RAP-536 treatment was associated with increased RBC life span (28 days vs 20 days in vehicle-treated mice), but RBC life span in RAP-536-treated mice was still well below that of wild-type mice (42 days). Decreased total serum bilirubin indicating reduced hemolysis and improved RBC morphology was also observed in RAP-536-treated mice. Smad 2/3 phosphorylation was decreased in the spleen sections of RAP-536-treated mice compared to vehicle-treated mice, indicating diminished Smad 2/3 activation.

Secondary Pharmacology

A panel consisting of 43 ligands from the TGF-β superfamily, including select heterodimers, were tested for binding to ACE-536 or RAP-536 by SPR. ACE-536 and RAP-536 stably bound GDF8, GDF11, BMP6, BMP10, and activin B with high affinity. All other members of the TGF-β superfamily did not bind ACE-536 or RAP-536, or bound transiently, non-specifically, and/or with low affinity.

Safety Pharmacology

No standalone safety pharmacology studies were conducted with ACE-536 or RAP-536. Safety pharmacology endpoints were incorporated into the 1- and 3-month toxicology studies in cynomolgus monkeys. There were no ACE-536-related adverse cardiovascular, respiratory, or neurologic findings.

## 5.4. ADME/PK

<table>
<tr><th>Type of study</th><th>Major findings</th></tr>
<tr><td>Absorption</td><td></td></tr>
<tr><td>4-week study in cynomolgus monkeys (study number WHH00067)</td><td>Bioavailability of ACE-536 following SC dosing was 76% (based on AUC comparison at 10 mg/kg SC and 10 mg/kg IV).</td></tr>
<tr><td>Distribution</td><td></td></tr>
<tr><td>Lacteal transfer study in rats (study number 8369726)</td><td>Pregnant female rats were administered a single dose of ACE-536 by SC injection; the mean lacteal transfer was 12%.</td></tr>
<tr><td>Metabolism</td><td>Metabolism studies were not conducted.</td></tr>
<tr><td>Excretion</td><td>Excretion studies were not conducted.</td></tr>
<tr><td>TK data from general toxicology studies</td><td></td></tr>
<tr><td>13-week study in rats (study number 20017484)</td><td>$t_{1/2}$: 69.7-215.1 hours<br>Accumulation ($AUC_{0\text{-}336h}$ after dosing on Day 85 relative to that after Day 1): modest accumulation (1.46-2.42x) at all dose levels.<br>Dose proportionality (based on $AUC_{0\text{-}336h}$): slightly less than or equal to dose proportional (0.52-0.92x) across the dosing range.<br>Immunogenicity: 64% of TK animals developed an ADA response; ADA-positive animals with compromised ACE-536 exposure were excluded from the TK analysis.<br><br>TK parameters on Day 1
<table>
<tr><th>Dose (mg/kg)</th><th>Age</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th></tr>
<tr><td>1</td><td rowspan="3">17 weeks</td><td>7.82</td><td>1615.18</td><td>152.09</td></tr>
<tr><td>3</td><td>21.16</td><td>4005.81</td><td>118.05</td></tr>
<tr><td>15</td><td>83.27</td><td>16659.58</td><td>78.01</td></tr>
<tr><td>15</td><td>8 weeks</td><td>136.01</td><td>25503.27</td><td>69.74</td></tr>
</table>
No sex-related differences were observed, data are sex-combined.<br>TK parameters on Day 85*
<table>
<tr><th>Dose (mg/kg)</th><th>Age</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th><th>R</th></tr>
<tr><td>1</td><td rowspan="3">17 weeks</td><td>12.64</td><td>3916.65</td><td>190.53</td><td>2.42</td></tr>
<tr><td>3</td><td>19.86</td><td>6681.52</td><td>215.10</td><td>1.67</td></tr>
<tr><td>15</td><td>100.67</td><td>30755.03</td><td>202.04</td><td>1.85</td></tr>
<tr><td>15</td><td>8 weeks</td><td>131.14</td><td>37252.34</td><td>183.66</td><td>1.46</td></tr>
</table>
* Excludes statistical outliers; R: accumulation; no sex-related differences were observed, data are sex-combined.</td></tr>
</table>

<table>
<tr><th>Type of study</th><th>Major findings</th></tr>
<tr><td>6-month study in cynomolgus monkeys (study number 20039148)</td><td>$t_{1/2}$: 128-175 hours<br>Accumulation ($AUC_{0\text{-}336h}$ after dosing on Day 183 relative to that after Day 1): modest accumulation (1.4-1.7x) at all dose levels.<br>Dose proportionality (based on $AUC_{0\text{-}336h}$): approximately dose proportional (0.82-1.07x) across the dosing range.<br>Immunogenicity: One animal (3.3%) developed an ADA response; this animal was excluded from the TK analysis.<br><br>TK parameters on Day 1<br><table><tr><th>Dose (mg/kg)</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th></tr><tr><td>0.3</td><td>3.87</td><td>866</td><td>170</td></tr><tr><td>1</td><td>12.2</td><td>2569</td><td>175</td></tr><tr><td>6</td><td>70.9</td><td>14891</td><td>174</td></tr></table>No sex-related differences were observed; data are sex-combined.<br>TK parameters on Day 183*<br><table><tr><th>Dose (mg/kg)</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th><th>R</th></tr><tr><td>0.3</td><td>5.40</td><td>1228</td><td>174</td><td>1.4</td></tr><tr><td>1</td><td>19.7</td><td>4412</td><td>152</td><td>1.7</td></tr><tr><td>6</td><td>111</td><td>21627</td><td>128</td><td>1.4</td></tr></table>* Excludes one ADA-positive animal; R: accumulation; no sex-related differences were observed, data are sex-combined.</td></tr>
<tr><td>TK data from reproductive toxicology studies</td><td></td></tr>
<tr><td>Embryo-fetal development study in rats (study number 20040548)</td><td>TK parameters on GD 10<br><table><tr><th>Dose (mg/kg)</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}168h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th></tr><tr><td>5*</td><td>58.2</td><td>6166</td><td>21.6</td></tr><tr><td>15</td><td>171</td><td>16128</td><td>18.1</td></tr><tr><td>30^</td><td>326</td><td>34488</td><td>25.2</td></tr></table>* Fetal NOAEL; ^ maternal NOAEL</td></tr>
<tr><td>Embryo-fetal development study in rabbits (study number 20040550)</td><td>TK parameters on GD 11<br><table><tr><th>Dose (mg/kg)</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}168h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th></tr><tr><td>5*</td><td>91.1</td><td>12420</td><td>55.0</td></tr><tr><td>20</td><td>279</td><td>36314</td><td>74.3</td></tr><tr><td>40</td><td>616</td><td>75690</td><td>46.7</td></tr></table>* Maternal and fetal NOAEL</td></tr>
<tr><td>Pre and postnatal development study in rats (study number 847-167)</td><td>Maternal to fetal ACE-536 concentration ratios, GD20<br><table><tr><th rowspan="2">Dose (mg/kg)</th><th colspan="2">Maternal to fetal ratio</th></tr><tr><th>8 hours</th><th>24 hours</th></tr><tr><td>3</td><td>24.8</td><td>8.6</td></tr><tr><td>10</td><td>21.2</td><td>9.8</td></tr><tr><td>30</td><td>7.9</td><td>8.2</td></tr></table></td></tr>
<tr><td>TK data from juvenile animal study</td><td></td></tr>
<tr><td>13-week study in juvenile rats (study number WIL-961003)</td><td>TK parameters on Day 91*<br><table><tr><th>Dose (mg/kg)</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th></tr><tr><td>1</td><td>10.4</td><td>2636</td><td>142</td></tr><tr><td>3</td><td>37.5</td><td>8486</td><td>168</td></tr><tr><td>10</td><td>94.1</td><td>20054</td><td>174</td></tr></table>* Excludes six ADA-positive animals; no sex-related differences were observed, data are sex-combined.</td></tr>
</table>

<table>
<tr><th>Type of study</th><th>Major findings</th></tr>
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<tr><td>Absorption</td><td></td></tr>
<tr><td>4-week study in cynomolgus monkeys (study number WHH00067)</td><td>Bioavailability of ACE-536 following SC dosing was 76% (based on AUC comparison at 10 mg/kg SC and 10 mg/kg IV).</td></tr>
<tr><td>Distribution</td><td></td></tr>
<tr><td>Lacteal transfer study in rats (study number 8369726)</td><td>Pregnant female rats were administered a single dose of ACE-536 by SC injection; the mean lacteal transfer was 12%.</td></tr>
<tr><td>Metabolism</td><td>Metabolism studies were not conducted.</td></tr>
<tr><td>Excretion</td><td>Excretion studies were not conducted.</td></tr>
<tr><td>TK data from general toxicology studies</td><td></td></tr>
<tr><td>13-week study in rats (study number 20017484)</td><td>$t_{1/2}$: 69.74-215.10 hours<br>Accumulation ($AUC_{0\text{-}336h}$ after dosing on Day 85 relative to that after Day 1): modest accumulation (1.46-2.42x) at all dose levels.<br>Dose proportionality (based on $AUC_{0\text{-}336h}$): slightly less than or equal to dose proportional (0.52-0.92x) across the dosing range.<br>Immunogenicity: 64% of TK animals developed an ADA response; ADA-positive animals with compromised ACE-536 exposure were excluded from the TK analysis.<br><br>TK parameters on Day 1
<table>
<tr><th>Dose (mg/kg)</th><th>Age</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th></tr>
<tr><td>1</td><td rowspan="3">17 weeks</td><td>7.82</td><td>1615.18</td><td>152.09</td></tr>
<tr><td>3</td><td>21.16</td><td>4005.81</td><td>118.05</td></tr>
<tr><td>15</td><td>83.27</td><td>16659.58</td><td>78.01</td></tr>
<tr><td>15</td><td>8 weeks</td><td>136.01</td><td>25503.27</td><td>69.74</td></tr>
</table>
No gender differences were observed, data are sex-combined.<br>TK parameters on Day 85*
<table>
<tr><th>Dose (mg/kg)</th><th>Age</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th><th>R</th></tr>
<tr><td>1</td><td rowspan="3">17 weeks</td><td>12.64</td><td>3916.65</td><td>190.53</td><td>2.42</td></tr>
<tr><td>3</td><td>19.86</td><td>6681.52</td><td>215.10</td><td>1.67</td></tr>
<tr><td>15</td><td>100.67</td><td>30755.03</td><td>202.04</td><td>1.85</td></tr>
<tr><td>15</td><td>8 weeks</td><td>131.14</td><td>37252.34</td><td>183.66</td><td>1.46</td></tr>
</table>
* Excludes statistical outliers; R: accumulation; no gender differences were observed, data are sex-combined.</td></tr>
</table>

<table>
<tr><th>Type of study</th><th>Major findings</th></tr>
<tr><td>6-month study in cynomolgus monkeys (study number 20039148)</td><td>$t_{1/2}$: 128-175 hours<br>Accumulation ($AUC_{0\text{-}336h}$ after dosing on Day 183 relative to that after Day 1): modest accumulation (1.4-1.7x) at all dose levels.<br>Dose proportionality (based on $AUC_{0\text{-}336h}$): approximately dose proportional (0.82-1.07x) across the dosing range.<br>Immunogenicity: One animal (3.3%) developed an ADA response; this animal was excluded from the TK analysis.<br><br>TK parameters on Day 1<br><table><tr><th>Dose (mg/kg)</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th></tr><tr><td>0.3</td><td>3.87</td><td>866</td><td>170</td></tr><tr><td>1</td><td>12.2</td><td>2569</td><td>175</td></tr><tr><td>6</td><td>70.9</td><td>14891</td><td>174</td></tr></table>No gender differences were observed; data are sex-combined.<br>TK parameters on Day 183*<br><table><tr><th>Dose (mg/kg)</th><th>$C_{max}$ (µg/mL)</th><th>$AUC_{0\text{-}336h}$ (µg*hr/mL)</th><th>$t_{1/2}$ (hour)</th><th>R</th></tr><tr><td>0.3</td><td>5.40</td><td>1228</td><td>174</td><td>1.4</td></tr><tr><td>1</td><td>19.7</td><td>4412</td><td>152</td><td>1.7</td></tr><tr><td>6</td><td>111</td><td>21627</td><td>128</td><td>1.4</td></tr></table>* Excludes one ADA-positive animal; R: accumulation; no gender differences were observed, data are sex-combined.</td></tr>
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</table>

## 5.5. Toxicology

### 5.5.1. General Toxicology

**A 13-week (q2wk x 7) Study of ACE-536 Administered by Subcutaneous Injection in Mature Adult and Young Adult Sprague Dawley Rats with a 10-week Recovery Period/ study number 20017484**

Key study findings

- There were no ACE-536-related mortalities, clinical signs, or effects on food consumption or body weights.
- Changes in hematology parameters consistent with the expected pharmacodynamic effects of ACE-536 were observed and partially reversed following the recovery period.
- ACE-536-related microscopic findings were observed in the adrenal glands, kidneys, and liver. Glomerulonephritis was accompanied by microscopic evidence of immune complex deposition.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

| | |
|---|---|
| Dose and frequency of dosing: | 0, 1, 3, or 15 mg/kg/dose<br>Q2W for 13 weeks |
| Route of administration: | SC injection |
| Formulation/Vehicle: | 10 mM TRIS-buffered saline, pH 7.2 |
| Species/Strain: | Rat/Sprague Dawley CRL:CD IGS |
| Number/Sex/Group: | Main study: 10/sex/group (mature and young)<br>Recovery: 5/sex/group (mature and young) |
| Age: | Mature: ~17 weeks<br>Young: ~8 weeks |
| Satellite groups/Unique design: | Additional animals (6/sex/group) of both age groups were included for TK and immunogenicity assessments. Parallel design with mature (0, 1, 3, and 15 mg/kg) and young adult animals (0 and 15 mg/kg only). |
| Deviation from study protocol affecting interpretation of results: | No |

**Observations and results**

| Parameters | Major findings |
|---|---|

| | |
|---|---|
| **Mortality** | There were no ACE-536-related mortalities.<br><br>One mature female in the high dose group was found dead on day 74; the death was attributed to disseminated pleomorphic lymphoma. |
| **Clinical signs** | There were no ACE-536-related clinical signs. |
| **Body weights** | Unremarkable |
| **Ophthalmoscopy** | Unremarkable |
| **Hematology** | ACE-536-related dose-dependent increases in indicators of circulating erythrocyte mass were observed in both mature and young adult animals. Platelets were affected in both mature and young adult animals, while total white blood cell, lymphocyte, monocyte and basophil counts were increased in young adult animals only. At the end of the recovery period indicators of circulating erythrocyte mass remained increased in mature adult females and total white blood cell count remained increased in young adult females (data not shown). |

**ACE-536-related changes in hematology parameters relative to controls, Day 93**

| Parameter | Dose (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 15 | 15^ | 1 | 3 | 15 | 15^ |
| | Males | | | | Females | | | |
| RBC | - | 1.13x* | 1.23x* | 1.24x* | - | 1.07x | 1.19x* | 1.35x* |
| HGB | - | 1.07x* | 1.14x* | 1.12x* | - | 1.06x* | 1.12x* | 1.22x* |
| HCT | - | 1.08x* | 1.17x* | 1.15x* | - | 1.07x* | 1.16x* | 1.28x* |
| RETIC | - | - | - | - | - | - | 1.45x* | - |
| PLT | - | - | 0.69x* | 0.77x* | - | - | - | 0.59x* |
| WBC | - | - | - | 1.24x* | - | - | - | 1.58x* |
| LYM | - | - | - | 1.29x* | - | - | - | 1.52x* |
| MONO | - | - | - | 1.27x | - | - | - | 1.98x* |
| BASO | - | - | - | 1.80x* | - | - | - | 2.64x* |

^ = Young adults; * p≤0.05

| | |
|---|---|
| **Clinical chemistry** | ACE-536-related changes in BUN, ALP, and serum glucose were observed in mature and/or young adult animals. Only increases in BUN were considered adverse, and there were no changes at the end of the recovery period. |

**ACE-536-related changes in clinical chemistry parameters relative to controls, Day 93**

| Parameter | Dose (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 15 | 15^ | 1 | 3 | 15 | 15^ |
| | Males | | | | Females | | | |
| BUN | - | - | 1.19x* | 1.17x* | - | - | 1.36x* | 1.46x* |
| ALP | - | - | 1.19x | 1.28x* | - | - | 1.05x | 1.74x* |
| Glucose | - | - | 0.86x* | 0.81x* | - | - | 0.96x | 0.71x* |

^ = Young adults; * p≤0.05

| | |
|---|---|
| **Urinalysis** | Unremarkable |
| **Gross pathology** | Unremarkable |

| | |
|---|---|
| **Organ weights** | ACE-536-related decreases in absolute and/or relative weights of the heart (≥3 mg/kg, males and females), liver (≥3 mg/kg males; 15 mg/kg females), kidney (≥3mg/kg males), lung (≥3 mg/kg mature males), and prostate (15 mg/kg young adult males) were observed at terminal necropsy. ACE-536-related increases in absolute and/or relative weight of the adrenal gland (15 mg/kg young adult females) was observed at terminal necropsy. Changes in the adrenal gland were accompanied by histopathological correlates.<br><br>At the end of the recovery period all organ weight changes resolved except for decreases in heart weight in mature males at ≥3 mg/kg. |
| **Histopathology**<br>Adequate battery: Yes<br>Peer review: Yes | ACE-536-related microscopic findings were observed in the adrenal glands, kidneys, and livers at terminal necropsy. At the end of the recovery period changes in the adrenal glands (necrosis), kidneys (glomerulonephritis), and livers (vacuolation) persisted at lower incidence and/or severity indicating partial and/or ongoing recovery (data not shown). |

**ACE-536-related histopathologic changes, Day 93**

| Tissue | | Males | | | | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **0** | **1** | **3** | **15** | **0^** | **15^** | **0** | **1** | **3** | **15** | **0^** | **15^** |
| **Adrenal gland** | **Congestion** (No. affected) | (0) | (2) | (3) | (4) | (0) | (1) | (0) | (8) | (9) | (8) | (1) | (4) |
| | Minimal | 0 | 2 | 3 | 4 | 0 | 1 | 0 | 7 | 7 | 7 | 1 | 2 |
| | Mild | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |
| | Moderate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | Marked | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | **Necrosis** (No. affected) | (0) | (0) | (0) | (0) | (0) | (1) | (0) | (1) | (2) | (0) | (0) | (4) |
| | Minimal | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 0 |
| | Moderate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | Marked | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| | **Mineralization** (No. affected) | (0) | (0) | (0) | (0) | (0) | (0) | (0) | (0) | (0) | (0) | (0) | (3) |
| | Moderate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| **Kidney** | **Glomerulonephritis** (No. affected) | (0) | (9) | (8) | (10) | (0) | (8) | (0) | (6) | (6) | (9) | (0) | (8) |
| | Minimal | 0 | 9 | 4 | 5 | 0 | 7 | 0 | 6 | 6 | 8 | 0 | 7 |
| | Mild | 0 | 0 | 4 | 5 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| **Liver** | **Vacuolation** (No. affected) | (0) | (3) | (1) | (3) | (0) | (0) | (0) | (2) | (1) | (4) | (0) | (4) |
| | Minimal | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 1 | 3 | 0 | 2 |
| | Mild | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| | **Necrosis** (No. affected) | (0) | (0) | (0) | (0) | (0) | (0) | (1) | (1) | (3) | (3) | (1) | (1) |
| | Minimal | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 3 | 1 | 1 |
| | Mild | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |

^ = Young adults;

| | |
|---|---|
| **Transcriptional profiling of heart tissue** | RNA expression levels of myosin heavy chain alpha isoform (α-MHC), myosin heavy chain beta isoform (β-MHC), and transcription activator Brg1 in heart tissue were evaluated by qRT-PCR.<br><br>There were no ACE-536-related changes in α-MHC, β-MHC, or Brg1 RNA levels. |

| | |
|---|---|
| **Immunohistochemistry analysis of IgG, IgM, and/or C3 in rats with glomerulonephritis** | Kidney sections of select rats with glomerulonephritis were stained for activin receptor IIB (interpreted as ACE-536), immunoglobulins (IgG and IgM), and complement component C3.<br><br>Activin receptor IIB, IgG, and/or C3-containing granular deposits were observed in the glomeruli of 3/4 mid-dose (3 mg/kg) and 7/8 high-dose (15 mg/kg) rats with membranoproliferative glomerulonephritis. No glomerular granular deposits were observed in low-dose (1 mg/kg) rats with membranoproliferative glomerulonephritis. |

**A 6-Month (q2wk x 14) Study of ACE-536 by Subcutaneous Injection in Cynomolgus Monkeys with a 3-Month Recovery Period/ study number 20039148**

Key study findings

- Changes in hematology parameters consistent with the expected pharmacodynamic effects of ACE-536 were observed and returned to normal following a 3-month recovery period.
- ACE-536-related microscopic findings were identified in the kidney, brain (choroid plexus), and lymph nodes, and partially or fully resolved following a 3-month recovery period.
- IgG, IgM, and/or C3-containing granular deposits were observed in the kidney and choroid plexus of select animals; however, the majority of the animals were ADA-negative, suggesting these observations were direct drug effects rather than evidence of immune complex deposition.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

| | |
|---|---|
| Dose and frequency of dosing: | 0, 0.3, 1, or 6 mg/kg/dose<br>Q2W for 6 months |
| Route of administration: | SC injection |
| Formulation/Vehicle: | 10 mM TRIS-buffered saline, pH 7.2 |
| Species/Strain: | Cynomolgus monkeys |
| Number/Sex/Group: | 5/sex/group (including 2/sex/group for recovery) |
| Age: | 2.3-6.4 years |
| Satellite groups/ unique design: | No |
| Deviation from study protocol affecting interpretation of results: | No |

**Observations and results**

| Parameters | Major findings |
|---|---|
| **Mortality** | None |

| | |
|---|---|
| **Clinical signs** | There were no ACE-536-related clinical signs. |
| **Body weights** | Unremarkable |
| **Ophthalmoscopy** | Unremarkable |
| **ECG** | Not conducted |
| **Hematology** | ACE-536-related changes in hematology parameters were observed at the ≥0.3 mg/kg dose levels. The magnitude of changes were similar on Days 29 and 196. All hematology parameters were similar to controls following a 3-month recovery period. |

**ACE-536-related changes in hematology parameters relative to controls, males**

| Parameter | Dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | 0.3 | 1 | 6 | 0.3 | 1 | 6 |
| | Day 29 | | | Day 196 | | |
| RBC | 1.08x | 1.05x | 1.16x* | 1.08x | 1.09x | 1.18x* |
| HGB | 1.04x | 1.03x | 1.11x* | 1.05x | 1.06x | 1.10x* |
| HCT | 1.03x | - | 1.08x* | 1.04x | 1.06x | 1.10x* |
| RETIC | - | - | - | - | - | 1.54x* |
| RDW | - | - | - | - | 1.05x | 1.04x |
| MCV | - | - | 0.93x* | - | - | 0.93x* |
| MCH | - | - | - | - | - | 0.93x |
| MONO | - | 1.21x | - | - | - | 1.38x |

* p≤0.05

**ACE-536-related changes in hematology parameters relative to controls, females**

| Parameter | Dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | 0.3 | 1 | 6 | 0.3 | 1 | 6 |
| | Day 29 | | | Day 196 | | |
| RBC | 1.08x | 1.14x* | 1.14x* | 1.10x | 1.15x | 1.26x* |
| HGB | 1.04x | 1.15x* | 1.11x* | 1.07x | 1.15x* | 1.17x* |
| HCT | 1.07x* | 1.14x* | 1.11x* | 1.08x | 1.16x* | 1.20x* |
| RETIC | - | - | 2.08x* | - | - | 2.46x* |
| RDW | - | - | - | - | - | 1.05x |
| MCV | - | - | - | - | - | - |
| MCH | - | - | - | - | - | 0.93x |
| MONO | - | - | - | - | 1.67x* | 1.60x |

* p≤0.05

| | |
|---|---|
| **Clinical chemistry** | ACE-536-related changes in clinical chemistry parameters were observed at the ≥1 mg/kg dose levels. Changes in BUN and creatinine correlated with histopathologic changes in the kidney. All clinical chemistry parameters were similar to controls following a 3-month recovery period, with the exception of increased ALP in one male at 6 mg/kg; there were no histopathologic findings suggestive of hepatic injury. |

**ACE-536-related changes in clinical chemistry parameters relative to controls, males**

| Parameter | Dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | 0.3 | 1 | 6 | 0.3 | 1 | 6 |
| | Day 29 | | | Day 196 | | |
| ALP | - | - | - | - | - | 1.43x |
| BUN | - | - | 1.26x | - | - | 1.19x |
| Creatinine | - | 1.17x | 1.34x* | - | 1.18x | 1.24x |
| Ferritin | - | - | 1.62x | - | - | 1.61x |

* p≤0.05

**ACE-536-related changes in clinical chemistry parameters relative to controls, females**

| Parameter | Dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | 0.3 | 1 | 6 | 0.3 | 1 | 6 |
| | Day 29 | | | Day 196 | | |
| ALP | - | 1.43x | - | - | 1.41x | - |
| BUN | - | - | 1.29x | - | - | 1.29x |
| Creatinine | - | 1.42x* | 1.69x* | - | 1.41x* | 1.55x* |
| Ferritin | - | - | 1.82x | - | - | 1.97x |

* p≤0.05

| | |
|---|---|
| **Urinalysis** | Unremarkable |
| **Gross pathology** | Unremarkable |
| **Organ weights** | Unremarkable |
| **Histopathology**<br>Adequate battery: Yes | At terminal sacrifice ACE-536-related microscopic findings were identified in the kidney, brain (choroid plexus), and lymph nodes. Findings in the kidney correlated with increases in BUN and creatinine. Findings in the kidney and choroid plexus were suggestive of immune complex deposition.<br><br>At recovery sacrifice kidney findings had partially resolved, and brain findings were limited to one male at the 6 mg/kg dose level and were considered indicative of healing and repair. There were no lymph node findings at recovery sacrifice. |

**ACE-536-related histopathologic changes, terminal sacrifice**

| Parameter | Dose (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0.3 | 1 | 6 | 0 | 0.3 | 1 | 6 |
| | Males | | | | Females | | | |
| **Number evaluated** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Kidney** | | | | | | | | |
| Membranoproliferative glomerulonephritis | | | | | | | | |
| Minimal | - | - | 3 | 1 | - | - | 1 | 1 |
| Mild | - | - | - | 1 | - | - | - | 2 |
| Moderate | - | - | - | 1 | - | - | - | - |
| Accumulation, protein, tubular epithelium (cytoplasmic) | | | | | | | | |
| Minimal | - | - | - | 1 | - | - | - | 1 |
| Tubular cast, protein | | | | | | | | |
| Minimal | - | - | - | 1 | - | - | - | 1 |
| Infiltrate, mixed cell, interstitial | | | | | | | | |
| Minimal | - | - | - | - | - | - | - | 1 |
| Mild | - | - | - | 1 | - | - | - | - |
| Fibrosis/fibroplasia, interstitium | | | | | | | | |
| Minimal | - | - | - | - | - | - | - | 2 |
| Moderate | - | - | - | 1 | - | - | - | - |
| Degeneration/atrophy, tubular, medullary | | | | | | | | |
| Minimal | - | - | - | - | - | - | - | 3 |
| Moderate | - | - | - | 1 | - | - | - | - |
| Vacuolation, interstitial cell and increased extracellular matrix, medullary | | | | | | | | |
| Minimal | - | - | 2 | - | - | - | 1 | 1 |
| Mild | - | - | - | - | - | - | - | 2 |
| Hemorrhage, acute, interstitial | | | | | | | | |
| Minimal | - | - | - | - | - | - | 1 | - |
| Mild | - | - | - | 1 | - | - | - | - |
| Hemorrhage, acute, tubular | | | | | | | | |
| Minimal | - | - | - | - | - | - | - | 1 |
| Pigment deposition (pigmentation), hemosiderin, interstitial | | | | | | | | |
| Minimal | - | - | - | 1 | - | - | - | - |
| **Brain (choroid plexus)** | | | | | | | | |
| Infiltrate, mixed cell | | | | | | | | |
| Minimal | - | - | 1 | 1 | - | - | 1 | 3 |
| Mild | - | - | - | 1 | - | - | - | - |
| Degeneration, vascular | | | | | | | | |
| Minimal | - | - | 2 | 1 | - | - | 1 | 3 |
| Mild | - | - | - | 1 | - | - | - | - |
| Deposition, protein, interstitial | | | | | | | | |
| Minimal | - | - | 1 | - | - | - | 1 | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mild | - | - | - | 2 | - | - | - | - |
| Infiltrate, foamy macrophages, interstitial | | | | | | | | |
| Minimal | - | - | - | 1 | - | - | 1 | 2 |
| Mild | - | - | - | - | - | - | - | 1 |
| Pigmented macrophages (presumptive hemosiderin), interstitial | | | | | | | | |
| Minimal | - | - | - | 2 | - | - | - | 1 |
| **Lymph node, mandibular** | | | | | | | | |
| Extramedullary hematopoiesis, medullary | | | | | | | | |
| Minimal | - | - | 1 | 1 | - | - | 2 | 1 |
| Mild | - | - | - | - | - | 1 | - | 1 |
| Moderate | - | - | - | - | - | 1 | - | 1 |
| **Lymph node, axillary** | | | | | | | | |
| Extramedullary hematopoiesis, medullary | | | | | | | | |
| Minimal | - | - | - | - | - | 1 | - | - |
| Mild | - | - | - | - | - | - | - | 1 |
| Moderate | - | - | - | - | - | - | - | 1 |

| | |
|---|---|
| **Immunohistochemistry analysis of IgG, IgM, and/or C3 in monkeys with glomerulonephritis and/or choroid plexus alterations** | Kidney and brain (choroid plexus) sections from monkeys with glomerulonephritis and/or choroid plexus alterations were stained for immunoglobulins (IgG and IgM) and complement component C3. Assay limitations precluded concurrent staining for ACE-536.<br><br>IgG, IgM, and/or C3-containing granular deposits were observed in the glomeruli of 8/10 animals with glomerulonephritis. C3 granularity was observed in the choroid plexus of 4/8 affected animals. |

**General toxicology; additional studies**

A 1-month (Q2W x 2) repeat-dose toxicity study of ACE-536 (0, 6, 20, or 60 mg/mg, SC injection) with a 2-month recovery period was conducted in Sprague Dawley rats; the results were generally consistent with the 13-week repeat-dose toxicity study of ACE-536 in rats.

Four-week and 13-week repeat-dose toxicity studies of ACE-536 were conducted in cynomolgus monkeys. In the 4-week (Q2W x 2) study ACE-536 was administered by SC injection (0, 0.4, 2, 10, or 30 mg/kg) or IV injection (10 mg/kg); bioavailability of ACE-536 by the SC route was 76% that of the IV route. In the 13-week (Q2W x 7) study ACE-536 was administered by SC injection (0, 1, 6, or 30 mg/kg). The results of the 4-week and 13-week studies were generally consistent with the 6-month repeat-dose toxicity study of ACE-536 in monkeys.

### 5.5.2. Genetic Toxicology

Not conducted per ICH S6(R1).

### 5.5.3. Carcinogenicity

Not conducted per ICH S6(R1).

### 5.5.4. Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

**Fertility and Early Embryonic Development to Implantation Administered Subcutaneously to Rats/ study number 20040551**

Key study findings

- At 15 mg/kg female hindlimbs were adversely affected and reduced mean number of corpora lutea (-22.9%), implantations (-19.3%), and viable embryos (-20.6%) were observed; these findings were observed at exposures 6.6-times the clinical exposure at the highest recommended human dose of 1.25 mg/kg.
- The NOAEL in males for general and reproductive toxicity was 15 mg/kg; the maternal NOAEL for general and reproductive toxicity was 3 mg/kg.

| | |
|---|---|
| Conducting laboratory and location: | (b) (4) |
| GLP compliance: | Yes |

Methods

| | |
|---|---|
| Dose and frequency of dosing: | 0, 1, 3, or 15 mg/kg/dose<br>Males were treated Q2W beginning 28 days before cohabitation, during cohabitation, and continuing through the day before sacrifice (total of four doses). Females were treated on study day (SD) 1, SD 15 (before cohabitation), and on presumed gestation day (GD) 3 (total of three doses). |
| Route of administration: | SC injection |
| Formulation/Vehicle: | 10 mM Tris Buffered Saline, 137 mM Sodium Chloride, and 2.7 mM Potassium Chloride, pH 7.2 |
| Species/Strain: | Rat/Sprague Dawley |
| Number/Sex/Group: | 25/sex/group |
| Satellite groups: | No |
| Study design: | Treated males were assigned 1:1 to cohabitation with untreated females. Treated females were assigned 1:1 to cohabitation with untreated males. The cohabitation period was ≤21 days. After mating was confirmed (GD 0) animals were housed individually. Males were |

| | |
|---|---|
| | sacrificed on SD 50 to SD 53, and females were sacrificed on GD 13. |
| Deviation from study protocol affecting interpretation of results: | No |

**Observations and results**

| Parameters | Major findings |
|---|---|
| Mortality | Males and females: none |
| Clinical signs | Males: one male at HD experienced ACE-536-related limited use of the hind limb.<br>Females: three females at HD experienced ACE-536-related swollen hindlimb, limited use of a hindlimb, and/or impaired or no grip reflex during the gestation period only. |
| Body weights | Males and females: unremarkable |
| Food consumption | Males and females: unremarkable |
| Estrous cycle, mating, and fertility indices | Males and females: unremarkable<br>**Number of pregnant rats (number of dams with any nonviable embryos)**<br>Dose level (mg/kg) / Treated males / Treated females<br>0: 23 (13) / 24 (15)<br>1: 24 (12) / 23 (13)<br>3: 22 (13) / 24 (14)<br>15: 25 (15) / 24 (13) |
| Sperm evaluations | Males: unremarkable |
| Necropsy findings | Males: no gross findings or ACE-536 related changes in organ weights.<br>Females: no gross findings; organ weights were not evaluated. |
| Caesarean section | Treated females: at the HD significant reductions ($p \leq 0.01$) in mean numbers of corpora lutea (-22.9%), implantations (-19.3%), and viable embryos (-20.6%) compared to the control group.<br>Untreated females: unremarkable |

LD: low dose; MD: mid dose; HD: high dose

**Study of Female Fertility and Early Embryonic Development to Implantation in Rats/ study number 847-211**

Key study findings

- At 15 mg/kg treatment-related reduction in mean ovarian (-11.3%) and uterus with cervix (-24.5%) weights, and reduction in mean number of implantations (-25.0%) and viable embryos (-25.9%) were observed after the treatment period, but not following a 14-week recovery period.

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|---|---|
| Conducting laboratory and location: | (b) (4) |
| GLP compliance: | Yes |

Methods

| | |
|---|---|
| Dose and frequency of dosing: | 0 or 15 mg/kg/dose<br>Females were treated on Days 1, 15, and 29.<br>Males were not treated. |
| Route of administration: | SC injection |
| Formulation/Vehicle: | (b) (4) sucrose, (b) (4) polysorbate 80 in a (b) (4) citrate buffer in Sterile Water for Injection, USP (b) (4) |
| Species/Strain: | Rat/CD® [Crl:CD®(SD)] |
| Number/Sex/Group: | 22/sex/group |
| Satellite groups: | No |
| Study design: | Treated females were assigned 1:1 to cohabitation with untreated males on Day 15 ("Treatment", Group 1 [control] and Group 2 [ACE-536]) or Day 127 ("Recovery", Group 3 [control] and Group 4 [ACE-536]). The cohabitation period was ≤21 days. After mating was confirmed (GD 0) animals were housed individually. Males were sacrificed at the end of the mating period and females were sacrificed on GD 13. |
| Deviation from study protocol affecting interpretation of results: | No |

**Observations and results**

| Parameters | Major findings |
|---|---|
| Mortality | Treatment and Recovery: none |
| Clinical signs | Treatment and Recovery: unremarkable |
| Body weights | Treatment and Recovery: unremarkable |
| Food consumption | Treatment and Recovery: unremarkable |
| Estrous cyclicity | Treatment and Recovery: unremarkable |
| Reproductive and fertility indices | Treatment: unremarkable<br>Recovery: decreased fertility and fecundity indices were attributed to the age of the recovery animals. |
| Necropsy findings | Treatment: no gross findings; significant reduction in mean ovarian weights (-11.3%, $p<0.05$) and uterus with cervix weights (-24.5%, $p<0.01$) compared to the control group.<br>Recovery: unremarkable |
| Caesarean section | Treatment: significant reductions ($p<0.01$) in mean number of implantations (-25.0%) and viable embryos (-25.9%) compared to the control group. |

| | |
|---|---|
| | Recovery: ovarian and uterine parameters were similar between ACE-536- and control-treated animals, but lower than historical control, which was attributed to the age of the recovery animals. |

Treatment: Groups 1 and 2; Recovery: Groups 3 and 4

Embryo-Fetal Development

**Subcutaneous Embryo-Fetal Development Study in Rats/ study number 20040548**

Key study findings

- ACE-536-related reduction in fetal weights (≥15 mg/kg), increase in skeletal variations (15 mg/kg), and an increase in embryo/fetal death (30 mg/kg) were observed; these findings were observed at exposures >12.5-times the clinical exposure at the highest recommended human dose.
- The fetal NOAEL was 5 mg/kg; no maternal toxicity was observed.

| | |
|---|---|
| Conducting laboratory and location: | (b) (4) |
| GLP compliance: | Yes |

Methods

| | |
|---|---|
| Dose and frequency of dosing: | 0, 5, 15, or 30 mg/kg/dose<br>Pregnant females were treated on presumed GD 3 and GD 10. |
| Route of administration: | SC injection |
| Formulation/Vehicle: | 10 mM Tris Buffered Saline, 137 mM Sodium Chloride, and 2.7 mM Potassium Chloride, pH 7.2 |
| Species/Strain: | Rat/CD® [Crl:CD®(SD)] |
| Number/Sex/Group: | 25/females/group |
| Satellite groups: | TK groups (n=3-6/females/group) |
| Study design: | Presumed pregnant females were treated on presumed GD 3 and GD 10. Blood samples from TK animals were collected after dosing on GD 3 and GD 10. Animals were sacrificed on GD 21; at necropsy main study animals and fetuses were examined. |
| Deviation from study protocol affecting interpretation of results: | No |

**Observations and results**

| Parameters | Major findings |
|---|---|
| Mortality | None |

| | |
|---|---|
| Clinical signs | Two animals at the HD experienced ACE-536-related slight discoloration, curled digits, limited use and/or swollen hindpaws and hindlimbs, impaired gripping reflex, hunched posture and/or mild dehydration. |
| Body weights | Mean body weight gains at the HD level were significantly reduced (-13.8%, p≤0.01) between GD 18 and GD 21; otherwise, unremarkable. |
| Necropsy findings<br>Cesarean section data | The number of pregnant rats in the HD group (21/25) was significantly reduced (p≤0.01) compared to the control (25/25), LD (24/25), and MD (25/25) groups. Reduction in the mean number of fetuses and live fetuses (-13.1%), increases in the mean number of resorptions per litter (+183.3%), and increases in mean post-implantation loss (+195.4%) in the HD group were not statistically significant but outside the historical control range. Two animals in the HD group had a clear fluid filled sac surrounding one kidney. |
| Necropsy findings<br>Offspring | LD: reduction in mean fetal body weights (p≤0.01), but inside the historical control range (therefore not considered related to ACE-536).<br>MD: reduction in mean fetal body weights (-6.1%, p≤0.01); significant (p≤0.01) increase in number of fetuses (n=4, 2.4%) and litters (n=3, 12.0%) with skeletal variations (asymmetric sternal centra).<br>HD: reduction in mean fetal body weights (-7.1%, p≤0.01); absence of malformations and/or variations may have been related to increased embryo/fetal death. |

LD: low dose; MD: mid dose; HD: high dose

**Subcutaneous Embryo-Fetal Development Study in New Zealand White Rabbits/ study number 20040550**

Key study findings

- ACE-536-related maternal observations included decreased mean body weights and food consumption (≥20 mg/kg), increases in the mean number of resorptions (40 mg/kg) and percent post-implantation loss (40 mg/kg), and reduction in mean litter size and live litter size (40 mg/kg).
- ACE-536-related fetal observations included reduction in mean fetal body weights (≥20 mg/kg) and increases in skeletal variations (40 mg/kg).
- Adverse maternal and developmental findings were observed at exposures >17.7-times the clinical exposure at the highest recommended human dose.
- The maternal and fetal NOAEL was 5 mg/kg.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing: 0, 5, 20, or 40 mg/kg/dose
Pregnant females were treated on presumed GD 4 and GD 11.
Route of administration: SC injection

| | |
|---|---|
| Formulation/Vehicle: | 10 mM Tris Buffered Saline, 137 mM Sodium Chloride, and 2.7 mM Potassium Chloride, pH 7.2 |
| Species/Strain: | Rabbit/New Zealand White [Hra:(NZW)SPF] |
| Number/Sex/Group: | 25/females/group |
| Satellite groups: | TK groups (n=3/females/group) |
| Study design: | Pregnant females were treated on presumed GD 4 and GD 11. Blood samples from TK animals were collected after dosing on GD 4 and GD 11. Animals were sacrificed on GD 29; at necropsy main study animals and fetuses were examined. |
| Deviation from study protocol affecting interpretation of results: | No |

**Observations and results**

| Parameters | Major findings |
|---|---|
| Mortality | None |
| Clinical signs | MD: scant feces, red eyes, swollen eyelids, and reduced food consumption.<br>HD: scant feces, red eyes, swollen eyelids, thin body condition, lacrimation, and reduced food consumption. |
| Body weights | MD: mean body weights were significantly reduced ($p \leq 0.05$) from GD 26 to GD 29; mean body weight gains were significantly reduced ($p \leq 0.01$) from GD 0 to GD 29.<br>HD: mean body weights were significantly reduced ($p \leq 0.05$ to $p \leq 0.01$) from GD 22 to GD 29; mean body weight gains were significantly reduced ($p \leq 0.01$) from GD 0 to GD 29. |
| Necropsy findings<br>Cesarean section data | HD: increases in the mean number of resorptions (1.6 vs. 0.1 in control, $p \leq 0.01$) and percent post-implantation loss (16.3% vs. 1.5% in control, $p \leq 0.01$); reduction in mean litter size and live litter size (-25.2%, $p \leq 0.01$). |
| Necropsy findings<br>Offspring | MD: reduction in mean fetal body weights (-10.4%, $p \leq 0.05$);<br>HD: reduction in mean fetal body weights (-14.5%, $p \leq 0.01$) and ossification site averages for forelimb phalanges ($p \leq 0.05$); increase in skeletal variations of the hyoid and sternebrae ($p \leq 0.01$). |

LD: low dose; MD: mid dose; HD: high dose

Prenatal and Postnatal Development

**Evaluation of Effects on Pre and Postnatal Development Including Maternal Function in Rats/ study number 847-167**

Key study findings

- ACE-536-related $F_1$ generation observations included decreased mean body weights, adverse kidney findings, and delayed male sexual maturation. Adverse developmental

findings were observed at exposures >1.6-times the clinical exposure at the highest recommended human dose.

- The NOAEL for general toxicity in the $F_1$ generation could not be determined. The fertility/reproductive NOAEL for the P and $F_1$ generations was 30 mg/kg.

| | |
|---|---|
| Conducting laboratory and location: | (b) (4) |
| GLP compliance: | Yes |

Methods

| | |
|---|---|
| Dose and frequency of dosing: | 0, 3, 10, or 30 mg/kg/dose<br>Pregnant females were treated Q2W on presumed GD 6, GD 20, and 14 days following GD 20. |
| Route of administration: | SC injection |
| Formulation/Vehicle: | (b) (4) sucrose, (b) (4) polysorbate 80 in a (b) (4) citrate buffer in sterile water for injection (b) (4) |
| Species/Strain: | Rat/CD® [Crl:CD®(SD)] |
| Number/Sex/Group: | 22/females/group |
| Satellite groups: | TK groups (n=6/females/group) |
| Study design: | Pregnant females (P generation) were treated Q2W from GD 6 to postnatal day (PND) 20 (GD 6, GD 20, and 14 days following GD 20); TK animals were treated on GD 6 and GD 20 only. On LD 4 all but eight randomly selected pups per litter ($F_1$ generation) were sacrificed and external evaluations were performed. On LD 21 P generation animals were sacrificed and necropsies were performed. On PND 28, offspring ($F_1$ generation) were randomly selected (22/sex/group) to continue on the study for assessment of sexual maturation and behavioral and reproductive performance; remaining $F_1$ generation animals were sacrificed and necropsies were performed. $F_1$ animals selected for further study were subject to reproductive and fertility assessments when at least 80 days of age. $F_1$ animals were sacrificed after the cohabitation period (males) or on GD 13 (females) and necropsies were performed. |

Deviation from study protocol affecting interpretation of results: No

**Observations and results**

| Generation | Major Findings |
|---|---|
| P generation | HD: on GD 12 one animal was prematurely sacrificed in moribund condition; ACE-536-related clinical signs included impaired limb function, purple discolored skin, and swelling of the right hind limb/foot. Decreased mean food consumption from LD 4 to LD 21 (-12.4%, p<0.01). |
| $F_1$ generation | LD: reduction in mean body weights (-8-14%) from LD 0 to PND 28 (p<0.01); during the reproductive and fertility assessments lower mean body weights persisted in males and recovered in females; adverse kidney findings at PND 28 (minimal membranoproliferative glomerulonephritis, minimal tubular atrophy/hypoplasia).<br>MD: reduction in mean body weights (-7-16%) from LD 0 to PND 28 (p<0.01); during the reproductive and fertility assessments lower mean body weights persisted in males and recovered in females; adverse kidney findings at PND 28 (minimal to mild tubular atrophy/hypoplasia, minimal vessel ectasia); delayed preputial separation (p<0.05).<br>HD: reduction in mean body weights (-11-22%) from LD 0 to PND 28 (p<0.01); during the reproductive and fertility assessments lower mean body weights persisted in males and recovered in females; adverse kidney findings at PND 28 (minimal membranoproliferative glomerulonephritis, minimal to mild tubular atrophy/hypoplasia, minimal to mild vessel ectasia); delayed preputial separation (+4.2 days, p<0.01). |
| $F_2$ generation ($F_1$ uterine examination) | Unremarkable |

LD: low dose; MD: mid dose; HD: high dose

### 5.5.5. Other Toxicology Studies

**A Juvenile (Postnatal Day 7) Toxicity Study of ACE-536 Administered Once Every Two Weeks (for a Total of 7 Doses) by Subcutaneous Injection in Juvenile Sprague Dawley Rats/ study number WIL-961003**

Key study findings

- ACE-536-related hindpaw/hindlimb findings were associated with moribundity; other notable premature deaths included one case of malignant lymphoma and one case of granulocytic leukemia. One case of lymphocytic leukemia was observed at scheduled necropsy. All malignancies were observed at 10 mg/kg, with exposures 8-times the clinical exposure at the highest recommended human dose.
- ACE-536-related delayed male sexual maturation and decreased male and female reproductive performance were observed.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Methods

| | |
|---|---|
| Dose and frequency of dosing: | 0, 1, 3, or 10 mg/kg/dose<br>Animals were treated Q2W for seven doses from PND 7 to 91. |
| Route of administration: | SC injection |
| Formulation/Vehicle: | 10 mM TRIS-buffered saline, pH 7.2 |
| Species/Strain: | Rat/Sprague Dawley [Crl:CD(SD)] |
| Number/Sex/Group: | Main study (subset 1): 10/sex/group<br>Recovery/reproductive study (subset 2): 22/sex/group<br>Immune assessment/recovery (subset 4): 20/sex/group |
| Age: | PND 7 |
| Satellite groups: | Additional animals (15-42/sex/group) were included for TK and immunogenicity assessments (subset 3). |
| Study design: | Juvenile rats were treated Q2W from PND 7 to 91 (all subsets), followed by an 18-week recovery period (subsets 2 and 4 only); recovery animals were mated with naïve animals (subset 2 only). |
| Deviation from study protocol affecting interpretation of results: | No |

**Observations and results**

| Parameters | Major findings |
|---|---|
| **Mortality** | Subset 1:<br><br>LD/MD/HD: 14 total unscheduled deaths, but none were attributed to ACE-536 (maternal neglect, accidental, undetermined, bacterial septicemia).<br><br>Subset 2:<br><br>HD: one male was prematurely sacrificed on PND 182 (granulocytic leukemia) while another with persistent hindlimb findings was found dead on PND 191 (undetermined).<br><br>Subset 3:<br><br>MD: one female with persistent hindlimb findings was prematurely sacrificed on PND 101 (ACE-536-related). |

| | |
|---|---|
| | HD: one female with persistent hindlimb findings was prematurely sacrificed on PND 77 (ACE-536-related); one male with no prior clinical findings was found dead on PND 7 (undetermined).<br><br>Subset 4:<br><br>LD: one male in poor clinical condition was prematurely sacrificed on PND 22 (undetermined) while another male with no prior clinical findings was found dead on PND 67 (undetermined).<br><br>HD: one female was prematurely sacrificed on PND 178 (malignant lymphoma); one male was found dead on PND 23 (accidental) and one female with no prior clinical findings was found dead on PND 16 (undetermined). |
| **Clinical signs** | Subset 1: adverse hindlimb findings (impaired use of, swollen, and/or reddened) were common at the HD, and rare at the LD and MD.<br><br>Subset 2: early in the recovery period adverse hindlimb findings (impaired use of and/or swollen) were common at the HD, and rare at the LD and MD; there were no hindlimb findings later in the recovery period.<br><br>Subset 3: adverse hindlimb findings (impaired use of, swollen, and/or reddened) were common at the MD and HD.<br><br>Subset 4: adverse hindlimb findings (impaired use of and/or swollen) were rare at the HD and were not observed after the recovery period. |
| **Body weights** | Subset 1 and 2: lower mean body weights and body weight gains were observed in males at the HD and were similar to control by the end of the recovery period. |
| **Immune assessments** | Subset 4: increases in absolute number of B and NK cells (MD and HD) and T cells (HD) were observed. |
| **Developmental indices** | Subset 1: delayed preputial separation at HD (+2.0 days, $p<0.01$). |
| **Reproductive performance** | Subset 2: when treated males were paired with naïve females, lower mean mating indices were noted at the MD and HD; when treated females were paired with naïve males, lower mean mating indices (LD, MD, HD) and lower mean fertility and conception indices (MD and HD) were observed. |
| **Hematology** | Subset 1: ACE-536-related increases in indicators of circulating erythrocyte mass were observed (LD, MD, HD); increase in mean LYM was observed in males (MD, HD), and decreases in mean PLT were observed in males (MD, HD) and females (HD).<br><br>Subset 2: after the recovery period increases in indicators of circulating erythrocyte mass were limited to males at the HD only. |
| **Clinical chemistry** | Subset 1: ACE-536-related decreases in mean total protein and albumin were observed in males at the MD and HD; increases in urea nitrogen (males, HD) and creatinine (females, HD) were observed.<br><br>Subset 2: after the recovery period decreases in total protein and albumin were observed in males at the MD and HD. |
| **Bone marrow cytology** | Subset 1: a homogenous population of round cells, consistent with lymphocytic leukemia, was observed in one male at the HD. |
| **Gross pathology** | Subset 1: gross lesions were observed in the adrenal glands of females (MD, HD). |

| | |
|---|---|
| | Subset 2: after the recovery period the adrenal gland was affected in one female at each the MD and HD. |
| **Organ weights** | Subset 1: ACE-536-related lower heart (males and females, LD, MD, HD), kidney (females, LD, MD, HD), and liver weights (females, HD) were observed.<br><br>Subset 2: after the recovery period, unremarkable. |
| **Histopathology**<br>Adequate battery: Yes<br>Peer review: Yes | Subset 1: ACE-536-related microscopic findings were observed in the adrenal glands, kidneys, paw, and glandular stomach (LD, MD, and/or HD).<br><br>Subset 2: after the recovery period, ACE-536-related microscopic findings were observed in the adrenal glands, kidneys, and glandular stomach (LD, MD, and/or HD). |

LD: low dose; MD: mid dose; HD: high dose
Subset 1: main study; subset 2: reproductive study; subset 3: TK study; subset 4: immune assessment

**A Renal Toxicity Study of ACE-011, ACE-536, RAP011, and RAP-536 in the 5/6 Nephrectomy or Unilateral Ureter Ligation Models in Female Sprague Dawley Rats/ study number CC-DISC-TOX-2024**

Key study findings

- Increases in urinary kidney injury biomarkers and glomerular and tubulointerstitial findings were observed in nephrectomized animals treated with ACE-536 or RAP-536 relative to vehicle-treated nephrectomized or sham-operated animals treated with ACE-536 or RAP-536.

Conducting laboratory and location: (b) (4)
GLP compliance: No

Methods

| | |
|---|---|
| Dose and frequency of dosing: | Vehicle control: QW for 13 weeks<br>ACE-536: 0.5 or 5 mg/kg/dose Q2W for 13 weeks<br>RAP-536: 10 mg/kg/dose Q2W for 13 weeks |
| Route of administration: | SC injection |
| Formulation/Vehicle: | 10 mM TRIS-buffered saline, pH 7.2 |
| Species/Strain: | Rat/Sprague Dawley [Crl:CD(SD)] |
| Number/Sex/Group: | Sham surgical procedure: n=3/females/group<br>Surgically altered: n=6/females/group |
| Age: | 9 weeks |
| Satellite groups: | No |
| Study design: | Rats were treated Q2W over a 13-week treatment period. During week 3 animals underwent 5/6 nephrectomy (n=6/group) or sham surgical procedure (n=3/group). During week 11 separate groups of animals underwent |

| | |
|---|---|
| | unilateral ureter ligation (n=6/group) or sham surgical procedure (n=3/group). |
| Deviation from study protocol affecting interpretation of results: | No |

**Observations and results**

| Parameters | Major findings |
|---|---|
| **Mortality** | None |
| **Clinical signs** | ACE-536 5 mg/kg: one animal experienced decreased activity, hunched posture, and red discharge. |
| **Body weights** | Unremarkable |
| **Urine biomarkers** | **Test article-related changes in urine biomarkers relative to controls** |

| Biomarker | 5/6 Nephrectomy | | | UUL | | |
|---|---|---|---|---|---|---|
| | ACE-536 0.5 mg/kg | ACE-536 5 mg/kg | RAP-536 10 mg/kg | ACE-536 0.5 mg/kg | ACE-536 5 mg/kg | RAP-536 10 mg/kg |
| Albumin | ↑ | ↑ | ↑ | - | - | - |
| TIM-1 (KIM-1) | - | ↑ | - | - | - | - |
| Lipocalin-2 | - | - | - | ↑ | ↑ | ↑ |
| Osteopontin | - | ↑ | ↑ | - | - | - |

↑: increase relative to vehicle control; changes shown reflect trends over course of study.

| | |
|---|---|
| **Histopathology** | 5/6 Nephrectomy: increased types and severity of glomerular and tubulointerstitial findings in ACE-536 and RAP-536 treatment groups, relative to vehicle-treated and sham-operated groups.<br><br>UUL: severe glomerular findings in the obstructed kidneys precluded the assessment of ACE-536 and RAP-536-related effects. |

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# 6 Clinical Pharmacology

## 6.1. Executive Summary

The Clinical Pharmacology Section of the BLA is supported by PK characterization, population PK (popPK), exposure-response (E-R) analyses, and immunogenicity assessment. The key review questions focus on the appropriateness of the proposed dosing regimen.

The Office of Clinical Pharmacology has reviewed the information contained in BLA 761136. This BLA is approvable from a clinical pharmacology perspective. The key review issues with specific recommendations and comments are summarized below:

| Review Issues | Recommendations and Comments |
|---|---|
| **Evidence of effectiveness** | A randomized, double-blind, placebo-controlled Phase 3 Study ACE-536-B-THAL-001 provides primary evidence. |
| **General Dosing instructions** | The recommended starting dose of REBLOYZL is 1.0 mg/kg once every 3 weeks by subcutaneous (SC) injection.<br>If a patient does not achieve a reduction in RBC transfusion burden after at least 2 consecutive doses (6 weeks) at the 1.0 mg/kg starting dose, increase the REBLOZYL dose to 1.25 mg/kg.<br>Do not increase the dose beyond the maximum dose of 1.25 mg/kg.<br>Patients must have their Hgb assessed and have results available prior to each administration. If an RBC transfusion occurred prior to dosing, the pretransfusion Hgb must be considered for dosing purposes.<br>If the pre-dose Hgb is greater than or equal to 11.5 g/dL and the Hgb level is not influenced by recent transfusion, delay dosing until the Hgb is less than or equal to 11.0 g/dL. |
| **Dosing in patient subgroups (intrinsic and extrinsic factors)** | No dose modification is needed for specific populations of age, sex, race, mild or moderate renal impairment, mild to severe hepatic impairment, and baseline disease characteristics. These factors were not found to be clinically significant covariates on luspatercept PK. (Section **Error! R eference source not found.**) |

There is no Post-Marketing Requirement (PMR) or Post-Marketing Commitment (PMC) from a clinical pharmacology perspective.

## 6.2.Summary of Clinical Pharmacology Assessment

### 6.2.1. Pharmacology and Clinical Pharmacokinetics

Luspatercept is a recombinant fusion protein that binds to select TGF-β superfamily ligands. By binding to specific endogenous ligands (e.g., GDF-11, activin B), luspatercept inhibits Smad2/3 signaling, resulting in erythroid maturation through differentiation of late-stage erythroid precursors (normoblasts) in the bone marrow. Smad2/3 signaling is abnormally high in disease models characterized by ineffective erythropoiesis, e.g., β-thalassemia.

Following SC administration of multiple doses of luspatercept every 3 weeks (Q3W) in patients with β-thalassemia, luspatercept drug exposures (i.e., $C_{max}$ & AUC) in serum increased proportionally to dose from 0.2 to 1.25 mg/kg. Following repeated dosing of the recommended Q3W dosing schedule, steady-state was reached after 3 doses with an accumulation ratio of 1.5 for the trough concentration. The geometric mean (%CV) was 7.1 L (26.7%) for apparent volume of distribution (Vd/F), 11 days (25.7%) for terminal half-life ($t_{1/2}$), and 0.44 L/day (38.5%) for clearance (CL).

Among 286 patients in Trial BELIEVE (ACE-536-B-THAL-001) who were treated with REBLOYZL at the recommended dosing regimen, 4 patients (1.4%) tested positive for treatment-emergent anti-drug antibodies (ADAs), including 2 patients (0.7%) developed neutralizing antibodies. Due to the limited number of patients developed neutralizing antibodies, effect on PK, efficacy or safety could not be concluded.

### 6.2.2. General Dosing and Therapeutic Individualization

**General Dosing**

The Applicant's proposed starting dose of luspatercept is 1.0 mg/kg once every 3 weeks administered via SC injection. Patient may escalate the dose to 1.25 mg/kg if (b) (4) reduction was achieved after at least 2 consecutive doses (6 weeks) compared to that at baseline. If the patient has pre-dose Hgb ≥ 11.5 g/dL and the Hgb level is not influenced by recent transfusion, delay dosing until Hgb ≤ 11.0 g/dL. This proposed dosing regimen appears to be effective and has a manageable safety profile in adult patients (n = 286) with β-thalassemia in Trial BELIEVE.

**Therapeutic Individualization**

Body weight-based dosing regimen is recommended based on population PK analysis, where the CL/F and Vd/F of luspatercept increased with body weight in patients with β-thalassemia.

Although effect of baseline albumin and baseline RBC-T burden on CL/F and baseline albumin and RBC-T burden on Vd/F were statistically significant in the population PK analysis, dose modification was not required as their impact on luspatercept exposure were limited and not clinically significant.

**Outstanding Issues**

There are no outstanding issues at this time.

## 6.3.Comprehensive Clinical Pharmacology Review

### 6.3.1. General Pharmacology and Pharmacokinetic Characteristics

The summary of clinical pharmacology, pharmacokinetics and ADME information of luspatercept is listed below.

| **Pharmacology** | |
|---|---|
| **Mechanism of Action** | Luspatercept is a recombinant fusion protein that binds select TGF-β superfamily ligands and inhibits Smad2/3 signaling, resulting in erythroid maturation. |
| **Active Moieties** | Luspatercept |
| **QT Prolongation** | At a dose 0.125 to 1.75 times the approved recommended dosage, luspatercept does not prolong the QT interval to any clinically relevant extent. See BLA 761136 DARRTS CONSULT REV-QTIRT-01 dated 06/20/2019 by ZHENG for details. |
| **General Information** | |
| **Bioanalysis** | Luspatercept was measured using validated Enzyme Linked Immunosorbent Assay (ELISA) method. |
| **Drug exposure after first dose** | Following SC dose of 1.0 mg/kg, $C_{max}$ was reached at 5.5 day at value of 5.64 [CV%: 25.1] µg/mL. |
| **Drug total exposure at steady state following the therapeutic dosing regimen** | Following multiple SC doses of 1.0 mg/kg Q3W, the steady state geometric mean $C_{max,ss}$ and $AUC_{ss}$ were 8.17 [CV%: 29.9] µg/mL and 126 [CV%: 35.9] day·µg/mL; following multiple SC dose of 1.25 mg/kg Q3W, the steady state geometric mean value were 10.2 [CV%: 29.9] µg/mL and 163 [CV%: 35.9] day·µg/mL for $C_{max,ss}$ and $AUC_{ss}$ respectively. |
| **Minimal effective dose or exposure** | 1.0 mg/kg administered via SC injection once every 3 weeks. |

| | |
|---|---|
| **Dose Proportionality** | Luspatercept serum exposure ($AUC_{ss}$ and $C_{max}$) increased approximately in a dose-proportional manner with SC dose from 0.2 to 1.25 mg/kg. |
| **Accumulation** | The accumulation ratio was approximately 1.5-fold. Steady sate was reached after 3 doses Q3W. |
| **Variability** | The %CV for $C_{max}$ was 25.1% after the first dose and 30.1% for $C_{min}$ at steady state. The %CV for $AUC_{ss}$ was 36.0%. |
| **Immunogenicity** | A total of 1.4% (4/284) patients tested positive for treatment-emergent anti-luspatercept antibodies, including 0.7% (2/284) patients had neutralizing antibodies. Luspatercept serum concentration tended to decrease in the presence of neutralizing antibodies. There was no discernible effect of ADAs on efficacy or safety. |
| **Distribution** | |
| **Volume of Distribution** | The volume of distribution (%CV) was 7.1 L (26.7%). |
| **Plasma Protein Binding** | Not evaluated. As a fusion protein with a molecular weight of 76 kDa, luspatercept is not expected to bind to plasma proteins. |
| **Blood to Plasma Ratio** | Not evaluated. |
| **Substrate transporter** | Not evaluated. As a fusion protein, luspatercept is not expected to be a substrate of metabolic transporters. |
| **Elimination** | |
| **Clearance** | The clearance (%CV) was 0.44 L/day (38.5%). |
| **Mean terminal elimination half-life** | The half-life (CV%) was 11 days (25.7%). |
| ***Metabolism*** | |
| **Primary metabolic pathway(s)** | No evaluated. Luspatercept is expected to be catabolized into amino acids by general protein degradation processes in multiple tissues, and thus its elimination is not dependent on a single organ. |
| **Inhibitor/Inducer** | Not evaluated. |
| ***Excretion*** | |
| **Primary excretion pathways (% dose) ± SD** | No evaluated. Luspatercept is not expected to be excreted into urine due to its large molecular mass (76 kDa) that is above the glomerular filtration cut-off threshold (~65 kDa). |

### 6.3.2. Clinical Pharmacology Questions

**Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?**

Yes. The proposed luspatercept starting dose and dose titration scheme is acceptable and supported by a generally favorable benefit/risk profile demonstrated in the BELIEVE trial. Specifically, the body weight based dosing reduced risk of over-exposure or under-exposure of luspatercept in patients with extreme body weight. The Q3W dosing schedule was able to maintain luspatercept trough concentration at steady state to ensure continuous target engagement.

**Body weight based dosing:** Body weight was a statistically significant covariate of luspatercept apparent CL/F and Vd/F in the population PK analysis. A total of 100 trials was simulated based on PK model to compare 3 dosing regimens in 394 patients: weight based dosing (1.25 mg/kg), modified weight-based dose (1.25 mg/kg up to 120 mg), and fixed dose (71 mg). Results predicted that the weight based dosing would perform better than the fixed dosing by limiting the exposure difference between light/heavy and normal weight patients to within 10% instead of the 25-30% predicted for the fixed dose.

**Q3W dosing schedule:** A Q3W dosing schedule is expected to maintain approximately 50% of the peak concentration at the end of a dosing interval as luspatercept has $T_{max}$ ~6 days and $t_{1/2}$ ~11 days in patients with β-thalassemia. Following the Q3W dosing schedule, the mean $C_{trough}$ at steady state (≥ 3.5 µg/mL or ≥ 46 nM) was far above the $K_d$ of luspatercept to bind GDF11 (0.71 nM) or the $IC_{50}$ of luspatercept to inhibit signaling through GDF11 (7.1 ng/mL) in *in-vitro* assays.

**Starting dose 1.0 mg/kg and dose titration to 1.25 mg/kg:**

- In the supportive phase 2 study A536-04, higher response rates in Hgb increase ≥ 1.5 g/dL sustained for ≥ 14 days and a reduction of ≥ 20% in RBC-T burden were observed at ≥1 mg/kg within the studied dose range of 0.2 - 1.25 mg/kg.
- The proposed titration-to-response dosing regimen (1-1.25 mg/kg) was confirmed to be effective in trial BELIEVE with both primary and secondary efficacy endpoints achieved. Refer to Section **Error! Reference source not found.** for details.
- Luspatercept dose levels up to 1.25 mg/kg were well tolerated in subjects with β-thalassemia. In the phase 2 studies, MTD was not reached at 1.25 mg/kg for up to 5 treatment cycles in Study A536-04, and no new safety concerns were identified during longer-term luspatercept use (17 treatment cycles or more) in Study A536-06. Refer to Section **Error! Reference source not found.** for details.
- Dose escalations were more frequently seen in subjects who had no splenectomy and higher baseline EPO (≥ 200 U/L), conditions known to be associated with more resistant anemia or more advanced disease.

**Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?**

No. Population PK analysis (n = 285) showed that patient demographics such as, age (18 - 66 years), sex (56.8% female/43.2% male), race (28.8% Asian/0.7% Black/63.5% White), hepatic impairment (18.6% normal/27.4% mild/40.4% moderate/13.7% severe based on NCI-ODWG criteria), and renal impairment (86% normal/13% mild/1.1% moderate based on eGFR), baseline serum erythropoietin (2.4 to 972 U/L), baseline albumin (30 - 56 g/L), baseline RBC-T burden (0 to 34 units/24 weeks), beta thalassemia genotype (β0/β0 vs. non-β0/β0), splenectomy, location of SC injection (i.e., upper arm, thigh, or abdomen), and concurrent iron chelation therapy did not have a statistically meaningful influence on PK of luspatercept after the dose was adjusted by body weight. The effect of severe renal impairment (eGFR <30 mL/min/1.73 $m^2$) is unknown. Refer to Appendix 19.5.3 Population PK Analysis for further detailed information.

**Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?**

Since luspatercept is administered via SC injection, food-drug interactions are not anticipated. Drug-drug interactions are not expected with Cytochrome P450 enzymes (CYPs), other metabolizing enzymes, or transporters, as luspatercept is a fusion protein with molecular weight of 76 kDa. Therefore, no drug-drug interaction studies were conducted in vitro or in vivo.

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# 7 Sources of Clinical Data and Review Strategy

## 7.1. Table of Clinical Studies

Details for the BELIEVE (ACE-536-B-THAL-001) trial conducted in patients with beta-thalassemia who require regular transfusions are provided in the table below.

**Table 1: Controlled Studies to Support Efficacy and Safety**

| Trial and Status | Trial Design | Regimen/Schedule/ Route | Primary Endpoint | Treatment Duration and Follow Up | No. of Patients Enrolled | Study Population | Countries and no. of sites |
|---|---|---|---|---|---|---|---|
| Controlled Studies to Support Efficacy and Safety | | | | | | | |
| **ACE-536-B-THAL-001 (BELIEVE)** | **Pivotal Phase III**<br><br>Prospective, multi-center, randomized (2:1), double-blind, placebo controlled | **Double-blind Treatment Period:** 48 weeks<br><br>Luspatercept (1.0 mg/kg starting dose level) administered subcutaneously (SC) every 3 weeks (Q3W)<br><br>**Long Term Double-blind Phase:** Up to 48 weeks post Dose 1 of the last patient randomized<br><br>Luspatercept SC Q3W<br><br>**Open-label:** Up to 5 years<br><br>Luspatercept SC Q3W | To determine the proportion of patients 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 | Double-blind phase 48 weeks;<br><br>Open label 5 years | **Double -blind:** N=336 | Patients with transfusion dependent beta-thalassemia | Australia, Bulgaria, Canada, France, Greece, Israel, Italy, Lebanon, Malaysia, Taiwan, Thailand, Tunisia, Turkey, UK, US<br><br>65 sites |
| **ACE-536-B-THAL-002** | **Phase II**<br><br>Prospective, multi-center, randomized, double-blind, placebo controlled | **Double-blind Treatment Period:** 48 weeks<br><br>Luspatercept (1.0 mg/kg starting dose level) administered subcutaneously (SC) every 3 weeks (Q3W) | To evaluate the effect of luspatercept versus placebo on anemia, as measured by mean hemoglobin concentration in the absence of transfusions over continuous 12- | Double-blind phase 48 weeks | **Double-blind:** N=17 | Patients with transfusion dependent beta thalassemia | Greece, Italy, Lebanon, Thailand, UK, US |

| Trial and Status | Trial Design | Regimen/Schedule/ Route | Primary Endpoint | Treatment Duration and Follow Up | No. of Patients Enrolled | Study Population | Countries and no. of sites |
|---|---|---|---|---|---|---|---|
| | | | week intervals, from Week 13 to Week 24, compared to baseline | | | | |

## 7.2. Review Strategy

The key materials used for the review of efficacy and safety included:

- BLA 761136 datasets (raw and derived), clinical study reports, and responses to the review team's information requests
- Relevant published literature
- Relevant information in the public domain

Clinical data was provided in the Clinical Data Interchange Standards Consortium (CDISC) Foundational Standards SDTM (Study Data Tabulation Model) and ADaM (Analysis Data Model Implementation). Also submitted were the define files for the variables and the corresponding SAS programs for the primary ADaM data derivation to document the analysis results. The clinical and statistical reviewers were able to duplicate the analysis results based on the applicant's submitted datasets.

This review was primarily based on efficacy and safety analyses of the pivotal Phase III BELIEVE (ACE-536-B-THAL-001) trial. Refer to the previous table for further details. For the purposes of this review, luspatercept-aamt refers to luspatercept.

Sections 6 and 7 of this review were performed by Dr. Laurel Menapace, MD and Dr. Weishi Yuan, PhD. Analysis by Dr. Yuan was performed using SAS 9.4 (SAS Institute, Inc.).

Analyses by Dr. Menapace were performed utilizing JMP 13.0 (SAS Institute, Inc.) and JMP Clinical 6.1. MedDRA Adverse Events Diagnostic (MAED) 1.3 (Clinical Trials and Surveys Corporation & FDA) was used to assess safety signals. Unless specifically referenced, all analyses and presentation of findings are the work of FDA reviewers.

Please note that this review address only the proposed indication of beta-thalassemia. The Sponsor submitted the original BLA for treatment of myelodysplastic syndrome associated anemia and adult patients with beta thalassemia associated anemia. (b) (4)

# 8 Statistical and Clinical and Evaluation

## 8.1. Review of Relevant Individual Trials Used to Support Efficacy

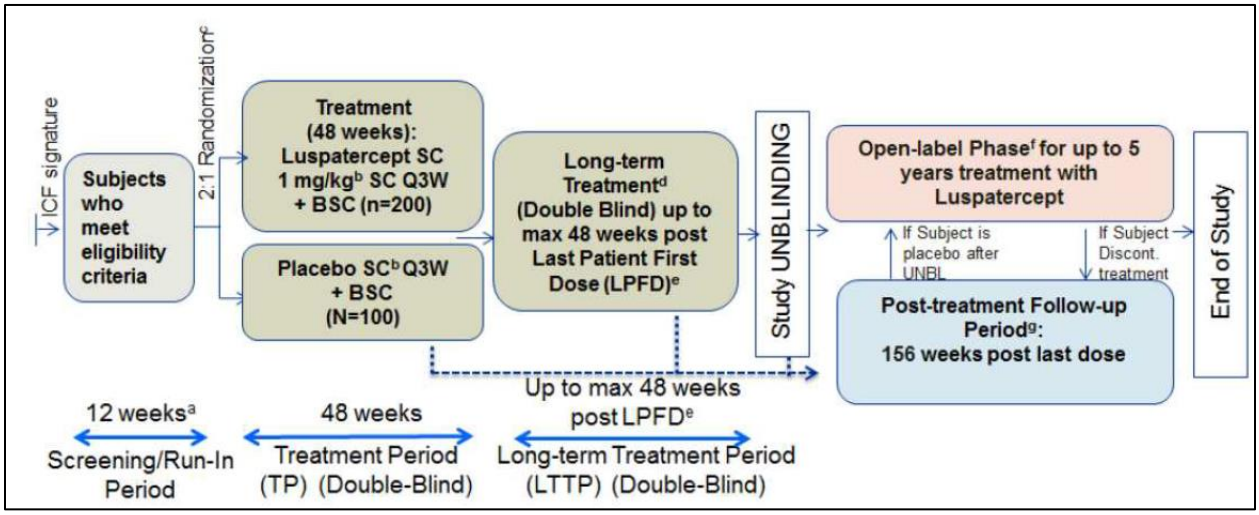
### 8.1.1. ACE-536-B-THAL-001

**Trial Design**

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 patients with β-thalassemia who require regular RBC transfusions. Consenting patients 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 documenting 12 weeks of historical retrospective transfusion history. Eligible patients were randomized to luspatercept combined with BSC or placebo combined with BSC in a 2:1 ratio. The study included a double-blind treatment period (Weeks 1 to 48), long-term treatment period (after Week 48; patients continued to receive the study drug to which they were initially randomized) and a post-treatment follow-up period of 156 weeks after the last dose of study treatment. After unblinding for the primary analysis, eligible patients including those randomized to placebo treatment were given the option of open-label luspatercept treatment for up to 5 years.

The following figure depicts the overall study design for ACE-536-B-THAL-001.

**Figure 8-1: Study Schema for ACE-536-B-THAL-001 Study**




During the screening/run-in period, patients underwent safety and other assessments to determine eligibility for the study. The transfused β-thalassemia patients must have had at least

24 weeks of documented transfusion history available (including Hb levels prior to each transfusion, the number of units transfused, and the transfusion date) prior to randomization. Twelve weeks of transfusion history was collected prospectively during the screening/run-in period, in addition to the 12-week historical retrospective data that were further recorded in each patient's electronic case report form (eCRF). The 24-week transfusion history prior to randomization into the double-blind treatment Period was used to determine the baseline RBC transfusion burden as well as the mean pretransfusion Hb value.

Patients were required to be diagnosed with β-thalassemia (including HbE/β-thalassemia), aged ≥ 18 years and be transfusion dependent. Transfusion dependence was defined in this study as receiving 6 to 20 RBC units during the 24 weeks prior to randomization, with no transfusion-free period for > 35 days. The minimum of 6 RBC units had been defined based on potential transfusion practices, including a minimum of 1 unit every 4 weeks (or 6 RBC units/24 weeks), which may be observed in countries with limited blood supply, as per feedback received from investigators. The maximum of 20 RBC units in the 24 weeks prior to randomization was determined based on the clinical data from Phase 2 studies with luspatercept in β-thalassemia.

Stratification was performed prior to randomization based on the following geographical regions: North America/Europe; Middle East/North Africa and Asia-Pacific.

Eligible patients were randomized at a ratio of 2:1 to luspatercept + BSC or placebo + BSC treatment at a starting dose level of 1.0 mg/kg administered subcutaneously (SQ) every 3 weeks (Q3W). The maximum total dose per administration was not to exceed 120 mg. Best supportive care was available to patients in both the luspatercept and placebo treatment arms. This included RBC transfusions; ICTs; antibiotic, antiviral, and antifungal therapies; and/or nutritional support as needed, thus minimizing the safety risks during the conduct of the study.

Dose levels of luspatercept were titrated (increased) stepwise up to a maximum of 1.25 mg/kg during the double-blind treatment period or were titrated during the double-blind long-term treatment period and open-label phase unless dose modification was required. Dose titration was based on erythroid response during the previous 2 dose cycles (approximately 6 weeks).

The dose 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

Dose titration rules are defined in the following table.

**Table 8-1: 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 |

Source: Sponsor generated table, Study Report Body

Treatment with hydroxyurea and anagrelide was not allowed during the double-blind treatment period. In addition, the use of hematopoietic growth factors was not allowed during the double-blind phase. However, any subject who required hematopoietic growth factor treatment during the double-blind treatment period was to be discontinued from the study, at which point hematopoietic growth factor treatment was permitted.

Anticoagulant therapies and platelet aggregation inhibitors were permitted for use on protocol. However, if these therapies were used due to a related AE that qualified for treatment discontinuation, the subject was to be discontinued from the study drug. Anticoagulant therapies used for prophylaxis, as well as aspirin and low-molecular-weight heparin, were also allowed on study.

Study drug was administered as an SC injection at the clinical site by the study staff. Monitoring for compliance with the treatment regimen was therefore unnecessary. Enrolled patients were also provided with diaries to record any RBC transfusions received outside their normal transfusion center.

The following tables outline the schedule of events and safety assessments performed in the screening/run-in and double-blind phases of the protocol.

## Table 8-2: Study Schedule of Events

| Assessments | Section of the CSP | Screening/ Run-in Period Wk -12 to Day -1 | TP (Double-blinded) | | | | LTTP (Double-blinded)[a] q3w Visits From Wk 49 (ie, Dose 17 if no Dose Delays) /Day 1 (± 5 Days) | OLP, q3w Visits From Unblinding (± 5 Days) | | Day 22 Post Last Dose/ Treatment D/C[b] | Posttreatment FU Period (± 7 Days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Luspatercept/ Placebo Dose 1 Schedule (+ 7 Days) | Luspatercept/Placebo Doses 2 up to Maximum 48 Wks Treatment (± 3 Days) | | | | | | | FU Wk 9 (From Last Dose)[c] | Only If[d] Early D/C – FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | All Subjects : FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | EOS[e]/ FU Wk 156 (From Last Dose)[c] |
| | | | Day 1 | Day 1 | Day 8 | Day 15 | | Dose 1 for Placebo, D/C[f] | Dose X Day 1[g] | | | | | |
| **Study Entry and General Assessments** | | | | | | | | | | | | | | |
| Informed Consent | 6.1 | X | - | - | - | - | - | - | - | - | - | - | - | - |
| Inclusion/Exclusion Criteria | 6.1 | X | - | - | - | - | - | X | - | - | - | - | - | - |
| Demographics | 6.1 | X | - | - | - | - | - | - | - | - | - | - | - | - |
| Medical History | 6.1 | X | - | - | - | - | - | - | - | - | - | - | - | - |
| β-thalassemia Genotype (ie, β- and α-globin Mutations, Only If Not Available in Subject Medical History)[h] | 6.1 | X | - | - | - | - | - | - | - | - | - | - | - | - |
| Hepatitis B and C[i] | 6.1 | X | - | - | - | - | - | - | - | - | - | - | - | - |
| Iron Chelation Therapy | 6.1 to 6.6 | X | Record on ongoing basis, until 9 weeks post last dose[j] | | | | | | | | | | | |
| Other Prior/Concomitant /Post (Disease-specific) Medications/Therapies | 6.1 to 6.6 | X | Record on ongoing basis, until 9 weeks post last dose[j] | | | | | | | | | | | |
| Prior/Concomitant/Post Procedures (eg, Surgery, Radiation Therapy) | 6.1 to 6.6 | X | Record on ongoing basis, until 9 weeks post last dose[j] | | | | | | | | | | | |
| Transfusion Assessment (≥ 24 Weeks of History Prior to Dose 1 Day 1) | 6.1 to 6.6 | X | Record on ongoing basis, until 9 weeks post last dose[j] | | | | | | | | | | | |

## Table 8-3: Study Schedule of Events, Continued

| Assessments | Section of the CSP | Screening/ Run-in Period Wk -12 to Day -1 | TP (Double-blinded) | | | | LTTP (Double-blinded)[a] q3w Visits From Wk 49 (ie, Dose 17 if no Dose Delays) /Day 1 (± 5 Days) | OLP, q3w Visits From Unblinding (± 5 Days) | | Day 22 Post Last Dose/ Treatment D/C[b] | Posttreatment FU Period (± 7 Days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Luspatercept/ Placebo Dose 1 Schedule (+ 7 Days) | Luspatercept/Placebo Doses 2 up to Maximum 48 Wks Treatment (± 3 Days) | | | | | | | FU Wk 9 (From Last Dose)[c] | Only If[d] Early D/C – FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | All Subjects : FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | EOS[e]/ FU Wk 156 (From Last Dose)[c] |
| | | | Day 1 | Day 1 | Day 8 | Day 15 | | Dose 1 for Placebo, D/C[f] | Dose X Day 1[g] | | | | | |
| Subject Transfusion – Information Collection[k] | 6.1 to 6.6 | X | Record on ongoing basis, until 9 weeks post last dose[j] | | | | | | | | | | | |
| **Safety Assessments** | | | | | | | | | | | | | | |
| Adverse Events | 6.1 to 6.7 | Continuous starting after informed consent signature, until 9 weeks post last dose, related AE to be reported until EOS | | | | | | | | | | | | |
| Malignancy and Premalignancy Reporting[l] (Section 9.5.1.2.3) | 6.1 to 6.7 | Continuous starting after informed consent signature, regardless of causality reporting occurrence of any case | | | | | | | | | | X[l] | X[l] | X[l] |
| Vital Signs[m] | 6.1 to 6.7 | X (within 4 wks prior Dose 1 Day 1) | X | X | - | - | X | X | X | X | X | - | - | - |
| Height (at Screening Only)/Weight | 6.1 to 6.7 | X (within 4 wks prior Dose 1 Day 1) | X | X | - | - | X | X | X | X | X | - | - | - |
| ECOG Performance Status | 6.1 and 6.4 | X (within 4 wks prior Dose 1 Day 1) | X | - | - | - | - | X | - | - | - | - | - | - |

| Assessments | Section of the CSP | Screening/ Run-in Period Wk -12 to Day -1 | TP (Double-blinded) | | | | LTTP (Double-blinded)[a] q3w Visits From Wk 49 (ie, Dose 17 if no Dose Delays) /Day 1 (± 5 Days) | OLP, q3w Visits From Unblinding (± 5 Days) | | Day 22 Post Last Dose/ Treatment D/C[b] | Posttreatment FU Period (± 7 Days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Luspatercept/ Placebo Dose 1 Schedule (+ 7 Days) | Luspatercept/Placebo Doses 2 up to Maximum 48 Wks Treatment (± 3 Days) | | | | | | | FU Wk 9 (From Last Dose)[c] | Only If[d] Early D/C – FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | All Subjects : FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | EOS[e]/ FU Wk 156 (From Last Dose)[c] |
| | | | Day 1 | Day 1 | Day 8 | Day 15 | | Dose 1 for Placebo, D/C[f] | Dose X Day 1[g] | | | | | |
| 12-Lead ECG – Read Locally | 6.1, 6.2, 6.4, and 6.5 | X (within 4 wks prior Dose 1 Day 1) | - | - | X (Dose 6 only) | - | - | X | - | X | - | - | - | - |
| Cardiac Doppler Echocardiography, MUGA or MRI[n, o]: LVEF | 6.1, 6.2, 6.3 | X | - | X (Wk 24, Wk 48 only) | - | - | X (Wk 96 only)[p] | - | - | - | - | - | - | - |
| Pregnancy Testing[q] | 6.1 to 6.6 | X (within 4 wks prior Dose 1 Day 1; serum only) | X | X | - | - | X | X | X | X | X | - | - | - |
| Menstrual Status (Females Only) | 6.1 to 6.6 | X | X | X | - | - | X | X | X | X | X | - | - | - |

## Table 8-4: Study Schedule of Events, Continued

| Assessments | Section of the CSP | Screening/ Run-in Period Wk -12 to Day -1 | TP (Double-blinded) | | | | LTTP (Double-blinded)[a] q3w Visits From Wk 49 (ie, Dose 17 if no Dose Delays) /Day 1 (± 5 Days) | OLP, q3w Visits From Unblinding (± 5 Days) | | Day 22 Post Last Dose/ Treatment D/C[b] | Posttreatment FU Period (± 7 Days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Luspatercept/ Placebo Dose 1 Schedule (+ 7 Days) | Luspatercept/Placebo Doses 2 up to Maximum 48 Wks Treatment (± 3 Days) | | | | Dose 1 for Placebo, D/C[f] | Dose X Day 1[g] | | FU Wk 9 (From Last Dose)[c] | Only If[d] Early D/C – FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | All Subjects : FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | EOS[e]/ FU Wk 156 (From Last Dose)[c] |
| | | | Day 1 | Day 1 | Day 8 | Day 15 | | | | | | | | |
| Hematology[r] (Central Laboratory; Use Local Laboratory for Predose Hb, in Case of AE, or Between Doses; Reticulocytes and Erythroblasts to Be Measured by Local Laboratory; in OLP – Use Local Laboratories) | 6.1 to 6.6 | X (within 4 wks prior Dose 1 Day 1) | X | X | X (Dose 6 only) | X (Dose 6 only) | X[s] | X | X | X | X | - | - | - |
| Serum Chemistry[t] (Predose: Central Laboratory; Use Local Laboratory in Case of AE, or Between Doses) | 6.1 to 6.6 | X (within 4 wks prior Dose 1 Day 1) | X | X | - | - | X[s] (every 4 doses) | X | - | X | X | - | - | - |
| Urinalysis: Microalbumin, Creatinine, Microalbumin/Creatinine Ratio (Morning Void; Central Laboratory) | 6.1.1 to 6.6 | X (within 4 wks prior Dose 1 Day 1) | - | X (every 4 doses) | - | - | X[s] (every 4 doses) | X | - | X | X | - | - | - |
| Serum Erythropoietin (Predose; Central Laboratory) | 6.1.1 to 6.5 | - | X | X (every 4 doses) | - | - | - | - | - | X | - | - | - | - |

## Table 8-5: Study Schedule of Events, Continued

| Assessments | Section of the CSP | Screening/ Run-in Period Wk -12 to Day -1 | TP (Double-blinded) | | | | LTTP (Double-blinded)[a] q3w Visits From Wk 49 (ie, Dose 17 if no Dose Delays) /Day 1 (± 5 Days) | OLP, q3w Visits From Unblinding (± 5 Days) | | Day 22 Post Last Dose/ Treatment D/C[b] | Posttreatment FU Period (± 7 Days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Luspatercept/ Placebo Dose 1 Schedule (+ 7 Days) | Luspatercept/Placebo Doses 2 up to Maximum 48 Wks Treatment (± 3 Days) | | | | | | | FU Wk 9 (From Last Dose)[c] | Only If[d] Early D/C – FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | All Subjects : FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | EOS[e]/ FU Wk 156 (From Last Dose)[c] |
| | | | Day 1 | Day 1 | Day 8 | Day 15 | | Dose 1 for Placebo, D/C[f] | Dose X Day 1[g] | | | | | |
| Serum PK (Predose If on Dosing Day; Central Laboratory)[u] | 6.8 | - | X | X (Doses 2, 3, 4, 5 6, 8, 10, 12, 14, and 16) | X (Dose 6 only) | X (Dose 6 only) | X (every 6 doses, eg, at Doses 22, 28, 34….) | - | - | - | - | - | - | - |
| Antidrug Antibody (Central Laboratory)[u, v] | 6.9 | - | X | X (Doses 2, 4, 6; 8, 12, and 16) | - | - | X (every 6 doses, eg, at Doses 22, 28, 34….)[u] | X (only if +ve at unblinding, every 6 doses up to 2 years of the Dose 1 Day 1 of the double-blind TP) | X (only if +ve at unblinding, every 6 doses up to 2 years of Dose 1 Day 1 of the double-blind TP)[u] | - | X | X (every 24 wks as applic-able)[u] | X (every 24 wks as applic-able)[u] | - |

## Table 8-6: Study Schedule of Events, Continued

| Assessments | Section of the CSP | Screening/ Run-in Period Wk -12 to Day -1 | TP (Double-blinded) | | | | LTTP (Double-blinded)[a] q3w Visits From Wk 49 (ie, Dose 17 if no Dose Delays) /Day 1 (± 5 Days) | OLP, q3w Visits From Unblinding (± 5 Days) | | Day 22 Post Last Dose/ Treatment D/C[b] | Posttreatment FU Period (± 7 Days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Luspatercept/ Placebo Dose 1 Schedule (+ 7 Days) | Luspatercept/Placebo Doses 2 up to Maximum 48 Wks Treatment (± 3 Days) | | | | Dose 1 for Placebo, D/C[f] | Dose X Day 1[g] | | FU Wk 9 (From Last Dose)[c] | Only If[d] Early D/C – FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | All Subjects : FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | EOS[e]/ FU Wk 156 (From Last Dose)[c] |
| | | | Day 1 | Day 1 | Day 8 | Day 15 | | | | | | | | |
| **Efficacy and Other Assessments** | | | | | | | | | | | | | | |
| MRI for LIC (With T2* or R2, mg/g dw)[n, w] | 6.1, 6.1.1, 6.2, 6.3, 6.4, 6.5 | X | - | X (Wks 24 and 48) | - | - | Wk 96 only, if applicable[p] | - | Wk 96 only, if applic -able[p] | X (if not performed within the last 12 wks) | - | X (Wk 48) post Dose 1) | - | - |
| MRI or Abdominal Ultrasound for Spleen Measurements for Spleen, Unless Splenectomized[n, w] | 6.1, 6.1.1, 6.2, 6.3, 6.4, 6.5 | X | - | X (Wks 24 and 48) | - | - | Wk 96 only, if applicable[p] | - | Wk 96 only, if applic -able[p] | X (if not performed within the last 12 wks) | - | X (Wk 48) post Dose 1) | - | - |
| MRI for Myocardial Iron (T2*; ms)[n, w] | 6.1, 6.1.1, 6.2, 6.3, 6.5 | X | - | X (Wk 48) | - | - | Wk 96 only, if applicable[p] | - | Wk 96 only, if applic -able[p] | X (if not performed within the last 12 wks) | - | X (Wk 48) post Dose 1) | - | - |
| DXA Scan[n, x] – Total Hip, Lumbar Spine (Read Locally) | 6.1, 6.1.1, 6.2, 6.3, 6.4, 6.5 | X | - | X (Wk 48) | - | - | Wk 96 only, if applicable[p] | - | Wk 96 only, if applic -able[p] | X (if not performed within the last 12 wks) | - | X (Wk 48) post Dose 1) | - | - |

## Table 8-7: Study Schedule of Events, Continued

| Assessments | Section of the CSP | Screening/ Run-in Period Wk -12 to Day -1 | TP (Double-blinded) | | | | LTTP (Double-blinded)[a] q3w Visits From Wk 49 (ie, Dose 17 if no Dose Delays) /Day 1 (± 5 Days) | OLP, q3w Visits From Unblinding (± 5 Days) | | Day 22 Post Last Dose/ Treatment D/C[b] | Posttreatment FU Period (± 7 Days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Luspatercept/ Placebo Dose 1 Schedule (+ 7 Days) | Luspatercept/Placebo Doses 2 up to Maximum 48 Wks Treatment (± 3 Days) | | | | | | | FU Wk 9 (From Last Dose)[c] | Only If[d] Early D/C – FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | All Subjects : FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | EOS[e]/ FU Wk 156 (From Last Dose)[c] |
| | | | Day 1 | Day 1 | Day 8 | Day 15 | | Dose 1 for Placebo, D/C[f] | Dose X Day 1[g] | | | | | |
| QoL Questionnaire (TranQoL, SF-36), Assessments to Be Performed Independent of Dose Delays | 6.1, 6.1.1, 6.2, 6.3, 6.11 | X (within 4 wks prior Dose 1 Day 1) | - | X (Wks 12, 24, 36, and 48) | - | - | every 12 wks | - | - | - | - | - | - | - |
| Healthcare Resource Utilization | 6.1 to 6.6 | Record on ongoing basis, until 9 weeks post last dose[j] | | | | | | | | | | | | |
| Serum Ferritin (Predose, Central Laboratory; Screening Value at Least 12 Wks Prior to Randomization) | 6.1 to 6.6 | X (within 4 wks prior Dose 1 Day 1) | X | X | - | - | X[s] (every 4 doses) | X | - | X | X | - | - | - |
| **Exploratory Assessments** | | | | | | | | | | | | | | |
| Serum GDF11 and Other Related Biomarkers (Predose, Central Laboratory) | 6.1.1, 6.4 | - | X | X (Doses 6, 8, and 16) | X (Dose 6 only) | X (Dose 6 only) | - | - | - | X (only if not performed at Dose 16 and not performed within the last 12 wks) | - | - | - | - |

| Assessments | Section of the CSP | Screening/ Run-in Period Wk -12 to Day -1 | TP (Double-blinded) | | | | LTTP (Double-blinded)[a] q3w Visits From Wk 49 (ie, Dose 17 if no Dose Delays) /Day 1 (± 5 Days) | OLP, q3w Visits From Unblinding (± 5 Days) | | Day 22 Post Last Dose/ Treatment D/C[b] | Posttreatment FU Period (± 7 Days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Luspatercept/ Placebo Dose 1 Schedule (+ 7 Days) | Luspatercept/Placebo Doses 2 up to Maximum 48 Wks Treatment (± 3 Days) | | | | | | | FU Wk 9 (From Last Dose)[c] | Only If[d] Early D/C – FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | All Subjects : FU Visits Wks 24, 48, 72, 96, 120, 144 (From Last Dose)[c] | EOS[e]/ FU Wk 156 (From Last Dose)[c] |
| | | | Day 1 | Day 1 | Day 8 | Day 15 | | Dose 1 for Placebo, D/C[f] | Dose X Day 1[g] | | | | | |
| Hb Electrophoresis for all Hb Fractions (Central Laboratory) | 6.1, 6.1.1, 6.4, 6.10 | X (within 4 wks prior Dose 1 Day 1) | - | X | X (Dose 6 only) | X (Dose 6 only) | - | - | - | X | - | - | - | - |
| **Investigational Product** | | | | | | | | | | | | | | |
| Administer Luspatercept/Placebo Perform Drug Accountability[y] | 6.1.1 | - | X | X | - | - | X | X | X | - | - | - | - | - |

+ve = positive; ADA = antidrug antibody; AE = adverse event; CSP = clinical study protocol; D/C = discontinuation; dw = dry weight; DXA = dual-energy x-ray absorptiometry; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EOS = end of study; FU = follow up; GDF = growth differentiation factor; Hb = hemoglobin; LIC = liver iron concentration; LTTP = Long-term Treatment Period; LVEF = left ventricular ejection fraction; MRI = magnetic resonance imaging; ms = millisecond; MUGA = multigated acquisition scan; OLP = Open-label Phase; PK = pharmacokinetic; q3w = every 3 weeks; QoL = quality of life; RBC = red blood cell; SF-36 = 36-item Short Form Health Survey; TP = Treatment Period; TranQoL = Transfusion-dependent QoL questionnaire; wk = week.

[a] The LTTP was to end when all subjects completed 48 weeks of double-blind treatment or discontinued before reaching 48 weeks of double-blind treatment, whichever was the earlier date, or at the time the study was unblinded (per data monitoring committee recommendation). Only subjects who did not discontinue for reasons as described in Section 9.3.3 were to enter the LTTP.

[b] Day 22 post last dose corresponded to the end of the double-blind TP.

[c] Follow-up visits at Weeks 9, 24, 48, 72, 96, 120, 144, and 156 post last dose were to be performed by all subjects, regardless of whether a subject had discontinued early or not.

[d] Only for subjects who discontinued prior to completion of 48 weeks in the double-blind TP.

[e] The EOS Visit could have occurred at any time up to at least 156 weeks post last dose.

**Study Endpoints**

The primary efficacy endpoint of this study, erythroid response, 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 to the 12-week interval prior to randomization for luspatercept plus BSC versus placebo plus BSC.

The key secondary endpoints were measured at Week 24 and Week 48 and were to be statistically tested in a sequential order at $\alpha = 0.05$ level:

1. Proportion of subjects with erythroid response, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48.

2. 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.

3. 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.

4. Mean change from baseline in transfusion burden from Week 13 to Week 24.

**Sample Size and Power Considerations**

The assumed targeted response rate for the primary endpoint was 40% in luspatercept group and 20% for the placebo group. A total sample size of 300 (200 in the luspatercept group, 100 in placebo group) would have 90% power to detect the difference between the luspatercept group and the placebo group with a 2-sided alpha of 0.05 and assumed 10% drop-out rate for each treatment group.

**Statistical Analysis Plan**

The efficacy analyses were to be performed on the ITT population. The primary efficacy analysis would be performed based on 24 weeks of data after all subjects had completed the double-blind 24-week treatment period phase or discontinued before reaching 24 weeks of double-

blind treatment period.

The primary endpoint response rate was calculated using the number of responders divided by all subjects in the ITT population. The Cochran Mantel-Haenszel (CMH) chi-square test would be performed with randomization factor as strata and 2-sided type 1 error rate of 0.05.

Gate-keeping methods would be used to control the overall Type 1 error rate for the key secondary endpoints. If the result from the primary efficacy analysis in the ITT population showed statistical significance, the key secondary endpoint 1 would be tested next. The key secondary endpoint 2 would be tested only if the test results for both primary endpoint and the key secondary endpoint 1 were significant. The key secondary endpoint 3 would be tested only if the test results for primary endpoint and the key secondary endpoints 1 and 2 were all significant. The 4th secondary endpoint was not included in the testing order.

No interim analysis was planned.

**Protocol Amendments**

There were 2 amendments to the original protocol for the ACE-536-B-THAL-001 study. A summary of significant changes made to the protocol is provided in the table listed below.

**Table 8-8: Protocol Amendments**

| Date | Amendment(s) |
|---|---|
| April 21, 2017 | **Protocol Amendment 1**<br>• Allow all subjects, regardless of the initial treatment to which they have been randomized in the double-blind treatment period, to receive active treatment in the Open-label Phase.<br>• Inclusion of the Open-label Extension study as part of the current study, i.e., the Open-label Phase and not a separate study<br>• Extended the Post-treatment Follow-up Period (post last dose follow-up) from 9 weeks to 156 weeks.<br>• Inclusion of malignancy and pre-malignancy reporting in Post-treatment follow-up given pre-clinical |

| Date | Amendment(s) |
|---|---|
| | animal data..<br>• Exclusion criterion on malignancy were created given pre-clinical animal data.<br>• Implementing subject diary for transfusion received outside of the regular transfusion center.<br>• Eastern Cooperative Oncology Group (ECOG) measurement during study treatment was removed; ECOG measurement at eligibility was not changed.<br>• Introduced threshold for dose adjustments in respect to the WBC counts compared to baseline WBC counts.<br>• Anticoagulant therapies used during the study treatment are allowed as long as the medications are not used due to a related adverse event (AE) that would qualify for treatment discontinuation.<br>• Anagrelide added as prohibited medication.<br>• Interim analysis (IA) removed from protocol due to high accrual rates.<br>• Treatment discontinuation should occur in the setting of any new malignancy. |
| December 11, 2018 | **Protocol Amendment 2**<br><br>• Additional criteria for dose adjustment have been introduced:<br>• Dose delay for condition of worsening of anemia and discontinuation if |

| Date | Amendment(s) |
|---|---|
| | confirmed hematologic malignancy<br>• Dose delay for condition of Grade ≥ 3 leukopenia, neutropenia and/or thrombocytopenia. |

### 8.1.2. Study Results

**Compliance with Good Clinical Practices**

The applicant provided attestation that this study was conducted in accordance with U.S. regulations governing the protection of human subjects, Institutional Review Boards, and the obligations of clinical investigators in accordance with good clinical practice (GCP).

**Financial Disclosure**

The applicant submitted financial disclosure information from all investigators and sub investigators for this trial. A total of 1 sub-investigator (b) (6) received a significant payment of $25,000 from Celgene on July 26, 2018 as an educational grant. It is unlikely that this payment influenced the observed study results given the relatively large number of investigators and study sites.

**Data Quality and Integrity**

No data quality or integrity issues were identified during the course of this review. Datasets and programming for the key study endpoints were included with sufficient details for statistical verification.

**Patient Disposition**

A total of 447 subjects were screened for inclusion and 336 were randomized to study treatment at 65 sites in 15 countries. Ninety-two percent(312 patients) completed 24 weeks of treatment with 210 (94%) completing treatment in the luspatercept+BSC treatment group and 102 patients (91%) in the placebo + BSC treatment group. The proportion of subjects who completed 48 weeks of treatment was similar with 89.3% in the luspatercept group and 86% in the placebo group, respectively.

A total of 66 (20%) patients discontinued study drug with 42 patients(19%) in the luspatercept + BSC treatment group and 24 patients (221%) in the placebo group. The most common reason for discontinuation was withdrawal by subject( 12% in the luspatercept group and 11% in the placebo group. More patients discontinued study drug due to AEs(5%) in the luspatercept group compared to 0.9% in the placebo group. The following table describes the disposition in

the ITT population.

**Table 9 Subject Disposition (ITT Population)**

| | Luspatercept + BSC N=224 n(%) | Placebo + BSC N=112 n(%) |
|---|---|---|
| Patients Randomized | 224 (100) | 112 (100) |
| Subjects Received Treatment | 223 (99.6) | 109 (97.3) |
| Treatment Ongoing | 181 (80.8) | 85 (75.9) |
| Treatment Discontinued | 42 (18.8) | 24 (21.4) |
| Completed 24 weeks of Treatment | 210 (93.8) | 102 (91.1) |
| Completed 48 weeks of Treatment | 200 (89.3) | 96 (85.7) |
| Reason for Treatment Discontinuation | | |
| Withdrawal by Subject | 26 (11.6) | 12 (10.7) |
| Adverse event/Other | 10 (4.5) | 1 (0.9) |
| Lack of Efficacy | 2 (0.9) | 8 (7.1) |
| Protocol Violation | 1 (0.4) | 0 (0) |
| Others | 3 (1.3) | 3 (2.7) |
| Discontinued Study | 26 (11.6) | 14 (12.5) |

Treatment ongoing at time of data cut-off of May 11, 2018. The most common reasons for study discontinuation for the overall population included withdrawal by patient (5.7%), adverse event (1.2%), other reasons to include personal reasons and moving to other city, and death (0.6%)

**Protocol Violations/Deviations**

A total of 24 (7%) of patients had at least one protocol violation before the data cutoff date. There were 19 patients (9%) in the luspatercept treatment group and 5 (5%) in the placebo group. The following table describes the summary of protocol violations.

**Table 10 Protocol Violations (ITT Population)**

| | Luspatercept + BSC N=224 n(%) | Placebo + BSC N=112 n(%) |
|---|---|---|
| Subjects with at least 1 Protocol Violation | 19 (8.5) | 5 (4.5) |
| Safety Reporting | 8 (3.6) | 0 |
| Entered study but Patient did not meet Entry Criteria | 4 (1.8) | 1 (0.9) |
| Laboratory Tests/Procedures | 2 (0.9) | 3 (2.7) |

| | Luspatercept + BSC N=224 n(%) | Placebo + BSC N=112 n(%) |
|---|---|---|
| Investigational Product issues | 3 (1.3) | 1 (0.9) |
| Other | 2 (0.9) | 0 |
| Visit Schedule | 1 (0.4) | 1 (0.9) |
| Concomitant Medication and or Procedure | 1 (0.4) | 0 |

*Reviewer Comment: None of the protocol violations appear to have an impact on the results or interpretation of the efficacy results.*

**Table of Demographic Characteristics**

The following table summarizes the demographics of the patients at baseline.

**Table 11. Patient Demographics for Study ACE-536-B-THAL-001**

| | | Luspatercept + BSC | Placebo + BSC |
|---|---|---|---|
| | | N=224 | N=112 |
| Age | Mean (SD) | 32.2 (10.7) | 31.9 (9.9) |
| | Median (Range) | 30 (18, 66) | 30 (18, 59) |
| | ≤32 | 129 (57.6%) | 63 (56.3%) |
| | >32 - ≤50 | 78 (34.8%) | 44 (39.3%) |
| | >50 | 17 (7.6%) | 5 (4.5%) |
| Sex | F | 132 (58.9%) | 63 (56.3%) |
| | M | 92 (41.1%) | 49 (43.8%) |
| Race | Asian | 81 (36.2%) | 36 (32.1%) |
| | Black or African American | 1 (0.4%) | 0 (0.0%) |
| | White | 122 (54.5%) | 60 (53.6%) |
| | Other | 15 (6.7%) | 11 (9.8%) |
| | Not Collected or Reported | 5 (2.2%) | 5 (4.5%) |
| Region | ASIA-PACIFIC | 72 (32.1%) | 35 (31.3%) |
| | MIDDLE EAST & NORTH AFRICA | 52 (23.2%) | 26 (23.2%) |
| | NORTH AMERICA & EUROPE | 100 (44.6%) | 51 (45.5%) |

*Reviewer Comment: The patient demographics appeared to be balanced between the two treatment arms.*

**Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)**

The following table summarizes the baseline characteristics of the patients.

**Table 12. Baseline Characteristics in Study ACE-536-B-THAL-001**

| | | Luspatercept + BSC | Placebo + BSC |
|---|---|---|---|
| | | N=224 | N=112 |
| ECOG | 0 | 176 (78.6%) | 91 (81.3%) |
| | 1 | 48 (21.4%) | 20 (17.9%) |
| | missing | 0 (0.0%) | 1 (0.9%) |
| B-thal Diagnosis | Beta-Thalassemia | 174 (77.7%) | 83 (74.1%) |
| | B-Thal comb w/Alpha-Thalassemia | 18 (8.0%) | 8 (7.1%) |
| | Hemoglobin E/Beta-Thalassemia | 31 (13.8%) | 21 (18.8%) |
| | missing | 1 (0.4%) | 0 (0.0%) |
| Baseline Trans. Burden Rate | <=6 units/12 weeks | 112 (50.0%) | 56 (50.0%) |
| | >6 units/12 weeks | 112 (50.0%) | 56 (50.0%) |
| Baseline LIC Category | <=3 mg/gr dry weight | 70 (31.3%) | 37 (33.0%) |
| | >3-<=7 mg/gr dry weight | 51 (22.8%) | 30 (26.8%) |
| | >7-<=15 mg/gr dry weight | 38 (17.0%) | 19 (17.0%) |
| | >15 mg/gr dry weight | 65 (29.0%) | 26 (23.2%) |
| Splenectomy | N | 95 (42.4%) | 47 (42.0%) |
| | Y | 129 (57.6%) | 65 (58.0%) |
| Baseline EPO | <200 (IU/L) | 197 (87.9%) | 93 (83.0%) |
| | >=200 to =<500 (IU/L) | 15 (6.7%) | 5 (4.5%) |
| | >500 (IU/L) | 3 (1.3%) | 0 (0.0%) |
| | missing | 9 (4.0%) | 14 (12.5%) |
| Renal Function | NORMAL | 189 (84.4%) | 90 (80.4%) |
| | MILD IMPAIRMENT | 32 (14.3%) | 19 (17.0%) |
| | MODERATE IMPAIRMENT | 2 (0.9%) | 0 (0.0%) |
| | missing | 1 (0.4%) | 3 (2.7%) |
| Hepatic Function | NORMAL | 51 (22.8%) | 23 (20.5%) |
| | MILD IMPAIRMENT | 68 (30.4%) | 40 (35.7%) |
| | MODERATE IMPAIRMENT | 82 (36.6%) | 37 (33.0%) |
| | SEVERE IMPAIRMENT | 22 (9.8%) | 9 (8.0%) |
| | missing | 1 (0.4%) | 3 (2.7%) |
| Gene Mutation | B0/B0 | 68 (30.4%) | 35 (31.3%) |
| | Non-B0/B0 | 155 (69.2%) | 77 (68.8%) |
| | missing | 1 (0.4%) | 0 (0.0%) |

*Reviewer Comment: The baseline characteristics appeared to be balanced between the two treatment arms.*

**Treatment Compliance, Concomitant Medications, and Rescue Medication Use**

Compliance was assessed by regular reviews of study drug administration data recorded in eCRFS, study drug accountability records and source documents.

A total of 97.3% of patients received at least one prior iron chelation therapy. The most frequently used iron chelation therapy was deferasirox with 62.3% of patients in the luspatercept arm and 57.8% of patients in the placebo arm receiving this therapy. The other most frequently used prior medications in the overall population included vitamin D and analogues(49.4%), folic acid and derivatives (45.2%), calcium (25%) and platelet aggregation inhibitors excluding heparin (15.7%).

During the study, all patients expect one in the luspatercept group used at least 1 iron chelation therapy during the study. The most frequently used iron chelation therapy was deferasirox (65%), deferiprone(41%), and deferoxamine mesylate/deferoxamine(33.7%).

A total of 95.8% of patients received at least one concomitant medication(95.5% in the luspatercept group and 96.3% in the placebo group). The most common concomitant medications most frequently used (> 20% of patients) included:

- Anilides(57%), mainly paracetamol (55%)
- Vitamin D and analogues (40%)
- Propionic Acid Derivatives (28%(, mainly ibuprofen (17%)
- Combination of penicillin's, including B-lactamase inhibitors (26%), mainly Augmentin (23%)

**Efficacy Results – Primary Endpoint**

For the primary endpoint, a greater proportion of subjects achieved ≥ 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 treatment group (4.5% of subjects) . The difference in the proportion of subjects with ≥ 33% reduction in RBC transfusion burden during the fixed Week 13 to Week 24 interval between the 2 treatment groups was 17.0%, with results favoring luspatercept + BSC over placebo + BSC, which was statistically significant ($p < 0.0001$).

**Table 13. Primary efficacy analysis in Study ACE-536-B-THAL-001**

| | **Luspatercept + BSC (N = 224)** | **Placebo + BSC (N = 112)** |
|---|---|---|
| Number of Responders, n (%) | 48 (21.4) | 5 (4.5) |
| 95% CI of response rate | (16.2, 27.4) | (1.5, 10.1) |
| Difference (%) (95% CI) | 17.0 (10.4, 23.6) | |
| p-value | < 0.0001 | |

**Efficacy Results – Secondary and other relevant endpoints**

For the first three secondary analysis, a greater proportion of subjects achieved ≥ 33% or 50% reduction in RBC transfusion burden during the fixed Week 37 to Week 48 interval or Week 1 to Week 24 interval in the luspatercept + BSC treatment group than in the placebo + BSC treatment group. The differences in the proportion of subjects between the 2 treatment groups were statistically significant.

**Table 14. Secondary efficacy analyses in Study ACE-536-B-THAL-001**

| | **Luspatercept + BSC (N = 224)** | **Placebo + BSC (N = 112)** |
|---|---|---|
| **≥33% reduction in RBC transfusion burden during the fixed Week 37 to Week 48** | | |
| Number of Responders, n (%) | 44 (19.6) | 4 (3.6) |
| 95% CI of response rate | (14.7, 25.5) | (1.0, 8.9) |
| Difference (%) (95% CI) | 16.1 (9.8, 22.4) | |
| p-value | < 0.0001 | |
| **≥ 50% reduction in RBC transfusion burden during the fixed Week 13 to Week 24** | | |
| Number of Responders, n (%) | 17 (7.6) | 2 (1.8) |
| 95% CI of response rate | (4.5, 11.9) | (0.2, 6.3) |
| Difference (%) (95% CI) | 5.8 (1.6, 10.1) | |
| p-value | 0.0303 | |
| **≥ 50% reduction in RBC transfusion burden during the fixed Week 37 to Week 48** | | |
| Number of Responders, n (%) | 23 (10.3) | 1 (0.9) |
| 95% CI of response rate | (6.6, 15.0) | (0.0, 4.9) |
| Difference (%) (95% CI) | 9.4 (5.0, 13.7) | |
| p-value | 0.0017 | |

***Reviewer's comment:***

*The analyses were also conducted to the following two exploratory endpoints:*

1. *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 red blood cells [RBCs]/time) with a reduction of at least 2 units, from Week 1 to Week 48 (the whole double-blind treatment period).*

2. *The proportion of subjects treated with luspatercept + best supportive care (BSC) versus placebo + BSC who achieved erythroid response, defined as ≥ 50% reduction from baseline in transfusion burden (units red blood cells [RBCs]/time) with a reduction of at least 2 units, from Week 1 to Week 48 (the whole double-blind treatment period).*

**Table 15. Reduction Transfusion Burden in Week 1 to Week 48**

| | **Luspatercept + BSC (N = 224)** | **Placebo + BSC (N = 112)** |
|---|---|---|
| **≥33% reduction in RBC transfusion burden during the fixed Week 1 to Week 48** | | |
| Number of Responders, n (%) | 41 (18.3) | 2 (1.8) |
| 95% CI of response rate | (13.5, 24.0) | (0.2, 6.3) |
| Difference (%) (95% CI) | 16.6 (10.9, 22.2) | |
| **≥ 50% reduction in RBC transfusion burden during the fixed Week 1 to Week 48** | | |
| Number of Responders, n (%) | 13 (5.8) | 1 (0.9) |
| 95% CI of response rate | (3.1, 9.7) | (0.0, 4.9) |
| Difference (%) (95% CI) | 5.0 (1.4, 8.5) | |

The results showed the same trend as those of primary and key secondary endpoints.

**Dose/Dose Response**

Please refer to the clinical pharmacology section 6 for discussions on general dosing and comprehensive clinical pharmacology review.

**Durability of Response**

The durability of response was assessed by both a 33% reduction or greater in RBC transfusion burden at weeks 13 to 24 and also a greater than or equal to 50% reduction in RBC transfusion burden at 13 to 24 weeks. Additionally, a reduction in transfusion burden was assessed from week 1 to week 48 as described in table 15. Overall, when evaluating primary endpoint of greater than or equal to 33% reduction in RBC transfusions at fixed interval of week 37 to week 48, 19.6% of patients in the luspatercept group had a reduction compared to 3.6% in the placebo group.

**Persistence of Effect**

Refer to the durability of response section above for brief discussion of the duration of

response.

**Efficacy Results – Subgroup Analysis**

Subgroup analyses were performed for the primary endpoint. The results are summarized below.

**Table 16. Subgroup analysis for the primary endpoint**

| | Responders/N | | ORR (95% CI) | |
|---|---|---|---|---|
| **Group** | **Luspatercept** | **Placebo** | **Luspatercept** | **Placebo** |
| **Primary Analysis** | 48 / 224 | 5 / 112 | 21.4 (16.2, 27.4) | 4.5 (1.5, 10.1) |
| **Age ≤ 32** | 22 / 129 | 4 / 63 | 17.1 (11.0, 24.7) | 6.3 (1.8, 15.5) |
| **Age > 32** | 26 / 95 | 1 / 49 | 27.4 (18.7, 37.5) | 2.0 (0.1, 10.9) |
| **Male** | 13 / 92 | 1 / 49 | 14.1 (7.7, 23.0) | 2.0 (0.1, 10.9) |
| **Female** | 35 / 132 | 4 / 63 | 26.5 (19.2, 34.9) | 6.3 (1.8, 15.5) |
| **White** | 30 / 122 | 2 / 60 | 24.6 (17.2, 33.2) | 3.3 (0.4, 11.5) |
| **Asian** | 12 / 81 | 2 / 36 | 14.8 (7.9, 24.4) | 5.6 (0.7, 18.7) |
| **Other Race** | 6 / 21 | 1 / 16 | 28.6 (11.3, 52.2) | 6.3 (0.2, 30.2) |
| **N Am & EU** | 23 / 100 | 1 / 51 | 23.0 (15.2, 32.5) | 2.0 (0.0, 10.4) |
| **ASIA-PACIFIC** | 14 / 72 | 2 / 35 | 19.4 (11.1, 30.5) | 5.7 (0.7, 19.2) |
| **MID EAST & N AFRICA** | 11 / 52 | 2 / 26 | 21.2 (11.1, 34.7) | 7.7 (0.9, 25.1) |

These subgroup analyses are considered to be exploratory. There is no outlier observed among these subgroup analyses.

**Additional Analyses Conducted on the Individual Trial**

Quality of Life was assessed in patients using the SF-36 and the TranQoL.

The study population, in general, had a reasonably good level of HRQoL at baseline compared to a general population and that this quality of life was maintained during the course of the 48 weeks on the study. However, since the study population had a good HRQoL at baseline, difficult to interpret any improvements from baseline. In addition, the QoL endpoints were descriptive only.

Change in Serum Ferritin Levels (ITT Population)
The mean baseline ferritin levels were 2096.91 ug/L and the median was 1441.25 ug/L (range 88, 6400) in the luspatercept group and the mean baseline ferritin level was 1845.05 ug/L and median of 1301 (range 135, 6400) in the placebo group. The postbaseline ferritin levels were a mean ferritin level of 1831.97 ug/L (median 1002.5, range 63.3, 6400) in the luspatercept-aamt group and mean ferritin of 1988.91 ug/L (median 1224.67, range 144.8, 6400) in the placebo group.

*Reviewer Comment: There may be a trend toward lower ferritin levels however difficult to interpret as the mean baseline ferritin level was lower in the placebo group.*

**Mean Change in Liver Iron Concentration**

The following table describes the mean change in derived liver iron concentration at week 48 in the ITT population. There was no significant reduction in the LIC level over the 48 week treatment period.

**Table 17 Mean Change in Derived Liver Iron Concentration at Week 48 (ITT Population)**

| | Luspatercept + BSC<br>N=224 | Placebo + BSC<br>N=112 |
|---|---|---|
| Baseline | | |
| N | 211 | 110 |
| Mean SD mg/g<br>Median (min, max) mg/g, dw | 9.62(9.963)<br>5.47 (0.8, 42) | 9.36 (10.241)<br>4.89 (0.2, 43) |
| Week 48<br>n | 202 | 103 |
| Mean SD mg/g<br>Median (min, max) mg/g, dw | 9.93 (10.194)<br>5.81 (0.8, 41.6) | 9.27 (10.357)<br>4.74 (0.8, 43) |

Mean change in Myocardial T2
At baseline the mean myocardial T2 was 33.52ms and 34.76ms in the luspatercept group and placebo groups, respectively. At week 48, the mean change in myocardial T2 from baseline was -18.3ms in the luspatercept group and + 0.02ms in the placebo group.

Most subjects in both treatment groups received iron chelation therapy at baseline and postbaseline (61% in each group). The use of iron chelation therapy at baseline and postbaseline period was reported in 23.2% and 19.6% of patients in the luspatercept and placebo groups, respectively. Of note, combo therapy was defined as more than 1 iron chelation drug taken by a subject during the specified baseline and post-baseline periods.

### 8.1.3 Integrated Review of Effectiveness

**Primary Endpoints**

A greater proportion of patients achieved the primary efficacy endpoint (subjects with ≥ 33% reduction in RBC transfusion burden during the fixed Week 13 to Week 24 interval) in the luspatercept + BSC treatment group (21.4% of patients) than in the placebo + BSC treatment

group (4.5% of patients). The difference in the proportion of subjects with ≥ 33% reduction in RBC transfusion burden during the fixed Week 13 to Week 24 interval between the 2 treatment groups was 17.0%, with results favoring luspatercept + BSC over placebo + BSC, which was statistically significant ($p < 0.0001$).

**Secondary and Other Endpoints**

A greater proportion of subjects achieved the first key secondary efficacy endpoint (subjects with ≥ 33% reduction in RBC transfusion burden during the fixed Week 37 to Week 48 interval) in the luspatercept + BSC treatment group (19.6% of subjects) than in the placebo + BSC treatment group (3.6% of subjects) (Table 18). The difference in the proportion of subjects with ≥ 33% reduction in RBC transfusion burden during the fixed Week 37 to Week 48 interval between the 2 treatment groups was 16.1%, with results favoring luspatercept + BSC over placebo + BSC, which was statistically significant ($p < 0.0001$).

When response was assessed over a rolling 12-week or rolling 24-week interval, which shows the proportion of subjects who achieve benefit from the treatment at any time and is more relevant to real-world clinical practice, the proportions of responders were 70.5% (12-week interval, ≥ 33% reduction from baseline and reduction of at least 2 RBC units) and 41.1% (24-week interval, ≥ 33% reduction from baseline) in the luspatercept + BSC group compared with 29.5% (12-week interval) and 2.7% (24-week interval) in the placebo + BSC group (nominal $p < 0.0001$ for both intervals). In the luspatercept + BSC group, 40.2% of subjects achieved a ≥ 50% reduction from baseline in RBC transfusion burden (and a decrease of at least 2 units) for any rolling 12-week interval compared with 6.3% of subjects in the placebo + BSC group (nominal $p < 0.0001$).

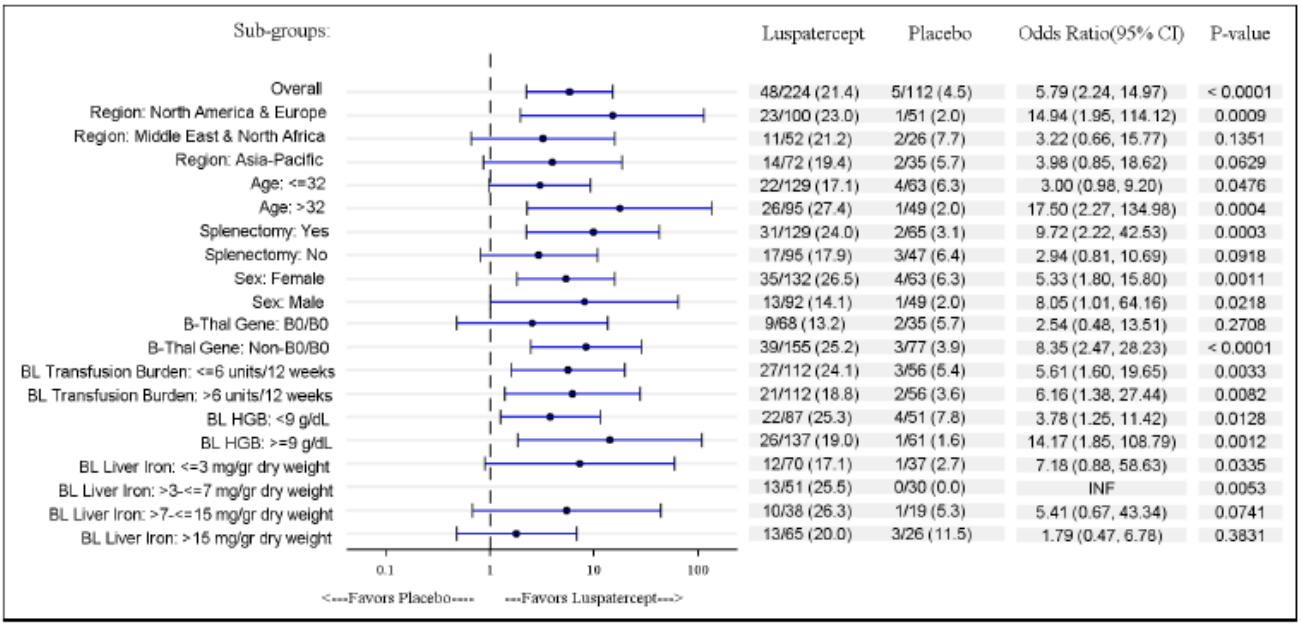
*Reviewer Comment: While a rolling 12 week and rolling 24 week interval response assessment was reviewed, the analyses results are considered to be exploratory since these endpoints were not included in the pre-specified testing procedure and no alpha was allocated.*

**Subpopulations**

For the primary efficacy endpoint of patients with greater than or equal 33% reduction in RBC transfusion burden during the fixed window of Week 13 to Week 24, a nominally significant was demonstrated in the luspatercept group versus placebo for all the subgroups evaluated. See the figure below(taken from the CSR Figure 8 page 124).

**Figure 2 Forest-Plot of RBC Transfusion Burden for the Subgroups.**

**Figure 8: 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.

Source: Figure 14.2.4.1.1.

**Reviewer Comment:** *Given small numbers of patients in each subgroup, no meaningful conclusions can be drawn. All analyses presented in Figure 8 are exploratory and all p-values are nominal. There was no pre-specified testing procedure for any of the subgroups presented in Figure 8.*

**Conducting a pooled subgroup analysis**

A pooled subgroup analysis was not performed.

**Additional Efficacy Considerations**

The analyses of red blood cell transfusion burden reduction in terms of red blood cell units was also evaluated. At the fixed interval from week 13 to Week 24 a reduction in the mean RBC transfusion burden in the luspatercept group ranged from 0.74 to 0.90 RBC units/12 weeks. In the patients in the luspatercept + BSC treatment arm with ≥ 33% reduction and ≥ 50% reduction in RBC transfusion burden during the fixed window (week 13 to Week 24), the mean RBC transfusion burden reduction per subject from baseline to Week 13 to Week 24 was 3.02 RBC units/12 weeks and 3.71 RBC units/12 weeks, respectively.

### 8.1.4 Integrated Assessment of Effectiveness

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 patients with transfusion dependent beta thalassemia who require regular RBC transfusions. Consenting patients 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 patients were randomized to luspatercept or placebo in a 2:1 ratio. The study included a double-blind treatment period (Weeks 1 to 48), long-term treatment period (after Week 48; patients continued to receive the study drug to which they were initially randomized]) and a post-treatment follow-up period of 156 weeks after the last dose of study treatment. After unblinding for the primary analysis, eligible patients (including those randomized to placebo) were given the option of open-label luspatercept treatment for up to 5 years .

The primary objective of this study is to determine the proportion of patients treated with luspatercept versus placebo who achieve erythroid response. Enrollment in the study is complete; however, long-term treatment and follow-up are ongoing.

Reduced survival in regularly transfused adult patients with beta thalassemia is largely due to the known iron overload complications in major organs, involving the heart, liver, and endocrine glands. In addition, blood transfusion exposes patients to a variety of risks (e.g., alloimmunization, infection), and may be affected by limited access to safe blood products and/or blood product shortages. Additionally, patients face the burden of transfusion time spent in the hospital, as well as a dependence on ICT to minimize the detrimental effects of iron overload. These present major challenges in managing patients who require regular/frequent transfusions for their entire lives in order to survive. Therefore, reduction in transfusion burden is expected to provide numerous benefits for these patients.

Reduction in transfusion burden in ACE-536-B-THAL-001 was assessed over fixed time periods as primary and key secondary response endpoints. The primary endpoint was based on a ≥ 33% reduction from baseline in RBC transfusion burden with reduction of at least 2 units in the fixed 12-week period from to Week 13 to Week 24.

The key secondary response endpoints evaluated ≥ 33% reduction from baseline with reduction of at least 2 units in the fixed 12-week period from to Week 37 to Week 48 and ≥ 50% reduction with reduction of at least 2 units in the fixed 12-week period from Week 13 to Week 24 and from Week 37 to Week 48. A 33% or greater reduction in transfusion burden is considered to be clinically meaningful for patients regularly transfused based on the decrease in the transfusion iron accumulation and related complications.

In addition to the protocol-defined primary and key secondary response endpoints, which measure RBC transfusion burden reduction between baseline and fixed intervals postbaseline, response endpoints (≥ 33% and ≥ 50% reduction) were also evaluated over 12- and 24-week rolling intervals in statistical analysis plan (SAP)-specified analyses (which were finalized prior to database lock and study unblinding). The rolling analyses show the proportion of subjects who achieve benefit from the treatment at any time and are more relevant to the real-world management of patients with beta thalassemia.

The pivotal study met the prespecified primary and key secondary response endpoints. Luspatercept + BSC was associated with a statistically significant reduction of RBC transfusion burden, as evident from the positive results across all of the analyses, regardless of the time interval or the reduction criteria (≥ 33% or ≥ 50% and a reduction of at least 2 units).

The response rates for a ≥ 33% reduction from baseline in transfusion burden and a reduction of at least 2 units from Week 13 to Week 24 (primary endpoint) were 21.4% (48/224) in the luspatercept + BSC group and 4.5% (5/112) in the placebo + BSC group ($p < 0.0001$; Table 5). When the response rate was assessed over Weeks 37 to 48 (key secondary endpoint), the percentage of subjects with a response demonstrated a sustained effect, remaining relatively consistent with the results from Week 13 to Week 24 (19.6%, 44/224 subjects in the luspatercept + BSC group and 3.6%, 4/112 subjects in the placebo + BSC group; $p < 0.0001$).

Similarly, a greater proportion of subjects in the luspatercept + BSC group achieved ≥ 50% reduction in transfusion burden (key secondary endpoint) during the fixed Week 13 to Week 24 interval (7.6%; 17/224) compared with the placebo + BSC group (1.8%; 2/112; $p = 0.0303$; Table 5). When the response rate was assessed over Weeks 37 to 48, the percentage of subjects with a ≥ 50% reduction in RBC transfusion burden improved slightly in the luspatercept + BSC group (10.3%, 23/224), again demonstrating a sustained effect; the response rate remained constant in the placebo + BSC groups (0.9%, 1/112). The difference between treatment groups was also statistically significant ($p = 0.0017$).

## 8.2 Review of Safety

### 8.2.1 Safety Review Approach

The pivotal BELIEVE trial (ACE-536-B-THAL-001) was included in the safety database for luspatercept as part of the BLA submission review. The safety population for ACE-536-B-THAL-001 was comprised of 332 patients with TD beta-thalassemia.

The majority of data presented in the following sections reflects the safety data submitted in the original BLA on April 4, 2019. Of note, a 120-Day Safety Update Report was submitted to the Agency on behalf of the applicant on July 8, 2018. Unless noted in the review, safety data presented reflects the original submission before the data cutoff.

### 8.2.2 Review of the Safety Database

**Overall Exposure**

A total of 332 patients (223 in the luspatercept + BSC treatment group and 109 in the placebo + BSC treatment group) received at least 1 dose of study drug and were included in the safety population.

The median treatment duration was similar between the luspatercept treatment arm (64.1 weeks) and the placebo treatment arm (64.0 weeks). This corresponds to a median of 21 doses of luspatercept in the luspatercept treatment arm and 21 doses of placebo in the placebo treatment arm (each dose was administered Q3W) until the data cutoff date.

Per study protocol, dose titration from the starting dose level of 1.0 to 1.25 mg/kg was permitted. Overall, 52.7% of subjects had their study drug dose titrated to 1.25 mg/kg at any time until the data cutoff date, with a lower proportion observed in the luspatercept treatment group (46.2%) than in the placebo treatment group (66.1%). Within the first 24 weeks of treatment, 24.7% of subjects in the luspatercept treatment group and 49.5% of subjects in the placebo treatment group had their dose titrated to 1.25 mg/kg.

Study drug dose reduction was allowed per study protocol. A total of 8.4% of patients (11.2% in the luspatercept treatment group and 2.8% in the placebo treatment group) had their dose reduced from 1.0 to 0.80 mg/kg. A second dose reduction (i.e., subsequent reduction of the dose from 0.80 to 0.60 mg/kg) occurred in 1.8% of patients in the luspatercept treatment arm and 0.9% of patients s in the placebo treatment arm. No enrolled patients had study drug dose reduced to 0.45 mg/kg during the study.

**Relevant Characteristics of Safety Population**

Safety population demographic characteristics for the pivotal BELIEVE trial are summarized by treatment in the table provided below. There were more females patients in both the luspatercept (n=132, 59.2%) and placebo (n=61, 55.9%) arms, respectively. The majority of patients in the safety population were white and identified as non-Hispanic/Latino. The mean age of patients in the luspatercept and placebo arms were 32.2 and 31.7 years, respectively.

**Table 18: Demographic Characteristics of Pooled Safety Population**

| | Luspatercept (N=223) | Placebo (N=109) |
|---|---|---|
| **Sex** | | |
| Female | 132 (59.2) | 61 (55.9) |
| Male | 91 (40.8) | 48 (44.0) |
| **Age** | | |

| | Luspatercept (N=223) | Placebo (N=109) |
|---|---|---|
| Mean | 32.2 | 31.7 |
| Median | 30 | 30 |
| Range (Min-Max) | 18-66 | 18-59 |
| **Age Group** | | |
| < 65 years of age | 222 (99.6) | 109 (100.0) |
| ≥ 65 years of age | 1 (0.44) | 0 (0.0) |
| **Race** | | |
| White | 121 (54.2) | 58 (53.2) |
| Black or African American | 1 (0.44) | 0 (0.0) |
| Asian | 81 (36.3) | 36 (33.0) |
| Other | 15 (6.7) | 10 (9.2) |
| Not Reported | 5 (2.2) | 5 (4.5) |
| **Ethnicity** | | |
| Hispanic or Latino | 5 (2.2) | 2 (1.8) |
| Not Hispanic or Latino | 217 (97.3) | 104 (95.4) |
| Missing | 1 (0.44) | 3 (2.7) |
| **Region** | | |
| North America & Europe | 99 (44.3) | 49 (44.9) |
| Asia Pacific | 72 (32.2) | 35 (32.1) |
| Middle East & North Africa | 52 (23.3) | 25 (22.9) |

Source: FDA Clinical Reviewer Analysis, JMP Clinical

*Clinical Reviewer Comment: The safety dataset was mostly comprised of Caucasian patients. Safety results are generalizable to the beta thalassemia patient population given that the majority of patients TD beta thalassemia are Caucasian. In addition, the safety population was comprised of younger patients with a mean age of 31-32 years. Because of the significant morbidities associated with beta thalassemia, many patients do not have a normal lifespan and suffer from premature death.*

**Adequacy of the safety database:**

The demographics of the safety population are consistent with those of the intended patient population. The safety database enrolled a heterogeneous population so that pooled safety results are generalizable to the intended patient population.

### 8.2.3 Adequacy of Applicant's Clinical Safety Assessments

**Issues Regarding Data Integrity and Submission Quality**

The quality of safety data submitted was adequate to permit substantial primary review. The

Applicant provided analysis-ready datasets for the pivotal study. Narrative for patient deaths, SAEs and discontinuations on the study were also provided at the time of the BLA submission. No issues regarding data integrity were identified during this review, or in the course of clinical investigational site inspections. Responses to Agency information requests were rapid and complete.

**Categorization of Adverse Events**

Summaries of AEs and other safety data were based on data through the data cutoff date (May 11, 2018) for patients who were randomized and received at least 1 dose of the study drug. Patients were analyzed according to the actual treatment they received.

Adverse events were analyzed in terms of TEAEs, which were defined as any AEs that occurred or worsened on or after the start of study drug through 63 days after the last dose of study drug. In addition, any AE with an onset date beyond this time frame and that was assessed by the investigator as related to study drug was considered a TEAE. All AEs were coded using MedDRA version 20.0.

The incidence of TEAEs was summarized by MedDRA SOC and PT. If a subject experienced multiple TEAEs under the same SOC or PT, then the subject was counted only once for that SOC or PT. The severity/intensity of TEAEs was graded 1 to 5 according to NCI CTCAE version 4.03.

**Routine Clinical Tests**

The schedule of safety evaluations and clinical assessments for the ACE-536-B-THAL-001 trial is described in Section 8.1.1. The frequency of monitoring was considered adequate and appropriate for the enrolled patient population. Refer to the study schedule of assessments for further detail.

### 8.2.4 Safety Results

**Deaths**

There were 2 patient deaths (1 in each treatment arm) as of the data cutoff date for the pivotal ACE-536-B-THAL-001 study. One patient (b) (6) in the luspatercept treatment arm died due to an SAE of urosepsis. The patient died 207 days after the last dose of study drug. The SAE of urosepsis was considered by the investigator to be not related to study drug. One patient in the placebo treatment arm (b) (6) died due to a serious TEAE of acute cholecystitis. The serious TEAE of cholecystitis acute was considered by the investigator to be not related to study drug.

In addition, there was 1 death reported in the 3-month safety follow up in a 26 year old male treated with luspatercept. The patient developed acute erythroleukemia with subsequent

neutropenic sepsis, pancytopenia and renal failure which resulted in death. The patient received induction chemotherapy for leukemia treatment prior to his death. Further details are provided in the patient narratives below.

**Patient (b) (6) Narrative**

The patient was a 19-year-old Asian male with an initial diagnosis of beta-thalassemia in 1997. The patient had received prior related beta-thalassemia treatment, which included calcium lactate, cholecalciferol, and folic acid (June 2015 to ongoing). Ongoing beta-thalassemia comorbidities included osteoporosis, clinically significant iron overload, and splenomegaly. The patient also had a history of hyperuricemia. The patient started study treatment on (b) (6) . The last dose of study treatment (Dose 12) was received on (b) (6) (Study Day 232). The total treatment duration for this patient was 253 days.

On (b) (6) (Study Day 253), the patient was discontinued from study treatment due to consent withdrawal. At the time of discontinuation, the patient was experiencing Grade 1 cough and Grade 1 sore throat.

The patient died on (b) (6). The reported cause of death was urosepsis. The investigator deemed the event not related to study drug.

*Clinical Reviewer Comment: There is insufficient data provided by the Sponsor to conclude the cause of death. No autopsy was performed. Given that the patient's death occurred nearly 7 months after study drug discontinuation, it appears unlikely that study drug was related directly to the death; however, no definitive conclusions can be drawn in this setting.*

**Patient (b) (6) Narrative**

The patient was a 24-year-old Asian male with an initial diagnosis of hemoglobin E/beta-thalassemia in 1992. The subject had received prior beta-thalassemia treatment, which included folic acid (June 2000 to ongoing). The patient's beta-thalassemia comorbidities included splenomegaly, clinically significant iron overload, osteopenia, and pulmonary hypertension (diagnosed in March 2016). The patient was receiving iron chelation therapy at study entry and throughout the study with deferiprone and deferoxamine.

The patient started study treatment with placebo on (b) (6) The last dose of study treatment (Dose 8) was received on (b) (6) (Study Day 147). Total treatment duration (placebo arm) for this patient was 153 days.

On (b) (6) (Study Day 152), the patient experienced acute Grade 5 cholecystitis . As per the safety report, the patient was admitted to the hospital for severe epigastric pain and fever (no temperature was reported). Diagnostic data and laboratory tests were not provided. No

ultrasonography was performed. The subject had received a RBC transfusion on (b) (6) for a pre-transfusion hemoglobin of 7.0 g/dL. Treatment for the event included ceftriaxone, metronidazole, and paracetamol. The study treatment was discontinued on (b) (6) due to this event.

On (b) (6), as per the safety report, the patient experienced hypotension and consequently cardiac arrest, and died on the same day. The reported cause of death was acute cholecystitis. As per the safety report, no autopsy report or death certificate was available.

*Clinical Reviewer Comment: This death occurred in a patient who received placebo treatment; therefore, this event is not drug-related.*

**Patient (b) (6) Narrative**

The patient was a 26-year-old Asian male with a diagnosis of beta-thalassemia combined with alpha-thalassemia. He had received prior beta-thalassemia treatment including calcium lactate and folic acid. The patient's ongoing beta-thalassemia comorbidities included splenomegaly, hypogonadism, osteoporosis, clinically significant iron overload and pulmonary hypertension. The patient received iron chelation therapy at study entry and throughout the study with deferiprone. As per safety reports, the patient was also receiving testosterone for hypogonadism.

The patient started study treatment on (b) (6) The last dose of study treatment (Dose 23) was received on (b) (6) (Study Day 469) as of the database cutoff.

The patient continued luspatercept treatment and received Dose 24 on (b) (6) in the double-blind treatment phase. The last dose of luspatercept (Dose 27) was received on (b) (6) (Study Day 554). The total duration of luspatercept was 574 days.

Per the safety report, since July 2018, the patient demonstrated increasing transfusion requirements (2-3 RBC transfusions/month) with subsequent development of persistent pancytopenia and severe neutropenia. The patient experienced the onset of neutropenia on (b) (6)

On (b) (6) the patient started experiencing abdominal symptoms. These were attributed to Grade 3 multiple splenic abscesses confirmed by abdominal ultrasound.
On (b) (6), the subject received his last dose of study treatment and presented with fever. The fever was attributed to neutropenic sepsis (Grade 3) secondary to deferiprone (suspected by the investigator). The subject was hospitalized subsequently. On admission, the subject was hypotensive and had an enlarged spleen. Routine blood cultures and blood films for parasitic infection were negative. Morphological analysis of blood did not identify any blasts or abnormal lymphoid cells. Treatment for neutropenic sepsis was initiated with paracetamol for

fever, IV Augmentin and ceftazidime, filgrastim, IV cefepime, ciprofloxacin, and ceftazidime IV was given to treat melioidosis and splenic abscess as well as IV meropenem to treat melioidosis only. Dengue serology (IgG and IgM) tested were both negative. Grade 4 neutropenic sepsis and Grade 3 thrombocytopenia were reported beginning on (b) (6).

On (b) (6) the subject exhibited Grade 3 pancytopenia; thus, a bone marrow aspirate and biopsy were performed. The bone marrow aspirate (BMA) was suboptimal; this demonstrated an erythroid series (75%) with few granulocytic cells, as well as few scattered megakaryocytes, no excess blasts, or abnormal cell clusters. The BM biopsy did not exhibit excess of blast cells. The trephine biopsy revealed hypercellular marrow with erythroid hyperplasia, with suspicious B-cell aggregates.

In October of 2018 the patient deteriorated once more developing a marked enlargement of the liver and spleen with concomitant neutropenic fever that was not subsiding despite steroid treatment and empiric antibiotic therapy. The patient deteriorated rapidly with persistent febrile neutropenia and pancytopenia.

The review from an independent expert regarding a peripheral blood smear performed on (b) (6) identified the presence of many red cell precursors and possible myeloid blasts, approximately 30% blastic cells in the setting many normoblasts. Most of the blastic cells appeared to be consistent with blasts of AML M6.

On (b) (6), blood tests undertaken on this date revealed a Hb of 8.9, platelets of 17,000, and a white blood count of 33.7. On the same day, the patient needed artificial ventilation due to type 2 respiratory failure and acute renal failure. The patient was transferred to an intensive care unit. A second bone marrow examination (aspirate and trephine) was performed. The BMA revealed the following: Differential count including erythroid cells 89, blast cells 1, myelo/metamyelocytes 1, neutrophils 7%, lymphocytes 2%, monocytes 1%. Morphology: WBC: leukocytosis with 4% blastic cells, left shift maturation; PLT: reduced. Granulopoiesis and megakaryopoiesis were both depressed. The immunophenotyping revealed morphologically hypercellular bone marrow with predominantly early stage erythroid series, many bare nuclei and depressed hematopoietic cell lineages. Flow cytometry revealed 52-58% of the cell population at CD45 negative low SCC gated and analyzed. Cells were positive for Gylcophorin-A, and negative for immature marker, specific-lineage markers (myeloid, B cell and T cell lymphoid marker). It was also reported that 3.3% of cell populations were CD45 intermediate with low SCC (side scatter) and expressed a similar phenotype. Other lymphoid markers tested negative.

The Investigator, acting on the local pathology report suggested the possibility of pure erythroid leukemia (FAB AML-M6) initiated chemotherapy with reduced dose daunorubicin and cytarabine (3+7 regimen, 30 mg/m$^2$, (b) (6)), under the rationale that the patient was in acute renal failure requiring hemodialysis and on mechanical

ventilation, and therefore deemed to have high risk of chemotherapy induced mortality if full dose chemotherapy was administered.

Peripheral blood was drawn on (b) (6) and cytomorphology, flow cytometry , cytogenetics, molecular genetics were performed and returned normal: no metaphases obtained, no evidence of immature myeloid cells. Cytogenetic analysis revealed no metaphases. The molecular genetic analysis revealed no mutation in ASXL1, CEBPA, DNMT3A, tyrosine-kinase 2 domain of FLT3, IDH1, IDH2, NPM1, RUNX1, TP53; no FLT3-ITD or KMT2A partial tandem duplication or mutations were detected. These findings did not allow to either prove or to rule out AML.

The patient subsequently died on (b) (6) in the setting of presumed AML status post dose-reduced induction chemotherapy, renal failure, and neutropenic sepsis. An autopsy was not performed.

*Clinical Reviewer Comment: From review of the safety reports, this death in a luspatercept treated patient appears to be possibly due to the development of an acute leukemia with resultant complications of neutropenic sepsis, acute renal failure requiring hemodialysis and respiratory failure. The Independent molecular genetic analysis was unable to detect mutations in any of the typical predetermined breaking points of genes known in AML. Typically, leukemia mutations present in at least one of the investigated genes are found in 90% of cases. However, chemotherapy had been administered and the initial aspirate and biopsy were not sufficient for cytogenetic analyses. The time course of onset of neutropenia in relation to luspatercept treatment is concerning in this case. A causal relationship between exposure to luspatercept and development of hematologic malignancy cannot be ruled out at this juncture.*

**Serious Adverse Events**

The overall incidence of serious TEAEs was higher in the luspatercept treatment arm (n=37, 16.6%) than in the placebo arm (n=6, 5.5%). The following table outlines SAEs by treatment group in the pivotal BELIEVE trial. The most frequently reported (in more than 1 patient) serious TEAEs in the luspatercept treatment group were anemia, cellulitis, cerebrovascular accident, cholangitis, deep vein thrombosis, and pyrexia. Serious TEAEs considered by the investigator to be related to study drug were reported in 6 (2.7%) patients in the luspatercept treatment group; no treatment-related serious TEAEs were reported in the placebo treatment arm.

**Table 19: SAEs in the Safety Population**

| Serious Adverse Event | Luspatercept (N=223) n(%) | Placebo (N=103) n(%) |
|---|---|---|
| Abdominal Pain | 2 (0.9) | 0 (0.0) |

| Serious Adverse Event | Luspatercept (N=223) n(%) | Placebo (N=103) n(%) |
|---|---|---|
| Acute Sinusitis | 1 (0.4) | 0 (0.0) |
| Anemia | 3 (1.3) | 0 (0.0) |
| Appendicitis | 1 (0.4) | 0 (0.0) |
| Back Pain | 1 (0.4) | 0 (0.0) |
| Blood Uric Acid Increased | 1 (0.4) | 0 (0.0) |
| Congestive Heart Failure | 1 (0.4) | 0 (0.0) |
| Cellulitis | 2 (0.9) | 0 (0.0) |
| Cerebrovascular Accident | 2 (0.9) | 0 (0.0) |
| Cholangitis | 2 (0.9) | 0 (0.0) |
| Cholecystitis | 1 (0.4) | 1 (0.9) |
| Deep Venous Thrombosis | 2 (0.9) | 0 (0.0) |
| Dengue fever | 2 (0.9) | 0 (0.0) |
| Drug Induced Liver Injury | 1 (0.4) | 0 (0.0) |
| EBV Infection | 0 (0.0) | 1 (0.9) |
| Febrile Neutropenia | 1 (0.4) | 0 (0.0) |
| Femur Fracture | 1 (0.4) | 0 (0.0) |
| Increased cardiac iron stores | 0 (0.0) | 1 (0.9) |
| Meningioma | 1 (0.4) | 0 (0.0) |
| Nephrolithiasis | 1 (0.4) | 0 (0.0) |
| Neuritis | 1 (0.4) | 0 (0.0) |
| Pain | 1 (0.4) | 0 (0.0) |
| Pan-reactive autoantibody | 0 (0.0) | 1 (0.9) |
| Pharyngitis Bacterial | 1 (0.4) | 0 (0.0) |
| Portal Vein Thrombosis | 1 (0.4) | 0 (0.0) |
| Pulmonary Embolism | 1 (0.4) | 0 (0.0) |
| Pulmonary Sepsis | 1 (0.4) | 0 (0.0) |
| Pyrexia | 3 (1.3) | 0 (0.0) |
| Renal Injury | 1 (0.4) | 0 (0.0) |
| Thermal burn | 1 (0.4) | 0 (0.0) |
| Tracheitis | 1 (0.4) | 0 (0.0) |
| Traumatic Fracture | 1 (0.4) | 0 (0.0) |
| Urosepsis | 1 (0.4) | 1 (0.9) |
| Urinary Tract Infection | 0 (0) | 1 (0.9) |
| Viral Infection | 1 (0.4) | 0 (0.0) |
| Viral URI | 1 (0.4) | 0 (0.0) |

Source: FDA Clinical Reviewer Analysis, JMP Clinical

**Dropouts and/or Discontinuations Due to Adverse Effects**

A total of 12 (5.4%) patients in the luspatercept treatment arm and 1 (0.9) patient in the placebo arm discontinued study drug due to a treatment-emergent adverse event (TEAE). All 12 patients in the treatment arm had a TEAE considered treatment-related as per the investigator.

The most common TEAEs leading to discontinuation in the luspatercept treatment arm included thrombotic events (n=4, 1.8%), including pulmonary embolism (n=1), deep venous thrombosis (n=2) and portal vein thrombosis (n=1). A total of 4 patients (1.8%) in the luspatercept treatment arm discontinued secondary to pain and/or myalgia. In addition, 1 patient (0.4%) in the luspatercept treatment arm discontinued treatment due to drug-induced liver injury.

*Clinical Reviewer Comment: There were an increased number of thrombotic events in the luspatercept treatment arm as compared to placebo, many of which led to study discontinuation. In addition, pain and/or myalgia were other common reasons for discontinuation of the study. Generalized pain, bony pain, and myalgia were seen more frequently in luspatercept-treated patients and also led to study drug discontinuation.*

**Significant Adverse Events**

In total, the number of patients on the luspatercept treatment arm who experienced at least 1 Grade 3 or higher TEAE was 67 (30.0%) versus 18 (16.5%) in the placebo arm.

Among the all-grade TEAEs that were reported by > 5% of subjects in the luspatercept + BSC treatment group, a total of 16 events were Grade 3 or higher. These included the TEAEs of bone pain (3), viral upper respiratory tract infection (1), headache (1), diarrhea (1), hypertension (4), and hyperuricemia (6). There were 4 Grade 4 events of hyperuricemia in the luspatercept arm. There was only 1 Grade 3 or higher event of headache in the placebo arm. Refer to the table on the following page for further details.

*Clinical Reviewer Comment: There was a significantly higher incidence of Grade 3 or higher TEAEs in the luspatercept treatment arm as compared to placebo. The most frequent Grade ≥ 3 events in the luspatercept arm included bone pain, hypertension, and hyperuricemia.*

**Treatment Emergent Adverse Events and Adverse Reactions**

The most common treatment-emergent AEs (all grades) that were reported by >5% of patients in the luspatercept treatment group and at a ≥ 1% higher incidence than in the placebo treatment group were bone pain (19.7% versus 8.3%, respectively), arthralgia (19.3% versus 11.9%, respectively), influenza, viral URI, cough, dizziness, headache, fatigue/asthenia, abdominal pain, nausea, diarrhea, hyperuricemia and hypertension.

Most of the TEAEs reported as of the data cutoff date were Grade 1 or 2 in severity. In both treatment groups, the majority of TEAEs were Grade 2 in severity (52.9% of patients in the luspatercept treatment group and 63.3% of patients in the placebo treatment group). Refer to

the table below for further details.

*Clinical Reviewer Comment: Luspatercept therapy was associated with a significantly higher incidence of bony pain, arthralgia, dizziness, infectious complications (influenza, viral upper respiratory infection), fatigue/asthenia (combined), hypertension, and hyperuricemia as compared to placebo. Health care professionals should consider the side effect profile of luspatercept when considering treatment. While luspatercept demonstrated efficacy in the pivotal BELIEVE trial the benefit versus risk and symptomatic burden of any intervention should be considered.*

**Table 20: Adverse Drug Reactions (>5%) with Treatment Difference of 1% between Treatment Arms**

| **Body System**<br><br>**Adverse Reaction** | **Luspatercept (N=223)** | | **Placebo (N=109)** | |
|---|---|---|---|---|
| | **All Grades**<br><br>**n (%)** | **Grades ≥3**<br><br>**n (%)** | **All Grades**<br><br>**n (%)** | **Grades ≥3**<br><br>**n (%)** |
| **Musculoskeletal and connective tissue disorders** | | | | |
| Bone Pain | 44 (19.7) | 3 (1.3) | 9 (8.3) | 0 (0.0) |
| Arthralgia | 43 (19.2) | 0 (0.0) | 13 (11.9) | 0 (0.0) |
| **Infections and Infestation** | | | | |
| Influenza | 19 (8.5) | 0 (0.0) | 6 (5.5) | 0 (0.0) |
| Viral Upper Respiratory Infection | 14 (6.2) | 1 (0.4) | 2 (1.8) | 0 (0.0) |
| **Nervous System Disorders** | | | | |
| Headache | 58 (26.0) | 1 (0.4) | 26 (23.8) | 1 (0.9) |
| Dizziness | 25 (11.2) | 0 (0.0) | 5 (4.6) | 0 (0.0) |
| **General Disorders and Administration Site Conditions** | | | | |
| Fatigue | 30 (13.4) | 0 (0.0) | 14 (12.8) | 0 (0.0) |
| Asthenia | 22 (9.8) | 0 (0.0) | 11 (10.0) | 0 (0.0) |
| **Gastrointestinal Disorders** | | | | |

| Body System<br><br>Adverse Reaction | Luspatercept (N=223) | | Placebo (N=109) | |
|---|---|---|---|---|
| | All Grades<br><br>n (%) | Grades ≥3<br><br>n (%) | All Grades<br><br>n (%) | Grades ≥3<br><br>n (%) |
| Abdominal Pain | 33 (14.7) | 0 (0.0) | 14 (12.8) | 0 (0.0) |
| Diarrhea | 27 (12.1) | 1 (0.4) | 11 (10.0) | 0 (0.0) |
| Nausea | 20 (8.9) | 0 (0.0) | 6 (5.5) | 0 (0.0) |
| **Vascular disorders** | | | | |
| Hypertension | 18 (8.0) | 4 (1.7) | 3 (2.7) | 0 (0.0) |
| **Metabolism and nutrition disorders** | | | | |
| Hyperuricemia | 16 (7.1) | 6 (2.6) | 0 (0.0) | 0 (0.0) |
| **Respiratory, thoracic and mediastinal disorders** | | | | |
| Cough | 32 (14.3) | 0 (0.0) | 12 (11.0) | 0 (0.0) |

Source: FDA Clinical Reviewer Analysis, JMP Clinical

**Laboratory Findings**

The incidence of patients exceeding predefined threshold for selected laboratory parameters (ALT, AST, direct bilirubin, creatinine clearance, serum creatinine, albumin/creatinine, and leukocytes) was presented in the Sponsor's submission.

Leukocyte Counts
Two patients in the luspatercept group had postbaseline leukocyte counts greater than or equal to 3x baseline the upper limit of normal. One subject had a leukocyte count of 22.53 x 109/L at Dose 14 Day 1. The increase in count was a single transient episode that started at day 10 after TEAE of influenza like illness. One subject had a leukocyte count of 24.24 x 109/L at Dose 5 Day1. Prior to the Dose 5 Day 1, the patient received an intramuscular treatment with betamethasone, vitamin B12 and diclofenac sodium.

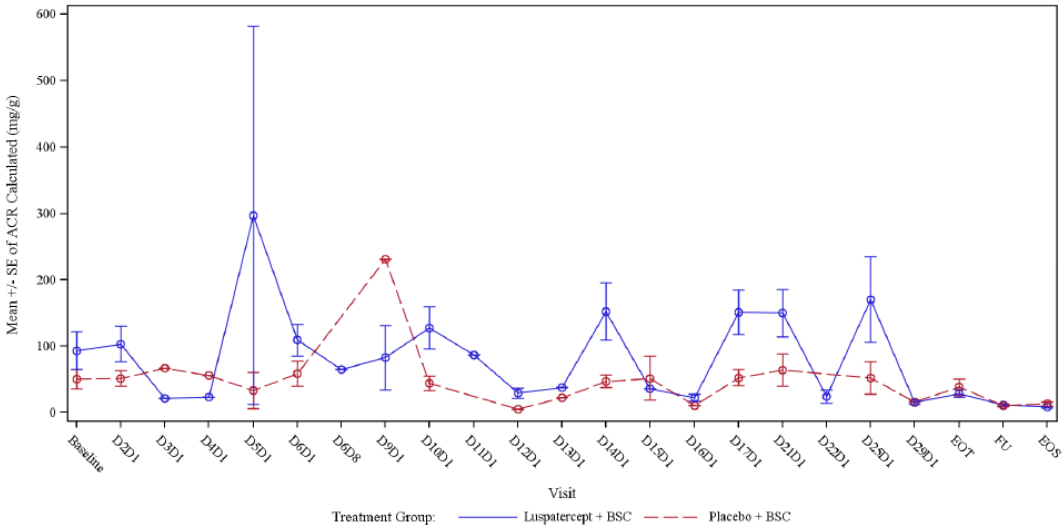
*Reviewer Comment: Both episodes of these elevated leukocyte counts could be explained by concomitant process. There were no grade 3 leukocyte count events.*

The mean albumin/creatinine value at baseline was higher in the luspatercept treatment group

than in the placebo group (93.338 mg/g versus 50.195mg/g, respectively). Overall, there were no sustained increases in mean albumin/creatinine values over time associated with luspatercept and the difference in mean albumin/creatinine values over time was not significant. See attached figure taken from the Clinical Study Report for ACE-536-B-THAL-001 on page 218.

**Figure 3 Mean Values for Albumin/Creatinine**

**Figure 22: Mean Values for Albumin/Creatinine (Urine) Calculated (mg/g) Over Time by Treatment (Safety Population)**




There was an increase uric acid reported in a few subjects in the luspatercept + BSC treatment group as evident from the occurrence of hyperuricemia and blood uric acid increased events. Hyperuricemia was reported in 16 (7.2%) in which 2 patients had Grade 3 events and 4 subjects had Grade 4 hyperuricemia. The events were considered by the investigator to be not related to the study drug. None of the reported events resulted in study drug discontinuation. Grade 3 or higher events of blood uric acid increase were reported in 3 patients with 2 subjects having a Grade 3 event and 1 subject with a Grade 4 event.

One of these occurrences, the patient had a grade 3 event of increased blood uric acid and was a considered a serious TEAE and also had serious TEAE of Grade 3 urine albumin/creatinine ratio which occurred as subject was fasting and dehydration. Both events resulted in study drug discontinuation and considered by investigator to be related to study drug.

The other two incidences (one grade 3 and one grade 4) of blood uric acid increase did not result in study drug discontinuation and were considered by investigator to be not related to study drug.

**Liver Function Tests**

A total of 4 patients in the luspatercept treatment arm and 1 patient in the placebo arm had aspartate aminotransferase values (AST) ≥ 3 x upper limit of normal (ULN) with direct bilirubin values ≥ 2 x ULN. None of these patient's met Hy's law given alternative etiologies for liver dysfunction including concomitant septic shock, cirrhosis and hepatitis C. This included 1 patient with elevated liver enzymes at baseline that did not worsen and 1 patient with a transient increase in AST coinciding with paracetamol treatment for dengue fever.

Aspartate aminotransferase values ≥ 3 x ULN were observed in 11.2% of patients in the luspatercept treatment group and 4.6% of patients in the placebo treatment group at any time postbaseline. Overall, as of the data cutoff date, incidences of aspartate aminotransferase increased were reported as a TEAE in 11 patients (9 in the luspatercept treatment arm and 2 in the placebo arm). None of these AST increases were reported as serious TEAEs, except in 1 patient in the treatment group. In this patient, an approximate increase in AST of 19 x ULN was recorded along with an increase in other hepatic parameters (total bilirubin 13 x ULN, ALT 13 x ULN, and alkaline phosphatase 2 x ULN). A serious TEAE of drug-induced liver injury (DILI) was reported in this patient, which was considered by the investigator to be related to luspatercept and concomitant clarithromycin therapy. Refer to the patient narrative below for further details.

**Patient Narrative** (b) (6)

The subject was a 29-year-old white male with a diagnosis of beta-thalassemia in August of 1987. No prior beta-thalassemia related treatments were reported. The subject had ongoing beta-thalassemia comorbidities including asthenia, extramedullary hematopoiesis (no diagnosis date), clinically significant iron overload (2007), osteopenia, splenectomy, cholecystectomy, and venous thrombosis (2013). The patient also had a history of ongoing glucose-6-phophate dehydrogenase deficiency (no diagnosis date), pancreatitis (no diagnosis date), gastritis, and esophagitis.

The patient received iron chelation therapy at study entry and during study with deferiprone (b) (6) and with deferoxamine mesilate ( (b) (6)

The patient started study treatment with luspatercept on (b) (6) The last dose of study treatment (Dose 19) was administered on (b) (6) (Study Day 428). Total treatment duration for this patient was 487 days. Baseline central labs included leukocytes 22.93 x $10^9$/L, relative nucleated erythrocytes 334%, and platelets 569 x $10^9$/L.

On (b) (6) (Study Day 190), as per the safety report, the patient experienced lower leg discomfort and was later diagnosed with Grade 2 thrombophlebitis superficial. Central analyses (b) (6) prior to the event included leukocytes 18.22 x $10^9$/L, relative nucleated erythrocytes 309%, and platelets 609 x $10^9$/L. On (b) (6) (Study Day 242), the patient

was admitted after a venous duplex ultrasound confirmed a Grade 2 deep vein thrombosis event requiring treatment with low molecular weight heparin and iron chelation. The patient had not been compliant with low molecular weight heparin treatment for superficial thrombophlebitis and the admission was to assure therapy compliance. The patient had received 10 doses prior to the occurrence of the event. The investigator indicated that the subject had multiple risk factors for thrombosis, which included thrombocytosis, sedentary life style, and lack of compliance with low molecular weight heparin. Study treatment (Dose 11) was interrupted due to the event and was administered on (b) (6)

As per the safety report, the patient had experienced upper gastric pain since (b) (6) the patient was diagnosed with Grade 1 gastritis erosive and on (b) (6) (Study Day 392), the patient was diagnosed with Grade 1 Helicobacter infection. There was no change in study dosing due to these events.

Dose 18 (Study Day 407) was interrupted due to investigator's availability and was administered on (b) (6) The delay from the previous dose, Dose 17 (b) (6) was 25 days. The patient's last RBC transfusion prior to Dose 18 occurred on (b) (6) with a pre-transfusion hemoglobin of 9.3 g/dL.

On (b) (6) clarithromycin, amoxicillin, esomeprazole and tinidazole were initiated for the treatment of Helicobacter pylori gastritis. The following day, on (b) (6) (Study Day 434), as per the safety report, the subject experienced significant right upper quadrant (RUQ) abdominal pain and the subject was hospitalized with Grade 3 drug-induced liver injury. Laboratory results showed SGOT 669, SGPT 984, bilirubin 14.0 and alkaline phosphatase 286 (units and normal ranges not reported). An abdominal ultrasound did not reveal any dilation of the biliary system or choledocholithiasis. Treatment for H. pylori was withheld with subsequent improvement noted. The subject recovered 4 days later. Corrective treatment included acetylcysteine, tramadol hydrochloride, and metamizole sodium. On (b) (6) the subject was discharged, and the drug-induced liver injury was considered resolved.

On (b) (6) (Study Day 444), 6 days after being discharged from the hospital, the patient was readmitted with recurrence of severe upper recurrence of severe RUQ abdominal pain, fever of 39.0ºC, and worsening of liver function with cholestasis features. The patient was diagnosed with Grade 3 ascending cholangitis and Grade 3 acute hepatitis. Laboratory results showed worsening liver function tests: SGOT 412, SGPT 500, bilirubin 19.0 and alkaline phosphatase 356 (units and normal ranges were not reported). An abdominal ultrasound showed no change from the previous results. The subject was treated symptomatically with sodium chloride, metamizole sodium, and pantoprazole.

On (b) (6) (Study Day 449), as per the safety report, during the hospitalization, the subject's condition had stabilized but subsequently deteriorated with a fever of 39ºC,

worsening abdominal pain, and laboratory results showing elevated white blood cell count 78,000/µL, liver enzymes of SGOT 169, SGPT 180, bilirubin 24.1 and alkaline phosphatase 334 (units and normal ranges not reported). The subject underwent CT angiography with insertion of a drainage tube followed by a percutaneous transhepatic cholangiography, which confirmed ongoing ascending cholangitis and life-threatening, Grade 4 septic shock. An unspecified bacteriologic examination revealed a systemic infection with Escherichia coli and Klebsiella pneumonia. Corrective treatment included ceftriaxone sodium, metronidazole, oxycodone hydrochloride, and acetylcysteine. The subject's condition improved. On (b) (6) the septic shock was considered resolved. On (b) (6) the patient underwent an endoscopic retrograde cholangiopancreatography and the drainage tube was removed. On (b) (6) the cholangitis and hepatitis acute were considered resolved. The erosive gastritis erosive remained ongoing.

On (b) (6) the patient was discontinued from study treatment due to the drug-induced liver injury. The patient was discharged from the hospital on (b) (6)

*Clinical Reviewer Comment: It cannot be conclusively determined that luspatercept did not have a direct hepatoxic effect in this case of DILI. While the patient was receiving concomitant antibiotics (specifically clarithromycin) which can induce hepatoxicity it is important to note that treatment with antibiotic therapy was initiated only 1 day prior to the observed LFT derangements. The patient was subsequently diagnosed with ascending cholangitis after the event of DILI; it is unclear whether the preceding LFT derangements were related to an evolving intrabiliary infection.*

**Vital Signs**

Descriptive statistics for vital signs (systolic blood pressure, diastolic blood pressure, body temperature, pulse rate, and body weight) including actual values and changes from baseline values by treatment and by visit, were provided by the Sponsor.

Patients in the luspatercept treatment arm were noted to have a higher incidence of hypertension per TEAEs as well as recorded vital signs as compared to patients receiving placebo. Hypertension was reported as a TEAE in 18 (8.1%) subjects in the luspatercept + BSC treatment group and 3 (2.7%) subjects in the placebo + BSC treatment group. Grade 3 hypertension was reported in 4 (1.8%) subjects in the luspatercept + BSC treatment group and no subjects in the placebo + BSC treatment group; no Grade 4, Grade 5, and/or serious TEAEs of hypertension were reported.

An approximate 3- to 6-mm Hg increase in mean SBP and DBP was noted in subjects receiving luspatercept versus placebo in the safety population and in those meeting the primary efficacy endpoint. The increase was much less marked or was not observed at all in several other responder groups including the subjects with ≥ 33% reduction in RBC transfusion burden during the fixed Week 37 to Week 48 interval and during any rolling 12-/24-week interval as well as

the subjects with ≥ 50% reduction in RBC transfusion burden during the fixed and rolling intervals. This observation is clinically important as the responders are more likely to receive long-term therapy with luspatercept.

The following tables describe post-baseline shifts in both SBP and DBP in the safety population.

**Table 21: Incidence of Subjects With Maximum Postbaseline Blood Pressure Change Exceeding Threshold (Safety Population)**

| Maximum Postbaseline Blood Pressure | Number (%) of Subjects | |
|---|---|---|
| | Luspatercept + BSC (N = 223) | Placebo + BSC (N = 109) |
| SBP Increase | | |
| No Increase in SBP | 5 (2.2) | 3 (2.8) |
| SBP Increased < 20 mm Hg | 115 (51.6) | 70 (64.2) |
| SBP Increased ≥ 20 mm Hg | 102 (45.7) | 36 (33.0) |
| SBP Increased ≥ 20 mm Hg and SBP ≥ 140 mm Hg | 27 (12.1) | 6 (5.5) |
| SBP Increased ≥ 20 mm Hg and SBP ≥ 150 mm Hg | 11 (4.9) | 2 (1.8) |
| Subjects With Only Baseline Values | 1 (0.4) | 0 |
| DBP Increase | | |
| No Increase in DBP | 10 (4.5) | 5 (4.6) |
| DBP Increased < 20 mm Hg | 116 (52.0) | 73 (67.0) |
| DBP Increased ≥ 20 mm Hg | 96 (43.0) | 31 (28.4) |
| DBP Increased ≥ 20 mm Hg and DBP ≥ 100 mm Hg | 14 (6.3) | 1 (0.9) |
| Subjects With Only Baseline Values | 1 (0.4) | 0 |

Source: Sponsor generated table, Study Report Body, Section 12.5.1

**Table 22: Systolic Blood Pressure Category - Shift From Baseline to Week 48 (Safety Population)**

| Treatment | SBP Category (mm Hg) | Baseline[a] n | Week 48[b] | | |
|---|---|---|---|---|---|
| | | | 0 - < 130 mm Hg n (%) | 130 - < 140 mm Hg n (%) | ≥ 140 mm Hg n (%) |
| Luspatercept + BSC | 0 - < 130 | 211 | 198 (93.8) | 10 (4.7) | 3 (1.4) |
| | 130 - < 140 | 9 | 9 (100.0) | 0 | 0 |
| | ≥ 140 | 3 | 1 (33.3) | 1 (33.3) | 1 (33.3) |
| | Total | 223 | 208 (93.3) | 11 (4.9) | 4 (1.8) |
| Placebo + BSC | 0 - < 130 | 103 | 102 (99.0) | 1 (1.0) | 0 |
| | 130 - < 140 | 5 | 3 (60.0) | 2 (40.0) | 0 |
| | ≥ 140 | 1 | 1 (100.0) | 0 | 0 |
| | Total | 109 | 106 (97.2) | 3 (2.8) | 0 |

Source: Sponsor generated table, Study Report Body, Section 12.5.1

**Table 23: Diastolic Blood Pressure Category - Shift From Baseline to Week 48 (Safety Population)**

| Treatment | DBP Category (mm Hg) | Baseline[a] n | Week 48[b] | | |
|---|---|---|---|---|---|
| | | | 0 - < 80 mm Hg n (%) | 80 - < 90 mm Hg n (%) | ≥ 90 mm Hg n (%) |
| Luspatercept + BSC | 0 - < 80 | 199 | 166 (83.4) | 27 (13.6) | 6 (3.0) |
| | 80 - < 90 | 23 | 14 (60.9) | 8 (34.8) | 1 (4.3) |
| | ≥ 90 | 1 | 0 | 1 (100.0) | 0 |
| | Total | 223 | 180 (80.7) | 36 (16.1) | 7 (3.1) |
| Placebo + BSC | 0 - < 80 | 102 | 102 (100.0) | 0 | 0 |
| | 80 - < 90 | 6 | 3 (50.0) | 3 (50.0) | 0 |
| | ≥ 90 | 1 | 1 (100.0) | 0 | 0 |
| | Total | 109 | 106 (97.2) | 3 (2.8) | 0 |

Source: Sponsor generated table, Study Report Body, Section 12.5.1

*Clinical Reviewer Comment: Luspatercept treatment was associated with development of hypertension in the pivotal BELIEVE trial. Patients should be counseled regarding this risk prior to initiation of treatment. Blood pressure should be monitored prior to every administration of luspatercept. New-onset hypertension or exacerbations of pre-existing hypertension should be treated with standard anti-hypertensive agents as per accepted societal guidelines. The long-term effects of luspatercept on the vascular system and chronicity of hypertension are unknown.*

**Electrocardiograms (ECGs)**

A 12-lead ECG was performed at baseline, Dose 6 Day 8, and EOT (for applicable patients). In general, there were no apparent clinically relevant mean changes from baseline to the time points assessed for ECG parameters (heart rate, QRS duration, RR interval, PR interval, QT interval, QT interval corrected for heart rate using Bazett's formula [QTcB] interval, and QT interval corrected for heart rate using Fridericia's formula [QTcF] interval) in either treatment arm.

Electrocardiogram abnormalities were reported as TEAEs in 3 patients. One patient in each treatment arm had a TEAE of Grade 2 atrial fibrillation. Both instances of atrial fibrillation were nonserious, did not result in study drug discontinuation, and were considered by the investigator to be not related to study drug. The events resolved within 1 to 2 days of onset. In addition, 1 patient in the placebo arm had a TEAE of Grade 1 electrocardiogram QT prolonged which was nonserious and resolved.

*Clinical Reviewer Comment: The instance of atrial fibrillation in a luspatercept treated patient does not appear related to study drug.*

**QT**

Seven (3.1%) patients (all in the luspatercept treatment arm) had a prolonged QTcF (> 480 msec) during study treatment. Of note, 2 of these 7 patients had a prolonged QTcF at baseline. For 1 patient, the QTcF remained prolonged from baseline to Dose 6 Day 8 (last assessment time point) and for the other patient, the QTcF remained prolonged from baseline to Dose 6 Day 8, but subsequently normalized (< 450 msec) at EOT.

Nineteen (8.5%) patients in the luspatercept treatment arm and 11 (10.1%) subjects in the placebo + BSC treatment arm had ≥ 30 msec increase in QTcF postbaseline.

Four (1.8%) patients in the luspatercept treatment group had a postbaseline QTcF > 480 msec and had QTcF increased by ≥ 60 msec from baseline. No cardiac events were reported in these 4 patients and the ECG overall interpretations for all 4 cases were considered to be normal.

**Immunogenicity**

In the safety population, 220 patients in the luspatercept treatment group and 107 patients in the placebo treatment arm were evaluated for antidrug antibodies (ADAs).

Treatment-emergent ADAs against luspatercept were observed in 4 (1.8%) patients in the luspatercept treatment arm, including 2 (0.9%) patients who developed neutralizing antibodies. In the luspatercept treatment group, the maximum treatment-emergent ADA titer among

patients ranged from 11.5 to 736.0. All 4 patients with treatment-emergent ADA tested positive within 64 days of the first dose, and only 1 patient had positive samples for more than 2 consecutive visits.

*Clinical Reviewer Comment: Luspatercept is associated with development of ADAs. Due to the limited number of ADA-positive patients in this trial, definitive conclusions cannot be drawn regarding the impact of ADAs on occurrence of TEAEs. Healthcare providers should monitor patients receiving luspatercept closely for loss of efficacy and assess for neutralizing ADAs if suspected.*

### 8.2.5 Analysis of Submission-Specific Safety Issues

#### 8.2.5.1 Malignancy

As previously highlighted in this review, toxicology and clinical safety data suggest that luspatercept therapy may be associated development of hematologic or solid tumor malignancy. In a repeat-dose toxicity study, juvenile rats were administered luspatercept at 1, 3, or 10 mg/kg once every 2 weeks from postnatal day 7 to 91; hematologic malignancies (granulocytic leukemia, lymphocytic leukemia, malignant lymphoma) were observed in 3 out of 168 rats examined in the definitive juvenile toxicity study. The occurrence of these tumors in young animals is unusual and of uncertain relationship to luspatercept treatment. Malignancies were observed at 10 mg/kg resulting in exposures (based on area under the curve [AUC]) approximately 8 times the maximum recommended human dose (MRHD) of 1.25 mg/kg. Refer to the non-clinical Toxicology section for further discussion of these findings.
Reports of both hematologic and solid tumor malignancies have been reported in the ongoing pivotal BELIEVE trial. There was 1 case of acute erythroleukemia in a 26 year old male patient receiving luspatercept who subsequently died from complications related to hematologic malignancy; the presentation was atypical for AML and a causal relationship to luspatercept cannot be ruled out. Please refer to Section 8.2.2 for the safety narrative of this case.
In addition, there 1 case of hepatocellular cancer (HCC) in a 56 year old male with beta thalassemia who received luspatercept treatment reported in the 3-month safety follow up after the data cut off. The patient had no prior history of cirrhosis, personal or familial malignancies, hepatitis B or C, hemochromatosis or ingestion of food contaminated with fungal aflatoxins (in subtropical regions). It is unknown if the patient had previous chemotherapy or environmental exposure to atmospheric pollutants or toxic chemicals. While HCC does occur at a higher incidence in the beta thalassemia population as compared to the general public, it typically is in the context of concomitant liver dysfunction such as cirrhosis or inflammatory conditions such as chronic hepatitis.

#### 8.2.5.2 Hepatoxicity

Please refer to Section 8.2.4 for further discussion of hepatotoxicity in the BELIEVE trial.

### 8.2.6 Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

There was no COA analyses performed informing safety and tolerability.

### 8.2.7 Safety Analyses by Demographic Subgroups

Safety analyses by demographic subgroups (age, gender, ethnicity) did not reveal any significant differences from overall safety findings that have already been presented.

Analysis of adverse events by splenectomy status was evaluated. In both treatment groups, the proportion of subjects with treatment emergent adverse events was similar for subjects who had a prior splenectomy and those who did not undergo a splenectomy prior to study entry. In the luspatercept + BSC treatment group, 95% of patients with prior history of splenectomy and 97% of patients with no history of splenectomy reported at least one treatment emergent adverse event. In the placebo + BSC treatment group, 90% of patients with prior history of splenectomy and 96% of patients without a history of splenectomy reported at least one treatment emergent adverse event. More patients who did not have a splenectomy in the luspatercept + BSC group reported arthralgia compared to those who had undergone a splenectomy (27% compared 13%, respectively).

### 8.2.8 Specific Safety Studies/Clinical Trials

### 8.2.9 Additional Safety Explorations

**Human Carcinogenicity or Tumor Development**

Please refer to the Toxicology section (Section 5.5) for further discussion of animal model findings in regard to tumor development and potential for carcinogenicity.

**Human Reproduction and Pregnancy**

Pregnant and lactating women were excluded from the study population and throughout the clinical development program. Female participants of childbearing potential (defined as sexually mature women who had not undergone hysterectomy or bilateral oophorectomy or were not naturally postmenopausal for at least 24 consecutive months) 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 cutoff date, there are no data regarding the clinical effects of luspatercept in pregnancy. There have been no pregnancies reported in female patients participating in

luspatercept clinical studies, and there was 1 pregnancy in the partner of a male patient 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.

**Pediatrics and Assessment of Effects on Growth**

No pediatric patients were enrolled on the clinical studies to support BLA 761136. Therefore, no pediatric assessments were conducted.

**Overdose, Drug Abuse Potential, Withdrawal, and Rebound**

In Study ACE-536-B-THAL-001, a protocol-defined overdose (referring to luspatercept only) was defined as an SC administration of a luspatercept dose at least 10% higher than the protocol-specified dose. In this study, 2 patients received a single dose of luspatercept that was higher than the protocol-specified dose (approximately 17% to 72% higher). No TEAEs were associated with the overdoses. There is no known abuse potential with luspatercept. There are no known withdrawal or rebound issues associated with luspatercept treatment.

### 8.2.10 Safety in the Postmarket Setting

**Safety Concerns Identified Through Postmarket Experience**

Luspatercept is currently not marketed in the U.S. or internationally.

**Expectations on Safety in the Postmarket Setting**

Vigilant safety reporting and follow up are anticipated in the postmarket setting and a PMC has been issued for this application. As highlighted in this review, while luspatercept treatment was effective in reducing RBC transfusion burden, there are concerns regarding development of malignancy on therapy, thrombotic risk, and hepatotoxicity.

### 8.2.11 Integrated Assessment of Safety

A total of 332 subjects randomized to the luspatercept-aamt (N = 223) and placebo (N = 109) treatment arms were included in the safety analysis. The median treatment duration was similar between the luspatercept treatment group (63.3 weeks) and the placebo treatment group (62.1 weeks).

The most frequently common adverse reactions (in ≥ 10% of subjects) in patients with beta-thalassemia treated with luspatercept were headache (26%), bone pain (20%), arthralgia (19%), fatigue (14%), cough (14%), abdominal pain (14%), diarrhea (12%), and dizziness(11%). Grade 3 and 4 treatment-emergent adverse events in the phase 3 trial occurred in 29% of patients in the luspatercept-aamt arm and 14.7% of patients in the placebo arm with the most common being bone pain (1.3%), anemia (3.1%), hyperuricemia (2.7%), and ALT increase (0.9%).

Serious TEAEs were higher in the luspatercept treatment arm (15.2% than in the placebo arm (5.5%). The most frequently reported (in more than 1 patient) serious TEAEs in the luspatercept treatment group were anemia, cellulitis, cerebrovascular accident, cholangitis, deep vein thrombosis, and pyrexia. Serious adverse reactions were reported in 1% of patients receiving luspatercept-aamt and included cerebrovascular accident and deep vein thrombosis. There was 1 death reported in the 3-month safety follow up in a 26-year-old male treated with luspatercept-aamt who developed neutropenic sepsis, pancytopenia and renal failure resulting in death due to an unconfirmed report of development of AML (M6) erythroleukemia.

Permanent discontinuation due to an adverse reaction (Grades 1-4) occurred in 5.4% of patients who received luspatercept-aamt. The most frequent adverse reactions leading to permanent discontinuation in patients who received luspatercept-aamt included arthralgia (1%), bone pain (< 1%) and headache (<1%). Dosage reductions due to an adverse reaction occurred in 2.7% of patients who received luspatercept-aamt and the most frequent adverse reactions requiring dosage reduction in > 0.5% of patients who received luspatercept-aamt included hypertension and headache. Dosage interruptions due to an adverse reaction occurred in 15.2% of patients who received luspatercept-aamt and the most frequent adverse reactions requiring dosage interruption in > 1% of patients included upper respiratory tract infections, ALT increase and cough.

Clinically relevant adverse reactions in < 5% of patients include vertigo/vertigo positional, syncope/presyncope, injection site reactions and hypersensitivity. Safety analyses of liver function tests demonstrated aspartate aminotransferase values ≥ 3 x ULN were observed in 11.2% of patients in the luspatercept treatment group and 4.6% of patients in the placebo treatment group at any time postbaseline. There was 1 serious TEAE of drug-induced liver injury (DILI) reported in a luspatercept treated patient, which was considered by the investigator to be related to luspatercept and concomitant clarithromycin therapy. Inclusion of liver function laboratory abnormalities in section 6 of the USPI is warranted to describe these findings.

Important identified safety findings with the use of luspatercept included thrombosis/thromboembolism and hypertension. In adult patients with beta thalassemia, thromboembolic events were reported in 8/223 (3.6%) treated patients. Reported thromboembolic events included deep vein thromboses, pulmonary embolus, portal vein thrombosis, and ischemic strokes. Patients with known risk factors for thromboembolism may be at further increased risk of thromboembolic conditions. Hypertension was reported in 10.7% (61/571) of luspatercept-aamt treated patients. The incidence of grade 3-4 hypertension ranged from 1.8% to 8.6% and reflects exposure to luspatercept-aamt as a single agent administered across a range of doses (0.125mg/kg to 1.75mg/kg) in 571 patients. In adult patients with beta thalassemia with normal baseline blood pressure, 13 (6.2%) patients developed systolic blood pressure (SBP)> 130mm Hg and 33 (16.6%) patients developed diastolic blood pressure (DBP) > 80mmg Hg.

In non-clinical data there appears to be a safety signal for carcinogenicity and renal toxicity. At all dose levels lower F1 pup body weights and adverse kidney findings such as membranoproliferative glomerulonephritis, tubular atrophy/hypoplasia and vessel ectasia) were observed. In addition, in repeat-dose toxicity studies, juvenile rats developed hematological malignancies at the 10mg/kg resulting in exposures (based on area under the curve) approximately 8 times the maximum recommended human dose of 1.25mg/kg. The mean urinary albumin/creatinine values at baseline and throughout the 48 week treatment period remained < 200mg/g which is in the lower microalbuminuria range albeit with periodic but transient fluctuations. There were decreases in renal function that were transient observed in the luspatercept-aamt group and generally occurred in patients with known risk factors for kidney injury. As luspatercept may be administered long-term to patients with beta-thalassemia the long-term safety and follow-up reports of ongoing studies will be important to assess the long-term safety of this product.

As with all therapeutic proteins, there is a potential for immunogenicity. Of the 284 patients with beta thalassemia treated with luspatercept-aamt and evaluable for the presence of anti-luspatercept aamt antibodies, 4 patients (1.4%) tested positive for treatment emergent anti-luspatercept-aamt antibodies including 2 (0.7%) who had neutralizing antibodies. There were no severe acute systemic hypersensitivity reactions reported for patients with anti-luspatercept-aamt antibodies in clinical trials and no association between hypersensitivity type reaction or injection site reaction and presence of anti-luspatercept-aamt antibodies.

## 8.3 Statistical Issues

The demographic and baseline characteristics of the two treatment arms are balanced. Patients in the luspatercept arm showed improvement over the placebo arm based on the primary and secondary endpoints pre-specified in the protocol. No outlier were observed in the subgroup analyses for the primary and key secondary endpoints.

## 8.4 Conclusions and Recommendations

In summary, patients with beta thalassemia who require regular transfusions have no available therapy and remains an area of high unmet medical need. Transfusions are a key component of the treatment of beta thalassemia however regular transfusions lead to iron overload in a disease with ineffective erythropoiesis. A reduction of RBC units that a patient receives is an important and clinically meaningful endpoint. The efficacy results from Study ACE-536-B-Thal-001 (BELIEVE Study) support the conclusion of effectiveness for luspatercept-aamt for the treatment of anemia in patients with beta thalassemia who require regular transfusions. The demonstration of a ≥33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units for 12 consecutive weeks (week 13- week 24) response rate of 21.4% (48/224) in the luspatercept + BSC group compared to 4.5% (5/112) in the placebo + BSC group ($p < 0.0001$) and response rate of 44% (19.6) for luspatercept-aamt + BSC compared to 4% (3.6) for

the placebo arm during Weeks 37 to 48 provides meaningful clinical benefit for patients with beta-thalassemia who require regular transfusions. The subgroup analysis demonstrated a consistent treatment effect for luspatercept-aamt across all the subgroups, albeit this is a descriptive finding only.

The overall efficacy and safety findings from the BELIEVE trial in patients with beta thalassemia who require red blood cell transfusions support regulatory approval for luspatercept-aamt for this indication.

Weishi (Vivian) Yuan, PhD
Primary Statistical Reviewer

Yeh-Fong Chen, PhD
Statistical Team Leader

Laurel Menapace, MD
Primary Clinical Reviewer

Tanya Wroblewski, MD
Clinical Team Leader

# 9 Advisory Committee Meeting and Other External Consultations

The content of this BLA was not presented at an Advisory Committee Meeting or to any external consultants.

# 10 Pediatrics

The safety and effectiveness of luspatercept in pediatric patients has not been evaluated. The applicant has orphan drug status for luspatercept for the indication of the treatment of beta thalassemia associated anemia. Hence, a pediatric assessment is not required for this application.

# 11 Labeling Recommendations

## 11.2 Prescription Drug Labeling

The following table provides a high level summary of changes made to the luspatercept prescription drug label.

**Table 24: Prescription Drug Labeling Summary of Changes**

| Section | Approved Labeling |
|---|---|
| Indications and Usage | Luspatercept described as an erythroid maturation agent |
| Indications and Usage | Indication reworded to "Reblozyl is indicated for the treatment of anemia in adult patients with beta thalassemia who require regular red blood cell (RBC) transfusions". |
| Warnings and Precautions | Included Warnings for Thrombosis/Thromboembolism, Hypertension, and Embryo-Fetal Toxicity. |
| Warnings and Precautions | Revised warning for thromboembolism to avoid exculpatory language. |
| Warnings and Precautions | Embryo-Fetal toxicity warning added to describe the risk of fetal harm. |
| Adverse Reactions | Revised/reordered section per current labeling approach for NMEs. Added sentence describing the patient population used to calculate rates in the W&P section as 2nd paragraph. Limited Adverse Reactions safety analysis to the Believe trial because placebo-controlled, randomized trials are the best source of safety data (per Adverse Reactions Guidance). Added description of serious adverse reactions, permanent discontinuations, dosage reductions, and dosage interruptions for the treatment arm only. AR table revised to limit ARs to events that occurred in more than 5% of Reblozyl-treated patients and at least 1% more frequently than the placebo arm (per Adverse Reactions guidance). |
| Clinical Trials Experience in Beta Thalassemia | Descriptive data regarding patient population demographics, drug exposure, adverse events and drug discontinuations/interruptions/reductions added. |
| Clinical Trials Experience in Beta Thalassemia | Table describing common TEAEs revised to add viral upper respiratory tract infection, cough, and abdominal pain as adverse reactions. |

| | |
|---|---|
| Clinical Trials Experience in Beta Thalassemia | Sponsor asked to combine TEAE terms of "fatigue" and "asthenia" for Common Adverse Reactions table to avoid splitting. Revised table formatting and footnotes to be consistent with current format for NMEs. |
| Immunogenicity | Text describing development of ADA and immunogenicity revised to be consistent with Biosimilar Product labeling guidance (also relevant to non-biosimilar biologics). |
| Use in Specific Populations | Revised sections 8.1-8.3 to be consistent with PLLR Guidance and Final Rule and internal PLLR Template (provided by DPMH).<br><br>Section 8.4 revised to reflect juvenile study findings implications for pediatric use.<br><br>Section 8.5 revised to reflect regulation requirements when inadequate numbers of geriatric patients treated. |
| Clinical Pharmacology | Revisions made to be consistent with Clinical Pharmacology Labeling Guidance. |

Virginia Kwitkowski, MS, ACNP-BC
Associate Director for Labeling

# 12 Risk Evaluation and Mitigation Strategies (REMS)

No safety issues were identified during the review of this application that warranted a REMS.

# 13 Postmarketing Requirements and Commitment

The following postmarketing requirements (PMRs) and commitments (PMCs) were issued for BLA 761136 Original 1:

**PMR-1**
Complete Study A536-06: An Open-Label Extension Study to Evaluate the Effects of ACE-536 in Patients with Beta-Thalassemia. Include all the patients from Study A536-04 who enrolled into the open-label extension study (A536-06). Include summary analysis and updated safety summary to include safety data on thrombosis, all malignancies (including AML), hepatic toxicity, and renal toxicity. Include updated assessments of ferritin levels, liver iron concentrations and use of chelators evaluations in the reports. Include updated safety and efficacy analysis and submit datasets at the time of final clinical study report submission.

| | | |
|---|---|---|
| PMR Desired Milestones: | Trial Completion: | 04/2020 |
| | Final Report Submission: | 10/2020 |

**PMR-2**
Complete Trial ACE-536-B-THAL-001: Phase 3, Randomized, Placebo-controlled, Multicenter Study to Determine the Efficacy and Safety of Luspatercept (ACE-536) in Adults who Require Regular Blood Transfusions. Evaluate safety and efficacy data for the patients who enrolled in the open-label treatment for up to five years. Include summary analysis and updated safety summary to include safety data on thrombosis, all malignancies (including AML), hepatic toxicity, and renal toxicity. Include updated assessments of ferritin levels, liver iron concentrations and use of chelators and any cardiac iron evaluations in the reports. Include updated safety and efficacy analysis and submit datasets at the time of final clinical study report submission.

| | | |
|---|---|---|
| PMR Desired Milestones: | Trial Completion: | 06/2020 |
| | Final Report Submission: | 12/2020 |

**PMR-3**
Complete Trial ACE-536-B-THAL-002: A Phase 2, Double-Blind, Randomized, Placebo-Controlled, Multicenter Study to Determine the Efficacy and Safety of Luspatercept (ACE-536) versus Placebo in Adults with non-Transfusion Dependent Beta (B) Thalassemia. Include summary analysis and updated safety summary to include safety data on thrombosis, all malignancies (including AML), hepatic toxicity, and renal toxicity. Include updated assessments of ferritin levels, liver iron concentrations and use of chelators and any cardiac iron evaluations in the reports. Include updated safety and efficacy analysis and submit datasets at the time of final clinical study report submission.

| | | |
|---|---|---|
| PMR Desired Milestones: | Interim Report (Preliminary Study Report): | 09/2021 |
| | Trial Completion | 03/2023 |
| | Final Report Submission: | 09/2023 |

**PMR-4**
Conduct an assessment of cases of secondary primary malignancies (and malignancies for B-thalassemia), to include hematological malignancies (AML, de-novo AML, transformation to AML), and solid tumors identified in sponsor-initiated and investigator-initiated clinical trials across the entire luspatercept development program for 5 years post approval.

PMR Desired Milestones

| | |
|---|---|
| Interim Report #1 (Annual Summary Safety Report) Submission: | 12/2020 |
| Interim Report #2 (Annual Summary Safety Report) Submission: | 12/2021 |
| Interim Report #3 (Annual Summary Safety Report) Submission: | 12/2022 |
| Interim Report #4 (Annual Summary Safety Report) Submission: | 12/2023 |
| Trial Completion: | 12/2023 |
| Final Report Submission: | 12/2024 |

**PMC-5**
Implement a test monitoring [redacted] (b) (4) oxidation and [redacted] (b) (4) isomerization into the luspatercept drug substance release and stability specification and drug product release specification. Submit the proposed release specification and supporting validation results following 21 CFR 601.12 (b).

| | | |
|---|---|---|
| PMC Desired Milestones: | Final Report Submission: | 12/2020 |

# 14 Division Director (DHOT) or designee

Haleh Saber, PhD
Deputy Director

# 15 Division Director (OCP)

Nam Atiqur Rahman, PhD
Division Director (OCP)

# 16 Division Director (OB) or designee

Thomas Gwise, PhD
Deputy Director (OB)

# 17 Division Director (Clinical) or designee Comments

(This Section depend in part on the reviews of the CDTL (Dr. Tanya Wroblewski) and Drs. Laurel Menapace and Weishi (Vivian) Yuan, PhD)

**Background**: On April 4, 2019, Celgene submitted BLA 761136, in which approval of luspatercept-aamt (Reblozyl) was requested for the treatment of "adult patients with beta-thalassemia associated anemia who require red blood cell (RBC) transfusions." Luspatercept-aamt is a fusion protein comprised of a sub-unit of the Activin IIB receptor (ActRIIB) linked to the human IgG1 Fc domain. This fusion protein binds to the natural ligand(s) for the ActRIIB receptor (GDF8, GDF11, BMP6, and activin B) and thereby prevents these ligands from binding to the Activin IIB receptor. Since prevention of engagement of the Activin IIB receptor by these ligands results in improvement of hematological parameters in a mouse model of ineffective erythropoiesis, one can predict that luspatercept-aamt will have a similar effect on the hematological profile of ineffective erythropoiesis in patients with transfusion dependent beta-thalassemia.

The request for approval of the Sponsor's proposed indication relied upon Study ACE-536-B-Thal-001 (the Believe trial) which was a Phase 3, double blind trial which randomized 336 patients with beta-thalassemia or HgbE/B-thalassemia between best supportive care (BSC) with placebo (n=112) vs BSC with luspatercept-aamt (n=224). These patients had a baseline transfusion of 6-20 units of RBC in the 24 weeks prior to the randomization. BSC was defined as iron-chelating agents, use of antibiotic, antiviral and antifungal therapy, nutritional support, and RBC transfusions as prespecified in the SAP. The primary endpoint was the proportion of patients on each arm exhibiting a ≥33% reduction from baseline of the RBC transfusion burden as well as a reduction of at least 2 units of RBCs administered from week 13 to week 24.

**Efficacy Results**: The Ace-536-B-Thal-001 (Believe) trial showed a statistically significant increase in the proportion of patients (21.4%) on the experimental arm which exhibited a ≥33% reduction from baseline in the RBC transfusion burden with a reduction of at least 2 units from week 13 to week 24 on the luspatercept-aamt + BSC arm (48/224), as compared to the percentage (4.5%) of patients in the placebo + BSC arm ($p<0.0001$). This response profile was durable and stable over the period of 37-48 weeks (19.6% or 44/224) in the luspatercept-aamt + BSC arm vs the placebo + BSC arm (3.6% or 4/112).

**Safety Results:** One patient on the luspatercept-aamt arm who was a 26 year old male was treated with induction chemotherapy for AML (7+3) for a diagnosis of M6 erythroleukemia. The patient presented with febrile neutropenia and hypotension following which the patient was admitted in shock to the ICU. Because of the discovery of 33% blasts in the peripheral blood, the patient was given 1 course of induction chemotherapy (7 days of cytarabine with 3 days daunomycin). The patient improved sufficiently to be discharged from the unit but later the leukemia recurred following which the patient deteriorated and died. Although the AML was never completely documented by cytogenetic or mutational analysis on the bone marrow, the occurrence of this problem raises concern given the proposed mechanism of action of luspatercept-aamt and the plan for long term therapy in responders.

Safety findings on the luspatercept-aamt arm included thromboembolic events in 3.6% (8/223) and hypertension in 10.7% (61/671). Grade 3-4 hypertension over 4 trials was 1.8%-8.6%. Four of the 284 patients treated with luspatercept-aamt and evaluable for the presence of antibodies were positive for anti-drug antibodies of whom 2 (0.7%) had neutralizing antibodies. There were no severe systemic hypersensitivity reactions reported. Although the safety profile was favorable, the follow up is short and the therapy plan is long term in responders.

**Benefit-Risk Discussion:** The benefits are significant and the toxicity manageable but the follow-up is short.

**Regulatory Recommendation of Albert Deisseroth:** Approval with a PMR for long term safety follow-up.

Albert Deisseroth, MD, PhD
Supervisory Associate Division Director
Division of Hematology Products (DHP)

# 18 Office Director (or designated signatory authority) Comments

*This application was reviewed by the Oncology Center of Excellence (OCE) per the OCE Intercenter Agreement. My signature below represents an approval recommendation for the clinical portion of this application under the OCE.*

Richard Pazdur, MD
Officer Director
Office of Hematology and Oncology Products (OHOP)

# 19 Appendices

## 19.2 References

Cappellini MD, Cohen A, Piga A, Bejaoui M, Perrotta S, Agaoglu L, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood. 2006;107(9):3455-62.

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## 19.3 Financial Disclosure

**Covered Clinical Study (Name and/or Number):** BELIEVE Trial (ACE-536-BTHAL-001)

| | | |
|---|---|---|
| Was a list of clinical investigators provided: | Yes ☒ | No ☐ (Request list from Applicant) |
| Total number of investigators identified: 279 (Principal Investigators and Sub-Investigators) | | |

| | | |
|---|---|---|
| Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0 | | |
| Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 1 | | |
| If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)):<br>Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 1<br>Significant payments of other sorts: 0<br>Proprietary interest in the product tested held by investigator: 0<br>Significant equity interest held by investigator in Sponsor: 0<br>Sponsor of covered study: 0 | | |
| Is an attachment provided with details of the disclosable financial interests/arrangements: | Yes ☒ | No ☐ (Request details from Applicant) |
| Is a description of the steps taken to minimize potential bias provided: | Yes ☒ | No ☐ (Request information from Applicant) |
| Number of investigators with certification of due diligence (Form FDA 3454, box 3) 0 | | |
| Is an attachment provided with the reason: | Yes ☐ | No ☐ (Request explanation from Applicant) |

## 19.4 Nonclinical Pharmacology/Toxicology

None

## 19.5 OCP Appendices (Technical documents supporting OCP recommendations)

### 19.5.1 Summary of Bioanalytical Method Validation and Performance

***Bioanalytical Method for Detection of Luspatercept***

Bioanalytical methods for the quantitative determination of luspatercept in human serum were developed and validated using an enzyme-linked immunosorbent assay (ELISA). The assay is designed to measure the total luspatercept in serum, with goat polyclonal anti-luspatercept

antibody (against the modified ECD of human ActRIIB) as the capture reagent. Table 19-1 summarizes the method performance parameters during method validation. The same method has been used to determine luspatercept serum concentrations in all clinical studies. The validation of the method and sample analysis were conducted in compliance with the appropriate regulations in place at the time of execution.

**Table 19-1. Performance parameters for luspatercept during method validation.**

| Type of assay | Enzyme linked immunosorbent assay | |
|---|---|---|
| Analytical facility | (b) (4) | |
| Capture reagent | Goat polyclonal anti-luspatercept antibody | |
| Detection reagent | Sheep polyclonal anti-human IgG1 HRP and TMB peroxidase substrate | |
| QC sample concentration | 50, 150, 250, 450, and 600 ng/mL, in 100% serum | |
| Calibration range | 50 to 600 ng/mL, in 100% serum | |
| Minimum dilution ratio | 1:20 | |
| Dilution integrity | 16000-fold dilution from 1 mg/mL | |
| Matrix effect | 9/10 normal lots met criteria; 9/10 disease (MDS) lots met criteria | |
| Precision | Inter-assay ≤ 12.0 % CV. Intra-assay ≤ 8.08 % CV. | |
| Accuracy | Inter-assay -2.33 to 7.60 % RE. Intra-assay -2.35 to 7.62 % RE. | |
| Stability (in serum) | Ambient temperature | Stable for at least 20.25 hours |
| | Freeze/thaw at -70ºC | Stable for at least 4 freeze/thaw cycles |
| | Long-term at -20ºC | Stable for at least 386 days |
| | Long-term at -70ºC | Stable for at least 1492 days |

%CV = % coefficient of variation; % RE = % relative error; HRP = horseradish peroxidase; MDS = myelodysplastic syndromes; TMB = tetramethylbenzidine; QC = quality control.
Source: EDR 2.7.1 Table 3.

***Bioanalytical Method for Detection of Anti-Drug Binding and Neutralizing Antibodies to Luspatercept***

Detection of ADAs

Bioanalytical methods for the quantitative determination of luspatercept binding ADA in human serum were developed and validated using an electrochemiluminescence (ECL) immunoassay. The method performance parameters during method validation are summarized in Table 19-2. Detection of ADA was based on the bivalent characteristics of the antibody. Anti-drug antibodies, if present, would form a “bridge” between the luspatercept coating on the plate and the biotinylated luspatercept added, and subsequently detected through addition of streptavidin-sulfotag binding to the biotin domain. Goat polyclonal anti-human ActRIIB antibody (directed against the ECD of human ActRIIB) was used as a positive control. The final confirmation of ADA positive was evaluated via immune-competition with luspatercept, ACE-536-his, and natural ECD of human ActRIIB.

**Table 19-2. Performance parameters for binding ADA assay during method validation.**

| Type of Assay | Electrochemiluminescent Immunoassay | |
|---|---|---|
| Analytical facility | (b) (4) | |
| Positive control (PC) | Goat polyclonal anti-human ActRIIB antibody | |
| Specificity test | Luspatercept | |
| | Modified ECD of human ActRIIB (receptor portion of luspatercept) | |
| | Natural ECD of human ActRIIB (cross reactivity) | |
| Minimum dilution ratio | 1:11.5 (including acidification step) | |
| QC sample concentration | 0.5 and 16 µg/mL, in 100% serum | |
| Cut point | Screening | 1.40 (multiplicative cut point factor) |
| | Confirmation with luspatercept [a] | 36.9% inhibition |
| | Specificity with ACE-536-His [a] | 32.3% inhibition |
| | Specificity with ActRIIB-ECD [b] | 26.8% inhibition |
| Sensitivity | 26.1 to 167 ng/mL PC, in 100% serum [c] | |
| Precision | Inter-assay ≤ 26.9 % CV. Intra-assay ≤ 4.10 % CV. | |
| Free drug interference | At 0.5 µg/mL PC | > 0.1 to > 1 µg/mL [d] |
| | At 16 µg/mL PC | > 10 to > 50 µg/mL [e] |

ACE-536-His = histidine-tagged modified ECD of human ActRIIB; ActRIIB = activin receptor type IIB; ActRIIB-ECD = natural ECD of human ActRIIB; CV% = % coefficient of variation; ECD = extracellular domain; PC = positive control; QC = quality control.
[a] Value derived from original report. Subsequent values from addendums differ but all values meet acceptance criteria.
[b] Value derived from Report 177374.
[c] 26.1 to 41.7 ng/mL in addendums 2 and 3 with multiple reagent lot changes, which have been used for all Phase 2 and Phase 3 studies.
[d] > 1 µg/mL in addendums 2 and 3 with multiple reagent lot changes, which have been used for all phase 2 and 3 studies.
[e] > 25 to 50 µg/mL in addendums 2 and 3 with multiple reagent lot changes, which have been used for all phase 2 and 3 studies.
Source: Report 174275 and addendums 1 to 3 (Report 177374, Report 180010, Report 183554).

Source: EDR 2.7.1 Table 11.

### Detection of NAbs

Confirmed ADA-positive serum samples were further evaluated in a neutralization assay to assess the ability to interfere with the luspatercept-ligand interaction by a validated ELISA method. This assay was designed to detect neutralizing antibodies (NAb) which bind to immobilized luspatercept and thereby block binding of biotinylated GDF11 to the drug. This method used chicken polyclonal anti-luspatercept antibody (directed against the modified ECD of human ActRIIB) as the positive control (Report #176678).

Serum samples identified as positive for cross-reactivity to the natural ECD of human ActRIIB in the specificity test were also examined in a second validated ELISA-based NAb assay. The second NAb assay was designed to detect NAb which binds to immobilized human ActRIIB-ECD-Fc fusion proteins (ACE-031) and thereby blocks binding of biotinylated activin A to the natural ECD of the ActRIIB-ECD-Fc protein, with mouse anti-ActRIIB monoclonal antibody (directed against ECD of human ActRIIB) as the positive control (Report #207-1001).

**Table 19-3. Performance parameters for neutralizing ADA assay during method validation.**

| Validation report | 176678 | 207-1001 |
|---|---|---|
| Type of assay | ELISA | ELISA |
| Analytical facility | [redacted] (b) (4) | |
| Analyte | Neutralizing luspatercept antibodies | Neutralizing AcRIIB-ECD-Fc antibodies (cross reactivity) |
| Positive control (PC) | Chicken polyclonal anti-luspatercept antibody | Mouse monoclonal anti-human ActRIIB antibody |
| Coated reagent | Luspatercept | ActRIIB-ECD-Fc fusion protein |
| Binding ligand | Biotinylated GDF11 | Biotinylated activin A |
| Minimum dilution ratio | 1:10 | 1:10 |
| QC sample concentration | 15 and 100 µg/mL, in 100% serum | 2.5, 6.25, 10, and 20 µg/mL in 100% serum |
| Cut point | 0.952 (multiplicative cut point factor) | 14.77 % inhibition |
| Sensitivity | 912 ng/mL PC, in 100% serum | 3681 ng/mL PC, in 100% serum |
| Precision | Inter-assay: ≤ 27.1 % CV | Inter-assay: ≤ 13.8 % CV [a] |
| | Intra-assay: ≤ 9.29 % CV | Intra-assay: ≤ 16.3 % CV [a] |
| Free drug interference | At 15 µg/mL PC: ≥ 0.01 µg/mL [b] | At 6.25 µg/mL PC: ≥ 50 µg/mL [c] |
| | At 100 µg/mL PC: ≥ 0.01 µg/mL [b] | |

ActRIIB = activin receptor type IIB; ActRIIB-ECD-Fc = a fusion protein joining the ECD of human ActRIIB to Fc portion of human Ig G; CV% = % coefficient of variation; ECD = extracellular domain; ELISA = enzyme linked immunosorbent assay; NAb = neutralizing antibodies; PC= positive control; QC = quality control.

[a] Results from positive control 2.5 µg/mg are excluded because it fails to meet the inter-assay precision %CV acceptance criteria of ≤ 30%.

[b] Presence of luspatercept as low as 0.01 µg/mL were found to interfere with the assay (reducing positivity signal). However, the overall response remains positive for the positive controls even in the presence of 100 µg/mL of luspatercept.

[c] Free drug interference for ACE-536 was done in Study ACE-536-B-Thal-001 (Report 155-1809).

Source: Report 176678, Report 207-1001.

Source: EDR 2.7.1 Table 12.

### 19.5.2 Clinical PK/PD and Immunogenicity Assessments

**PK Assessment**

Blood samples were collected for characterization of luspatercept PK in patients with β-thalassemia, including Phase 2 Studies A536-04 and A536-06, and Phase 3 Study ACE-536-B-THAL-001. Table 19-4 summarizes the clinical studies with the dosing regimen, drug product, and visits for PK sampling:

**Table 19-4. Summary of clinical studies in patients with β-thalassemia.**

| Study number (Cutoff date for interim report) | Dose regimen and drug product | Visit for PK sampling | No. of subjects included |
|---|---|---|---|
| **A536-04:** A Phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 in patients with β-thalassemia. | Dose escalation cohorts:<br>0.2, 0.4, 0.6, 0.8, 1, and 1.25 mg/kg, SC, Q3W.<br>Expansion cohort:<br>Starting dose = 0.8 mg/kg, SC, Q3W, with intra-subject dose escalation to 1 and 1.25 mg/kg allowed.<br>Drug product:<br>25 mg frozen liquid (Process I/II drug substance). | C1D1, C1D8, C1D11, C1D15, C2D1, C2D8, C4D1, C4D8, C4D15, C5D1, C5D8, C5D15, EOT, and EOS. | 64 |
| **A536-06:** An open-label extension study to evaluate the long-term effects of ACE-536 in patients with β-thalassemia previously enrolled in study A536-04.<br>(31 Aug 2017) | Subjects without treatment interruption:<br>Starting dose was the same as their last dose in Study A536-04, with intra-subject dose escalation to 1 and 1.25 mg/kg allowed.<br>Subjects with treatment interruption:<br>Starting dose = 0.8 mg/kg, SC, Q3W, with intra-subject dose escalation to 1 and 1.25 mg/kg allowed.<br>Drug product:<br>Switched from 25 mg frozen liquid to 50 mg lyophilized powder (Process II) on a site-by-site base. | C1D1, C1D8, C2D1, C9D1, C9D8, C10D1, C17D1, and C17D8.<br>Also collected in parallel with ADA. | 51 |
| **ACE-536-B-THAL-001:** A Phase 3, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of luspatercept (ACE-536) versus placebo in adults who require regular red blood cell transfusions due to beta (β)-thalassemia.<br>(11 May 2018) | Starting dose = 1 mg/kg, SC, Q3W, with intra-subject dose escalation to 1.25 mg/kg allowed.<br>Drug product:<br>25 and 75 mg lyophilized powder (Process III drug substance). | On treatment: C1D1, C2D1, C3D1, C4D1, C5D1, C6D1, C6D8, C6D15, C8D1, C10D1, C12D1, C14D1, C16D1; then, once every 6 doses. | Active: 221<br>Placebo: 109 |

ADA = anti-drug antibodies; C = cycle (or dose); D = day; EOS = end of study; EOT = end of treatment; PK = pharmacokinetics; Q3W = once every three weeks; SC = subcutaneous injection.

Source: EDR 5.3.3.5 ACE-536-MPK-001 CSR Table 2.

**In Study A536-04, noncompartmental PK analysis was conducted to describe individual luspatercept serum concentration-time profiles following the first dose (Table 19-5). Results show that increase in mean $C_{max}$ and AUC from time zero to 21 days ($AUC_{0-21d}$) was approximately proportional to dose from 0.2 to 1.25 mg/kg, and $C_{max}$ was observed at approximately 7 days. Moreover, a preliminary one-compartment PK model was utilized with first-order absorption and elimination to describe the individual luspatercept serum concentration-time profiles upon multiple dosing for all dose levels (**

Table 19-6). Results show that steady state was reached after 3 doses. Increases of both AUC at steady state ($AUC_{ss}$) and $C_{max,ss}$ were approximately proportional to dose from 0.2 to 1.25 mg/kg. The interindividual variability (IIV) of $AUC_{ss}$ was approximately 36% based on data from the expansion cohort (N = 29).

**Table 19-5. Summary of noncompartmental PK parameters following first dose of luspatercept in Study A536-04.**

| Starting Dose (mg/kg) | N | $C_{max}$ (µg/mL) | $T_{max}$ (day) | $AUC_{0-21d}$ (day•µg/mL) |
|---|---|---|---|---|
| 0.2 | 6 | 0.84(20.0) | 7 (7-10) | 11.7 (20.5) |
| 0.4 | 6 | 1.52 (21.9) | 7 (7-9) | 20.7 (22.8) |
| 0.6 | 6 | 2.47 (9.51) | 7.5 (6-10) | 31.4 (12.2) |
| 0.8 | 6 | 4.04 (27.5) | 7 (6-10) | 54.3 (26.7) |
| 1.0 | 6 | 5.73 (29.9) | 7 (6-7) | 70.4 (34.8) |
| 1.25 | 5 | 6.85 (21.4) | 7(6-8) | 93.8 (23.8) |
| Expansion [a] | 29 | 4.78 (23.6) | 7 (6-10) | 62.7 (29.6) |

AUC = area under the concentration-time curve; $AUC_{0-21d}$ = AUC from time zero to 21 days; $C_{max}$ = maximum concentration observed in first treatment cycle; N = number of subjects; $T_{max}$ = time to reach $C_{max}$.
[a] Starting dose = 0.8 mg/kg.
Median (minimum – maximum) data are presented for $T_{max}$ and geometric mean (geometric CV%) data are presented for other parameters.
Source: Report A536-04, Table 14.2.15.

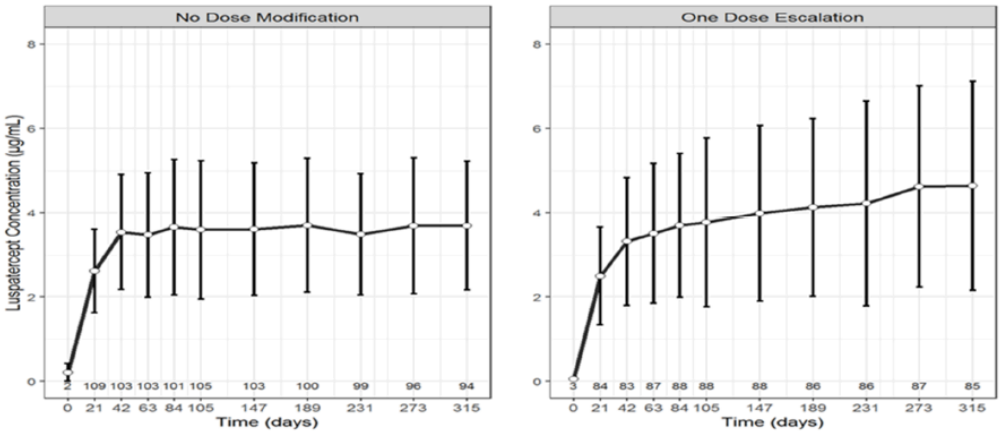
**Table 19-6. Summary of one-compartmental PK parameters following repeated doses of luspatercept in Study A536-04.**

| Starting Dose (mg/kg) | n | $K_a$ (1/day) | $C_{max}$ (µg/mL) | $T_{max}$ (day) | $AUC_{ss}$ (day•µg/mL) | $t_{1/2}$ (day) | CL/F (L/day) | V1/F (L) |
|---|---|---|---|---|---|---|---|---|
| 0.2 | 6 | 0.39 (141) | 0.94 (17.0) | 6 (2-9) | 19.5 (23.3) | 8.62 (43.4) | 0.659 (34.8) | 8.19 (40.7) |
| 0.4 | 6 | 0.34 (78.6) | 1.62 (32.0) | 6 (3-10) | 35.4 (35.6) | 9.58 (39.7) | 0.726 (30.9) | 10.0 (27.1) |
| 0.6 | 6 | 0.84 (86.1) | 3.10 (20.8) | 4 (2-6) | 66.2 (24.2) | 11.9 (34.9) | 0.581 (26.2) | 9.97 (12.5) |
| 0.8 | 6 | 0.73 (128) | 5.09 (23.7) | 4 (2-7) | 96.3 (27.1) | 9.73 (17.4) | 0.492 (28.5) | 6.91 (30.3) |
| 1.0 | 6 | 0.62 (93.4) | 6.60 (26.4) | 4 (2-7) | 117 (35.1) | 8.76 (27.0) | 0.589 (29.2) | 7.44 (29.1) |
| 1.25 | 5 | 0.91 (174) | 8.82 (26.8) | 2 (2-7) | 186 (42.1) | 10.7 (62.0) | 0.398 (51.7) | 6.13 (50.5) |
| Expansion [a] | 29 | 0.79 (94.5) | 5.79 (21.8) | 4 (2-7) | 115 (36.4) | 10.5 (36.7) | 0.445 (38.0) | 6.72 (20.4) |

$AUC_{ss}$ = area under the concentration-time curve at steady state for the starting dose; $C_{max}$ = maximum concentration for the starting dose; CL/F = apparent clearance; CV = coefficient of variation; $K_a$ = absorption rate constant; N = number of subjects; $t_{1/2}$ = elimination half-life; $T_{max}$ = time to reach $C_{max}$; V1/F = apparent volume of distribution of the central compartment.
[a] Starting dose = 0.8 mg/kg.
Median (minimum - maximum) data are presented for $T_{max}$ and geometric mean (geometric CV%) data are presented for other parameters.
Source: Report A536-04, Table 14.2.16.

The overall PK characteristics of luspatercept was assessed by population PK methodology with data from **Study ACE-536-B-THAL-001** in combination with data from Study A536-04. In Study ACE-536-B-THAL-001, observed data shows that in patients remaining on 1 mg/kg, mean $C_{trough}$ was stable from Day 42 to more than 300 days; in patients with dose escalation to 1.25 mg/kg, mean $C_{trough}$ increased by approximately 20% at later times (Day > 231) compared with patients who had no dose modifications (Figure 19-1. Mean trough serum concentration for luspatercept versus time in ACE-536-B-THAL-001.). The model-predicted results in this Phase 3 study were consistent with those observed in Study A536-04, with median $T_{max}$ as approximately 5.5 days and mean $t_{1/2}$ in serum as 11 days. The individual variability in overall exposure ($AUC_{ss}$) was 36%. See section below for summary of population PK report.

**Figure 19-1. Mean trough serum concentration for luspatercept versus time in ACE-536-B-THAL-001.**




Numbers at the bottom of each panel indicate the number of subjects at each time point.
Source: Report ACE-536-MPK-001, Figure 28.

**Table 19-7. Summary of luspatercept PK parameters by Bayesian estimation in Study ACE-536-B-THAL-001.**

| Parameter | Responders (N = 158) [a] | Non-Responders (N = 63) | Total (N = 221) |
|---|---|---|---|
| CL/F (L/day) | 0.430 (34.4) | 0.455 (47.5) | 0.437 (38.5) |
| V1/F (L) | 7.09 (27.8) | 7.06 (24.0) | 7.08 (26.7) |
| $t_{1/2}$ (day) | 11.4 (23.9) | 10.8 (29.4) | 11.2 (25.7) |
| $T_{max}$ (day) | 5.51 (4.23 – 7.74) | 5.41 (3.35 – 6.23) | 5.48 (3.35 – 7.74) |
| $C_{max}$ (µg/mL) | 5.67 (24.8) | 5.58 (26.0) | 5.64 (25.1) |
| $C_{max,ss}$ (µg/mL) | 8.41 (27.9) | 8.07 (35.1) | 8.31 (30.1) |
| $AUC_{ss}$ (day•µg/mL) | 131 (32.2) | 123 (44.6) | 129 (36.0) |

AUC = area under the concentration-time curve; $AUC_{ss}$ = AUC at steady state for the starting dose; $C_{max}$ = maximum concentration for the first dose; $C_{max,ss}$ = $C_{max}$ at steady state for the starting dose; CL/F = apparent clearance; CV = coefficient of variation; N = number of subjects; $t_{1/2}$ = elimination half-life; $T_{max}$ = time to reach $C_{max}$; V1/F = apparent volume of distribution of the central compartment.
Median (minimum - maximum) data are presented for $T_{max}$; geometric mean (geometric CV%) data are presented for other parameters.
[a] Responders are defined as subjects who achieved ≥ 33% reduction in red blood cell transfusion with a reduction of at least 2 units during any consecutive 12-week interval.
Source: Report ACE-536-MPK-001, Table 15.

**PD (Erythroid Response) Assessment**

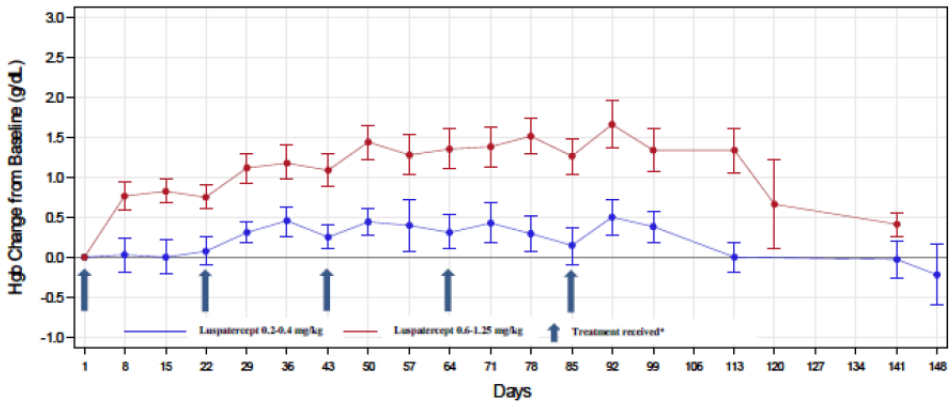
Blood samples were collected prior to dosing of luspatercept to obtain the hemoglobin level. For any RBC transfusions received during the study, collect hemoglobin value just prior to transfusion.

**In Study A536-04, change of hemoglobin level from baseline was assessed for non-transfusion-dependent (NTD) patients, while RBC-T reduction was directly used to assess the erythroid response in transfusion-dependent (TD) patients. In NTD patients, dose-dependent**

**increase from baseline in Hgb was observed. The mean increase was consistently higher in the 0.6 to 1.25 mg/kg group than in the 0.2 to 0.4 mg/kg group for the duration of the study (Figure 19-2). The increase in Hgb was sustained through end of treatment with the Q3W dosing schedule. See**

Table 19-8. Erythroid response during any consecutive 12-week interval by luspatercept dose group in Study A536-04. for erythroid response during a consecutive 12-week interval for the study.

**Figure 19-2. Mean (SE) change from baseline in hemoglobin in NTD patients in Study A536-04.**




Hgb = hemoglobin.
Note: Baseline is defined as mean of pretreatment Hgb values between Day -28 and Day 1. Hemoglobin values within 14 days of a transfusion are excluded from the summary. Arrows show the dosing time.
Source: Report A536-04, Figure 2.

**Table 19-8. Erythroid response during any consecutive 12-week interval by luspatercept dose group in Study A536-04.**

| Study Population | Erythroid Response | 0.2 to 0.6 mg/kg | 0.8 to 1.25 mg/kg |
|---|---|---|---|
| Non-transfusion dependent | N | 17 | 16 |
| | Mean Hgb increase ≥ 1.0 g/dL, n (%) | 5 (29.4) | 9 (56.3) |
| | Mean Hgb increase ≥ 1.5 g/dL, n (%) | 0 (0) | 7 (43.8) |
| Transfusion-dependent | N | 1 | 30 |
| | RBC-T reduction ≥ 50%, n (%) | 1 (100) | 16 (53.3) |

Hgb = hemoglobin; N = number of subjects per treatment group; n = number of responders; RBC-T = red blood cell transfusion.
Source: Report A536-04, Table 15 and Table 16.

**Immunogenicity Assessment**

Blood samples for assessment of ADA in serum were collected from all subjects in all clinical studies at the following visit timepoints. Time-matched PK samples were collected to assist in the interpretation of ADA results.

- Study A536-04: Pre-dose on C1D1 and C4D1; EOT, and EOS. Additional follow-up if applicable.
- Study A536-06: Pre-dose once every 4 cycles (C1D1, C5D1, C9D1, C13D1, C17D1, etc.), EOT, and EOS. Additional follow-up if applicable.
- Study ACE-536-B-THAL-001: Pre-dose on C1D1, C2D1, C4D1, C6D1, C8D1, C12D1, C16D1, once every 6 doses thereafter. At posttreatment, collect sample once every 24 weeks if applicable with up to 2 years of sampling.

A total of 391 patients with β-thalassemia provided evaluable ADA samples, including 284 luspatercept-treated patients and 107 placebo-treated patients. Results show that only patients in Study ACE-536-B-THAL-001 developed TEADA against luspatercept, with 1.41% developed binding antibodies and 0.7% developed neutralizing antibodies. The incidence of TEADA was similar between luspatercept- and placebo-treated patients. See Table 19-9 for details.

**Table 19-9. Incidence of TEADA in patients with β-thalassemia.**

| | Luspatercept, n (%) | | | Placebo, n (%) |
|---|---|---|---|---|
| Anti-drug antibodies | A536-04/06 (N = 64) | ACE-536-B-THAL-001 (N = 220) | Total (N = 284) | ACE-536-B-THAL-001 (N = 107) |
| ADA against luspatercept | 0 (0) | 4 (1.81) | 4 (1.41) | 2 (1.87) |
| Specificity for ACE-mECD | 0 (0) | 1 (0.45) | 1 (0.35) | 0 (0) |
| Specificity for ActRIIB ECD | 0 (0) | 1 (0.45) | 1 (0.35) | 0 (0) |
| Neutralizing luspatercept | 0 (0) | 2 (0.90) | 2 (0.70) | 0 (0) |
| Neutralizing ActRIIB-ECD-Fc | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

ActRIIB = activin receptor type IIB; ACE-mECD: modified ECD of human ActRIIB on luspatercept; ActRIIB ECD = natural ECD of human ActRIIB; ActRIIB-ECD-Fc = a fusion protein joining the natural ECD of human ActRIIB to Fc portion of human Ig G; ADA = anti-drug antibodies; ECD = extracellular domain; N = total number of subjects providing evaluable ADA sample; n = number of subjects with treatment-emergent ADA.
Source: Report ACE-536-B-THAL-001 Table 14.3.8.1, Appendix 13.1.5.

Source: EDR 5.3.3.5 ACE-536-MPK-004 CSR Table 10.

Luspatercept dose-normalized trough concentration was analyzed and compared for patients with ADA negative, pre-existing ADA, and NAb-negative TEADA and NAb positive. Results show that $C_{trough}$ tended to be lower in patients with TEADAs (2.19 µg/mL) compared to ADA negative (3.38 µg/mL). A 56% reduction in mean $C_{trough}$ was observed in patients with neutralizing TEADA (1.48 µg/mL) compared to ADA negative. However, given the number of patients developed TEADA was small, no statistically meaningful comparison could be conducted to draw a conclusion. There was no marked difference in luspatercept exposure between ADA negative patients and patients with pre-existing ADA.

**Table 19-10. Summary of dose-normalized luspatercept trough concentration in serum by ADA status.**

| Dose-normalized trough concentration (µg/mL) | Negative (N = 272) | Preexisting (N = 6) | Treatment-emergent | | |
|---|---|---|---|---|---|
| | | | NAb negative (N = 2) | NAb positive (N = 2) | Total (N = 4) |
| Mean (CV%) | 3.38 (41.0) | 2.64 (34.4) | 2.90 (26.3) | 1.48 (31.9) | 2.19 (44.3) |
| 90% CI | 3.24-3.52 | 1.90-3.39 | ND | ND | 1.05-3.33 |

CI = confidence interval; CV = coefficient of variation; N = number of subjects; NAb = neutralizing anti-drug antibodies against luspatercept; ND = not determined.
Source: Appendix 13.1.5.

Source: EDR 5.3.3.5 ACE-536-MPK-004 CSR Table 14.

### 19.5.3 Population PK Analysis

Population PK analysis was conducted based on 3680 evaluable luspatercept concentrations in 285 patients from Trials A536-04 and ACE-536-B-THAL-001. Summary statistics of the continuous and categorical covariates that were evaluated in the population PK analysis are shown in **Error! Reference source not found.** and Table 19-12, respectively.

**Table 19-11: Summary Statistics for the Continuous Covariates in the Population PK Analysis**

| Characteristics | A536-04 N = 64 | | ACE-536-B-THAL-001 N = 221 | | Total N = 285 | |
|---|---|---|---|---|---|---|
| | Mean (CV%) | Median [Min, Max] | Mean (CV%) | Median [Min, Max] | Mean (CV%) | Median [Min, Max] |
| Age (years) | 38.1 (27.7) | 38.5 [20.0, 62.0] | 32.1 (33.2) | 29.0 [18.0, 66.0] | 33.4 (32.6) | 32.0 [18.0, 66.0] |
| Weight (kg) | 64.3 (16.0) | 62.5 [47.5, 97.0] | 57.0 (17.9) | 56.0 [34.1, 91.0] | 58.6 (18.2) | 57.1 [34.1, 97.0] |
| Erythropoietin (U/L)[a] | 92.6 (104.5) | 57.7 [8.80, 499] | 93.8 (120.9) | 60.7 [2.40, 972] | 93.5 (117.2) | 60.5 [2.40, 972] |
| Transfusion Burden (units/24 weeks) | 8.74 (105.9) | 6.00 [0.00, 34.0] | 15.0 (26.0) | 15.0 [6.00, 25.1] | 13.6 (45.1) | 14.1 [0.00, 34.0] |
| Total Bilirubin (µmol/L) | 52.0 (73.8) | 40.8 [10.6, 246] | 35.5 (65.5) | 29.0 [5.00, 195] | 39.2 (71.8) | 32.8 [5.00, 246] |
| Albumin (g/L) | 44.9 (10.5) | 45.3 [30.6, 53.5] | 46.0 (7.3) | 46.0 [30.0, 56.0] | 45.8 (8.2) | 46.0 [30.0, 56.0] |
| Alkaline Phosphatase (U/L) | 70.2 (25.3) | 70.5 [39.0, 121] | 93.9 (41.0) | 85.0 [31.0, 262] | 88.6 (40.9) | 80.0 [31.0, 262] |
| Alanine Transaminase (U/L) | 22.6 (65.6) | 18.5 [6.00, 102] | 29.8 (85.7) | 21.0 [6.00, 166] | 28.2 (84.2) | 20.0 [6.00, 166] |
| Aspartate Transaminase (U/L) | 26.4 (47.6) | 22.0 [11.0, 67.0] | 29.2 (64.9) | 22.0 [10.0, 116] | 28.6 (62.0) | 22.0 [10.0, 116] |
| Lactate Dehydrogenase (U/L) | 397 (58.9) | 336 [89.0, 1500] | 194 (46.2) | 170 [75.0, 739] | 240 (66.7) | 190 [75.0, 1500] |
| Estimated Glomerular Filtration Rate (mL/min/1.73 $m^2$) | 126 (28.0) | 117 [59.2, 223] | 130 (33.5) | 120 [53.7, 314] | 129 (32.4) | 120 [53.7, 314] |

Source: Clinical PK/PD Report ACE-536-MPK-001, Table 7.

**Table 19-12: Summary Statistics for the Categorical Covariates in the Population PK Analysis**

| Characteristics | Number (%) of Subjects | | |
|---|---|---|---|
| | A536-04 N = 64 | ACE-536-B-THAL-001 N = 221 | Total N = 285 |
| **Baseline Characteristics** | | | |
| **Sex** | | | |
| Female | 31 (48.4%) | 131 (59.3%) | 162 (56.8%) |
| Male | 33 (51.6%) | 90 (40.7%) | 123 (43.2%) |
| **Age Category** | | | |
| ≥ 32 Years | 44 (68.8%) | 100 (45.2%) | 144 (50.5%) |
| < 32 Years | 20 (31.2%) | 121 (54.8%) | 141 (49.5%) |
| **Race** | | | |
| White | 62 (96.9%) | 119 (53.8%) | 181 (63.5%) |
| Black | 1 (1.6%) | 1 (0.5%) | 2 (0.7%) |
| Asian | 1 (1.6%) | 81 (36.7%) | 82 (28.8%) |
| Other (including uncollected or unreported) | 0 (0%) | 20 (9.0%) | 20 (7.0%) |
| **Transfusion Dependence** | | | |
| Dependent (≥ 2 RBC units/8 weeks) | 33 (51.6%) | 221 (100%) | 254 (89.1%) |
| Independent (< 2 RBC units/8 weeks) | 31 (48.4%) | 0 (0%) | 31 (10.9%) |
| **Hepatic function categories** | | | |
| Normal | 3 (4.7%) | 50 (22.6%) | 53 (18.6%) |
| Mild | 10 (15.6%) | 68 (30.8%) | 78 (27.4%) |
| Moderate | 34 (53.1%) | 81 (36.7%) | 115 (40.4%) |
| Severe | 17 (26.6%) | 22 (10.0%) | 39 (13.7%) |
| **Renal function categories** | | | |
| Normal | 57 (89.1%) | 188 (85.1%) | 245 (86.0%) |
| Mild | 6 (9.4%) | 31 (14.0%) | 37 (13.0%) |
| Moderate | 1 (1.6%) | 2 (0.9%) | 3 (1.1%) |
| **Baseline Splenectomy** | | | |
| Yes | 43 (67.2%) | 127 (57.5%) | 170 (59.6%) |
| No | 21 (32.8%) | 94 (42.5%) | 115 (40.4%) |
| **Disease Category** | | | |
| β-Thalassemia | 64 (100%) | 172 (77.8%) | 236 (82.8%) |
| β-Thalassemia combined with α-Thalassemia | 0 (0%) | 17 (7.7%) | 17 (6.0%) |
| Hemoglobin E/β-Thalassemia | 0 (0%) | 31 (14.0%) | 31 (10.9%) |
| Missing | 0 (0%) | 1 (0.5%) | 1 (0.4%) |

| | | | |
|---|---|---|---|
| **Genotype** | | | |
| β0/β0 | 0 (0%) | 67 (30.3%) | 67 (23.5%) |
| Non-β0/β0 | 0 (0%) | 153 (69.2%) | 153 (53.7%) |
| Missing | 64 (100%) | 1 (0.5%) | 65 (22.8%) |
| **On Treatment Characteristics** | | | |
| **Drug Product** | | | |
| 25 mg frozen liquid (Process I/II) | 64 (100%) | 0 (0%) | 64 (22.5%) |
| 25 or 75 mg lyophilized powder (Process III) | 0 (0%) | 221 (100%) | 221 (77.5%) |
| **Concurrent Use of Iron Chelation Therapy** | | | |
| Yes | 43 (67.2%) | 199 (90.0%) | 242 (84.9%) |
| No | 21 (32.8%) | 0 (0%) | 21 (7.4%) |
| Missing | 0 (0%) | 22 (10.0%) | 22 (7.7%) |
| **Antidrug Antibodies Status** | | | |
| Negative | 60 (93.8%) | 214 (96.8%) | 274 (96.1%) |
| Pre-existing | 4 (6.2%) | 2 (0.9%) | 6 (2.1%) |
| Treatment-emergent | 0 (0%) | 4 (1.8%) | 4 (1.4%) |
| Missing | 0 (0%) | 1 (0.5) | 1 (0.4%) |

Source: Clinical PK/PD Report ACE-536-MPK-001, Table 6.

The PK of luspatercept was best characterized by a one-compartment model with first order absorption rate constant (ka) and linear elimination. The inter-individual variability (IIV) was estimated as log-normally distributed with a non-zero covariance on apparent clearance (CL/F), apparent volume of distribution (V/F), with a block omega on CL/F and V/F.

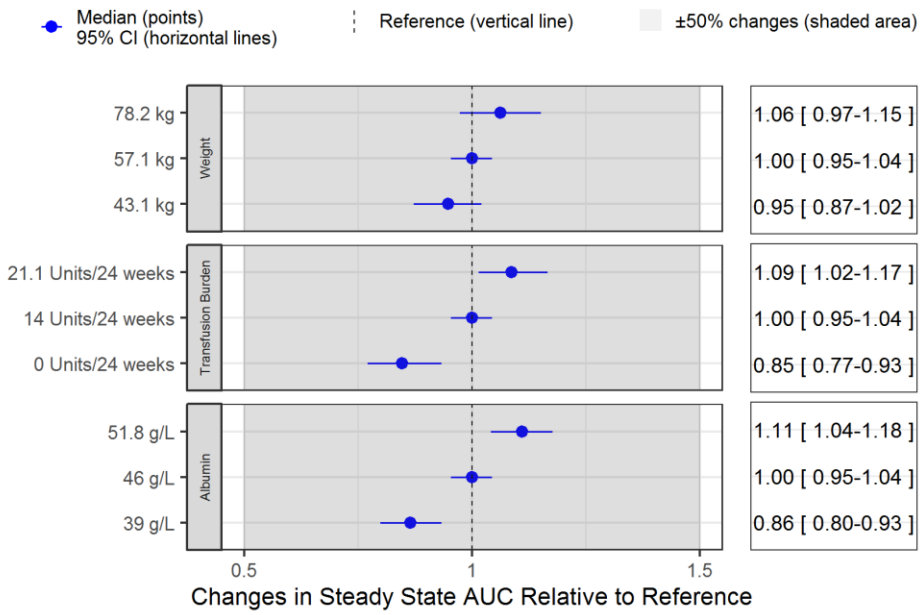
Covariate analysis identified 3 statistically significant and clinically relevant covariates, including the effect of weight, albumin and baseline transfusion burden on CL/F, and the effect of weight and baseline transfusion burden on V/F. Age, sex, race (Asian vs. non-Asian), mild to moderate renal impairment, mild to severe hepatic impairment defined by NCI-ODWG criteria, baseline liver enzymes (AST and ALT), baseline total bilirubin, baseline albumin, baseline EPO, β-thalassemia genotype, splenectomy, location of SC injection (i.e., upper arm, thigh, or abdomen), drug substance manufacturing process and drug product formulation (Process I/II frozen liquid vs. Process III lyophilized powder), and concurrent iron chelation therapy had no clinically meaningful effect on luspatercept PK. The equations of the final covariate models are the following:

$$\text{CL/F (L/day)} = 0.532 \times \left(\frac{\text{Weight}}{70}\right)^{0.806} \times \left(\frac{\text{Albumin}}{46}\right)^{-0.881} \times e^{(-0.0118 \times [\text{RBC}-\text{T}-14])}$$

$$\text{V1/F (L)} = 8.39 \times \left(\frac{\text{Weight}}{70}\right)^{0.705} \times e^{(-0.0141 \times [\text{RBC}-\text{T}-14])}$$

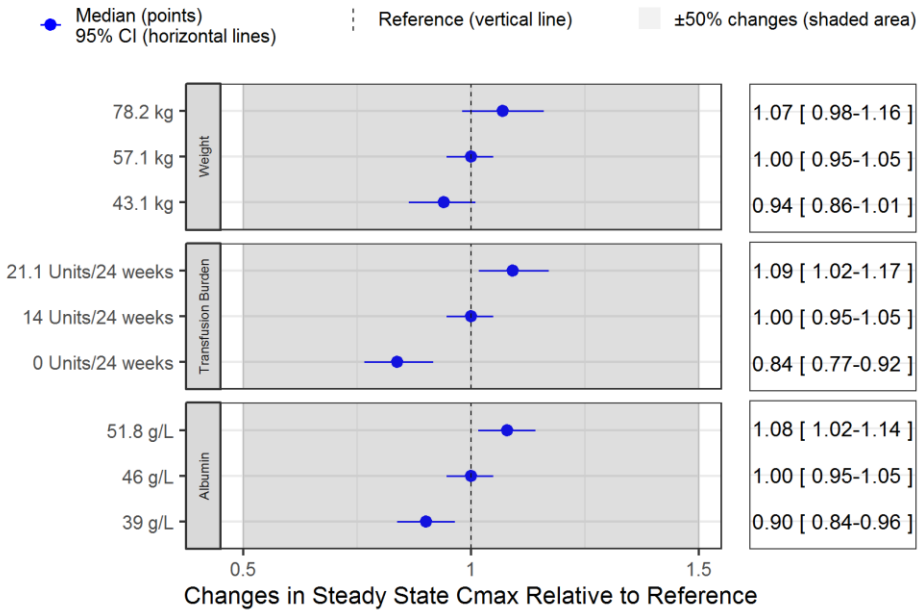
The effects of each covariate retained in the final PK model on steady-state AUC and $C_{max}$ are presented in Figure 19-3 and Figure 19-4, respectively. At the body weight-based dose of 1 mg/kg, no clinically significant difference (< 25%) was expected in the median exposure level between the 5$^{th}$ or 95$^{th}$ quantile of each covariate and the reference value.

**Figure 19-3 Forest Plot of Significant Covariates on Steady State AUC in the Final Model**




Source: Clinical PK/PD Report ACE-536-MPK-001, Appendix A, Section 4.27.

**Figure 19-4 Forest Plot of Significant Covariates on steady State Cmax in the Final Model**




Source: Clinical PK/PD Report ACE-536-MPK-001, Appendix A, Section 4.28.

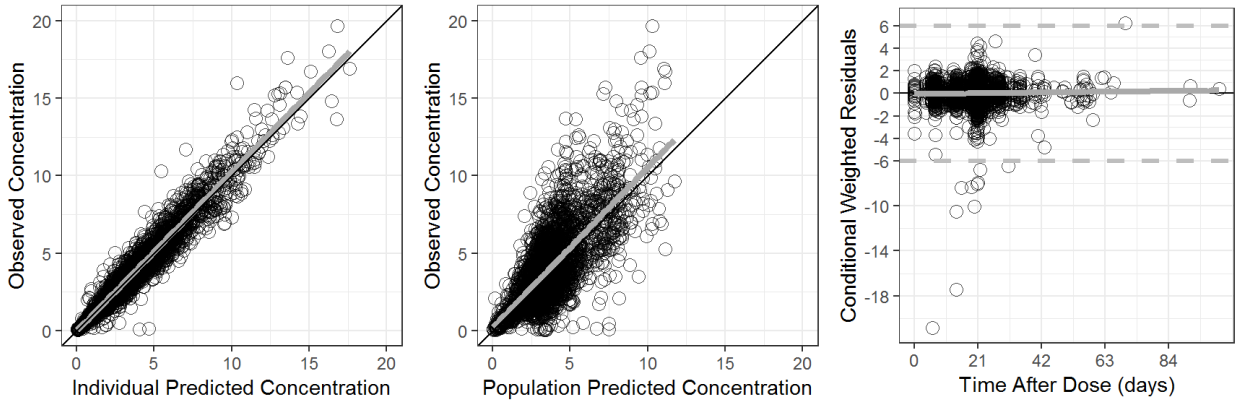
The final luspatercept PK model parameter estimates and the corresponding 95% confidence interval (CI) from Bootstrap are presented in Table 19-13. The goodness-of-fit plots for the final luspatercept PK model are presented Figure 19-3. The prediction-corrected visual predictive check (pcVPC) stratified by study (Figure 19-4) illustrated the prediction percentiles and corresponding 95% CI of simulated concentrations overlaid on the observed luspatercept concentrations and the corresponding 5$^{th}$ and 95$^{th}$ percentiles.

**Table 19-13 Population PK Parameters of Luspatercept from the Final PK Model and Bootstrap**

| Parameter | Model Term | Estimate | Asymptotic | | Bootstrap | |
|---|---|---|---|---|---|---|
| | | | RSE (%) | 95% CI | Median | 95% CI |
| **CL/F (L/day)** | $\theta$ | 0.532 | 3.18 | 0.498 – 0.565 | 0.532 | 0.500 – 0.569 |
| Weight (kg) | $\times (WT/70)^{\theta}$ | 0.806 | 13.9 | 0.586 – 1.03 | 0.809 | 0.594 – 1.04 |
| RBC-T burden (units/24 weeks) | $\times \exp(\theta\,(RBC\text{-}T - 14))$ | -0.0118 | 28.0 | -0.0183 – -0.00533 | -0.0120 | -0.0186 – -0.00544 |
| Albumin (g/L) | $\times (ALB/46)^{\theta}$ | -0.881 | 19.5 | -1.22 – -0.544 | -0.886 | -1.21 – -0.519 |
| **V1/F (L)** | $\theta$ | 8.39 | 2.68 | 7.95 – 8.83 | 8.39 | 7.97 – 8.85 |
| Weight | $\times (WT/70)^{\theta}$ | 0.705 | 15.2 | 0.496 – 0.915 | 0.718 | 0.495 – 0.916 |
| RBC-T burden (units/24 weeks) | $\times \exp(\theta\,(RBC\text{-}T - 14))$ | -0.0141 | 17.2 | -0.0188 – -0.00932 | -0.0141 | -0.0189 – -0.00921 |
| **$K_a$ ($day^{-1}$)** | $\theta$ | 0.409 | 7.56 | 0.349 – 0.470 | 0.410 | 0.354 – 0.481 |
| **Interindividual Variability** | | | | | | |
| On CL/F | $\omega = SD(\eta_{CL,i})$ | 0.337 | 10.9 | 0.265 – 0.408 | 0.332 | 0.277 – 0.424 |
| On V1/F | $\omega = SD(\eta_{V1,i})$ | 0.271 | 15.1 | 0.191 – 0.352 | 0.267 | 0.180 – 0.351 |
| Correlation CL/F, V1/F | $\omega = Corr(\eta_{CL,i}, \eta_{V1,i})$ | 0.616 | 15.1 | 0.434 – 0.798 | 0.630 | 0.436 – 0.803 |
| **Residual Variability** | | | | | | |
| Log-additive error | $\sigma = SD(\varepsilon_{i,j})$ | 0.206 | 9.90 | 0.166 – 0.246 | 0.204 | 0.168 – 0.248 |

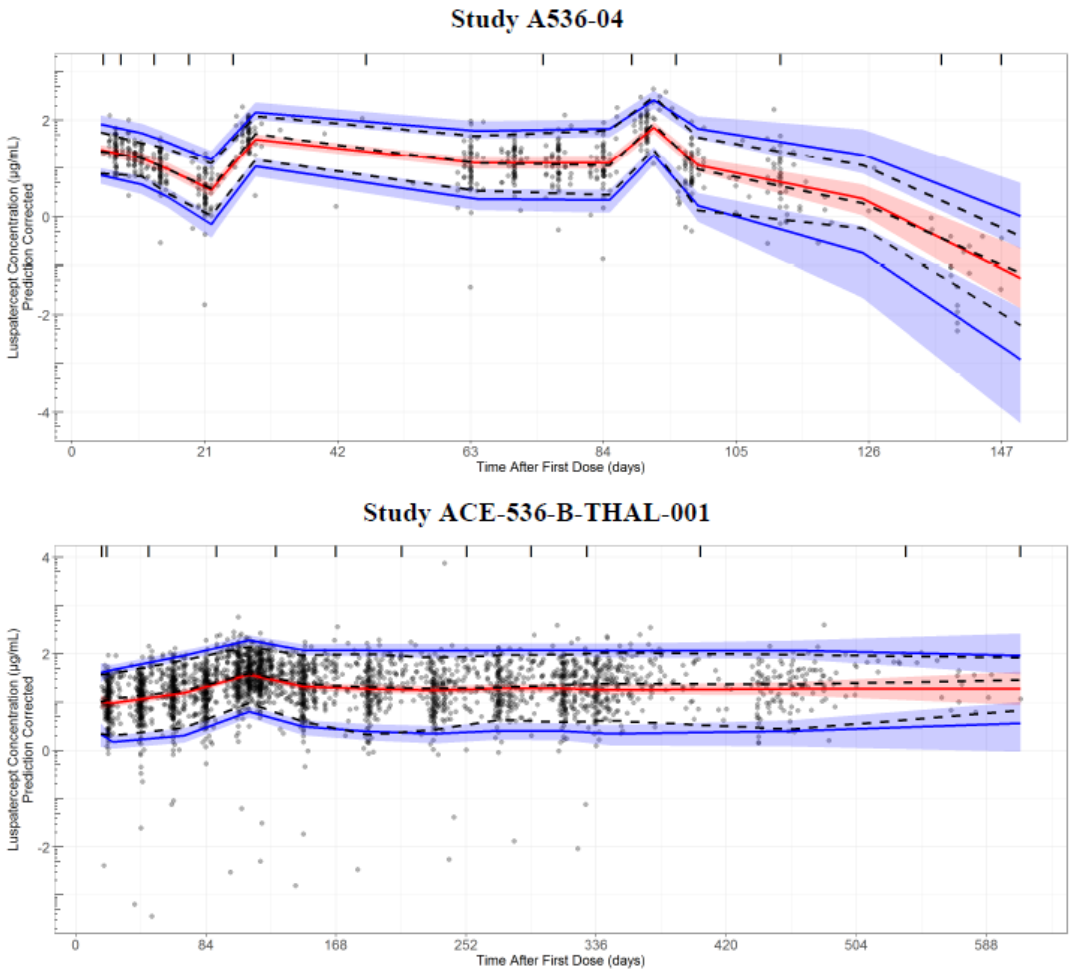
Source: Clinical PK/PD Report ACE-536-MPK-001, Table 8.

**Figure 19-5 Goodness-of-fit Plots for the Final Population PK Model for Luspatercept**




Source: Clinical PK/PD Report ACE-536-MPK-001, Figure 11.

**Figure 19-6 Prediction Corrected Visual Predictive Check for the Final Luspatercept Model**




Source: Clinical PK/PD Report ACE-536-MPK-001, Figure 12.

***Reviewer's Comments:*** *The Applicant's population PK model appears adequate to describe the luspatercept serum concentration-time profiles following the administration of luspatercept ranged from 0.2 mg/kg to 1.25 mg/kg SC every three weeks in patients with β-thalassemia. The shrinkage value was 2.7% for IIV on CL/F, 22.4% for IIV on V/F, and 6.0% for residual variability, indicating there was no obvious bias in the parameter estimates. Therefore, the PK model is acceptable for simulating post-hoc exposure metrics, e.g. average AUC from Week 1 to Week 15 ($AUC_{avg15}$) and average AUC to the first AE event ($AUC_{avg}$) of luspatercept for exposure-response analyses for efficacy and safety measurements.*

*Covariate analysis identified three statistically significant covariates: body weight, baseline albumin and baseline RBC-T burden on CL/F, as well as body weight and RBC-T burden on Vd/F. The significant impact of body weight on luspatercept CL/F and Vd/F supported the body-weight-based dosing regimen. Simulations utilizing the final population PK model revealed that the impact of albumin and RBC-T burden on luspatercept serum exposure was limited with < 20% alterations with body weight-based dosing and hence not clinically significant. In addition, the*

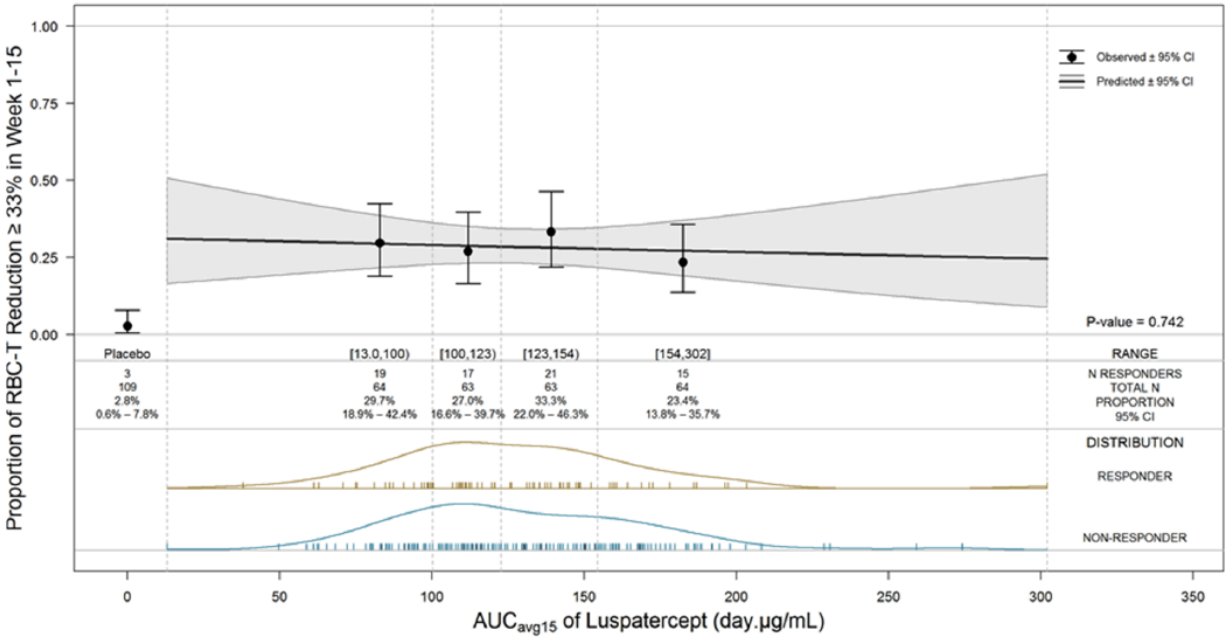
*luspatercept exposure was not clinically significantly altered by age, sex, race, mild to severe hepatic impairment, mild to moderate renal impairment, baseline serum erythropoietin, beta thalassemia genotype, splenectomy, location of SC injection, and concurrent iron chelation therapy after the dose was adjusted by body weight. Therefore, no dose adjustment is needed for the above-mentioned specific populations.*

### 19.5.4 Exposure-Response Analysis

**Applicant's Exposure-Response for Efficacy**

The exposure-response (E-R) analysis was conducted for achievement of ≥33% reduction from baseline in RBC transfusion (RBC-T) in Week 1 to Week 15 in 249 patients with β-thalassemia and regular transfusions of ≥ 6 RBC units/24 weeks at baseline from Trials A536-04 and ACE-536-B-THAL-001. The dose of luspatercept ranged from 0.6 to 1.25 mg/kg for the pooled population. However, the proportion of patients who started with doses < 1 mg/kg was small, with only 0.4% (1/254) receiving 0.6 mg/kg and 9% (24/254) receiving 0.8 mg/kg. As such, the exposure range was narrow (mainly 1 to 1.25 mg/kg) for the integrated analysis. The PK metrics used for E-R analysis was $AUC_{avg15}$. The Applicant concluded that no exposure-dependent ($AUC_{avg15}$) trend was observed for RBC-T reduction ≥ 33% during the first 15 weeks in luspatercept-treated patients (Figure 19-7).

**Figure 19-7 Relationship between Luspatercept Serum Exposure and Probability of RBC-T Reduction ≥ 33% in Week 1 to Week 15**



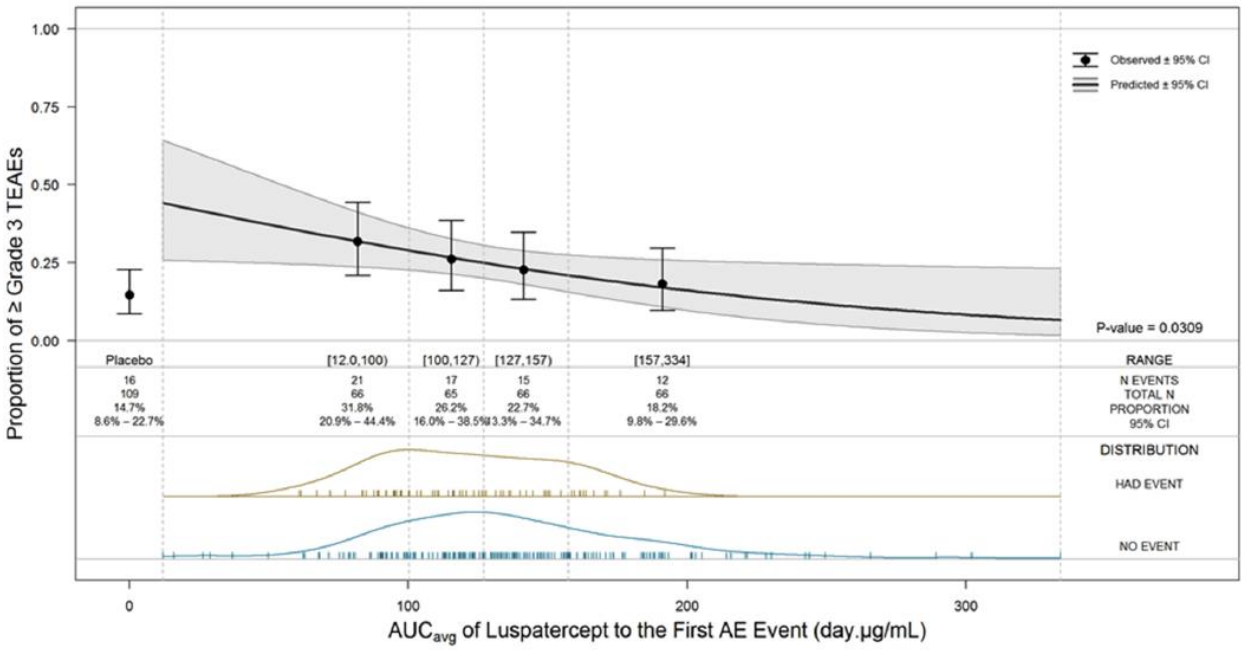

$AUC_{avg15}$ = average area under the concentration-time curve from Week 1 to Week 15; CI = confidence interval; N = number of subjects; RBC-T = red blood cell transfusion.
Source: Clinical PK/PD Report ACE-536-MPK-001, Figure 15.

***Reviewer's Comments:*** *The pooled exposure-efficacy analysis was confounded by dose titration design implemented in both Trials A536-04 and ACE-536-B-THAL-001, since the titration was based on individual patient's response. Based on the dose escalation part in Trial A536-04, higher response rate was observed with higher doses (0.2-1.5 mg/kg Q3W).*

**Applicant's Exposure-Response for Safety**

The exposure-safety analysis was conducted in 372 (109 placebo and 263 luspatercept) patients with β-thalassemia and regular transfusions of ≥ 6 RBC units/24 weeks at baseline from Trials A536-04, A536-06 and ACE-536-B-THAL-001. The dose of luspatercept ranged from 0.6 to 1.25 mg/kg for the pooled population. The PK metrics used for E-R analysis was $AUC_{avg15}$ based on starting dose level. The Applicant concluded that there was a statistically significant (p = 0.03) inverse relationship between luspatercept AUCavg and Grade ≥3 TEAEs in luspatercept-treated patients (Figure 19-8).

**Figure 19-8 Relationship between Luspatercept Serum Exposure and Probability of Experiencing TEAEs ≥ Grade 3**

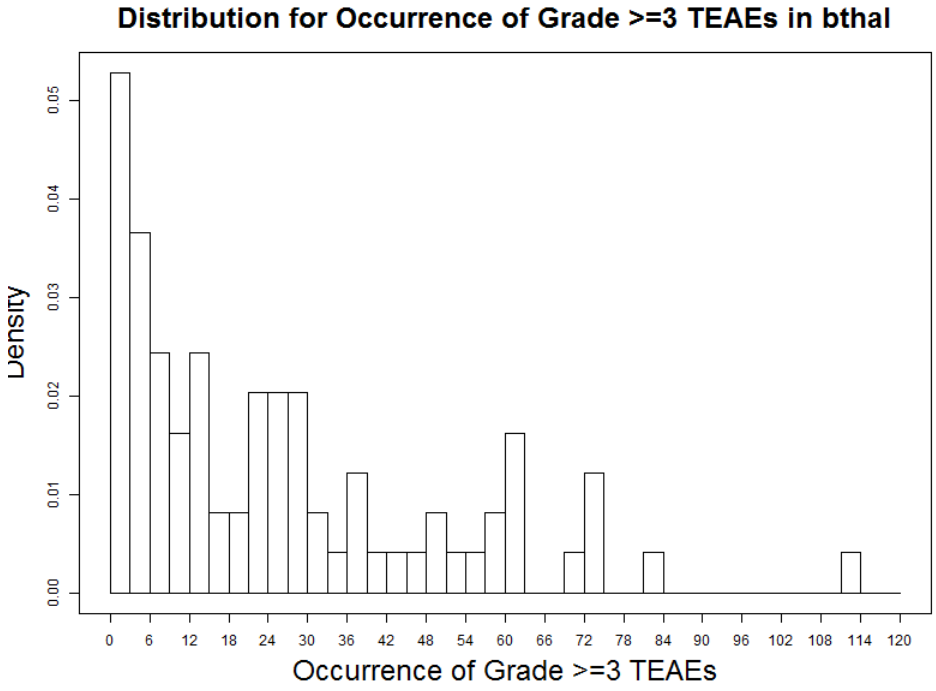



AE = adverse event; $AUC_{avg}$ = average area under the concentration-time curve to the first event; CI = confidence interval; N = number of subjects; TEAE = treatment emergent adverse events.
Source: Clinical PK/PD Report ACE-536-MPK-001, Figure 19.

***Reviewer's Comments:*** *The Applicant's inverse exposure-safety relationship was potentially confounded by the timing of TEAE occurrence and dose escalation scheme. This was corrected by the reviewer's sensitivity analysis which confirmed that the relationship between exposure and safety is flat.*

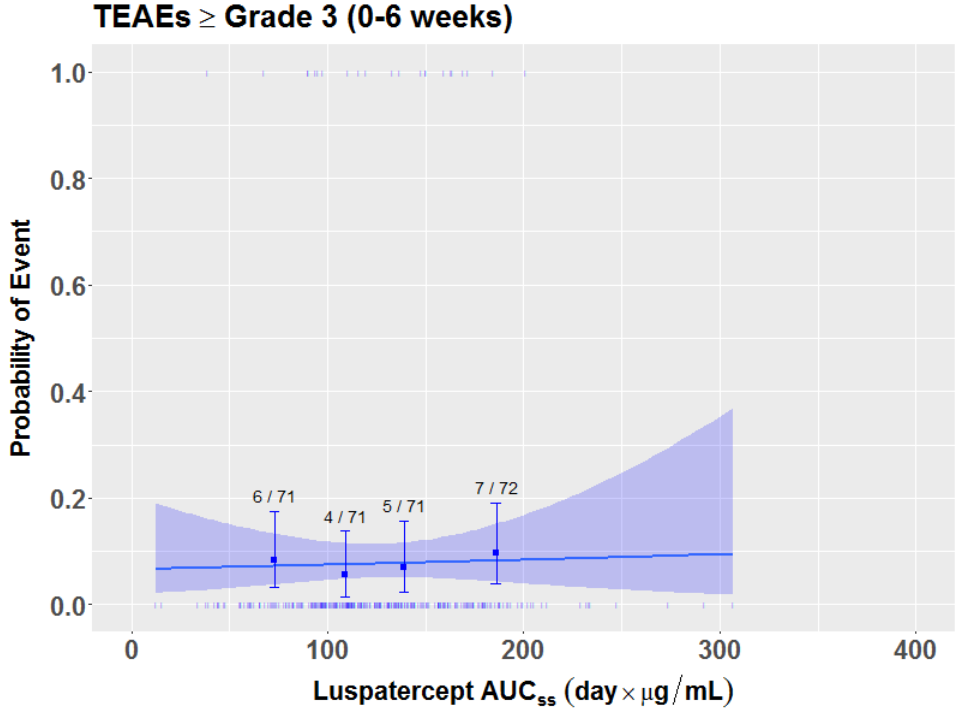
*The reviewer noted that most Grade ≥3 TEAEs occurred in the first two treatment cycles (0-6 weeks) prior to dose escalation (Figure 19-9), where the patients were still on the starting dose and the concentrations associated with these events would be lower compared to that in the later phase. Therefore, the analyses for safety were confounded by the trial design (dose titration) and may not represent the true E-R relationship. Accordingly, the reviewer conducted sensitivity analysis to explore the E-R for safety by different time period. The biased relationship was then corrected and the results showed that the E-R relationships were generally flat for Grade ≥3 TEAEs before dose escalation at Weeks 0-6 (Figure 19-10) and during dose escalation after Week 6 (Figure 19-11), suggesting that increase of luspatercept exposure up to 1.25 mg/kg was not associated the occurrence of these AEs. For each subject, only the occurrence of the first event was included in the analysis.*

**Figure 19-9 Distribution of occurrence of Grade ≥3 TEAEs**



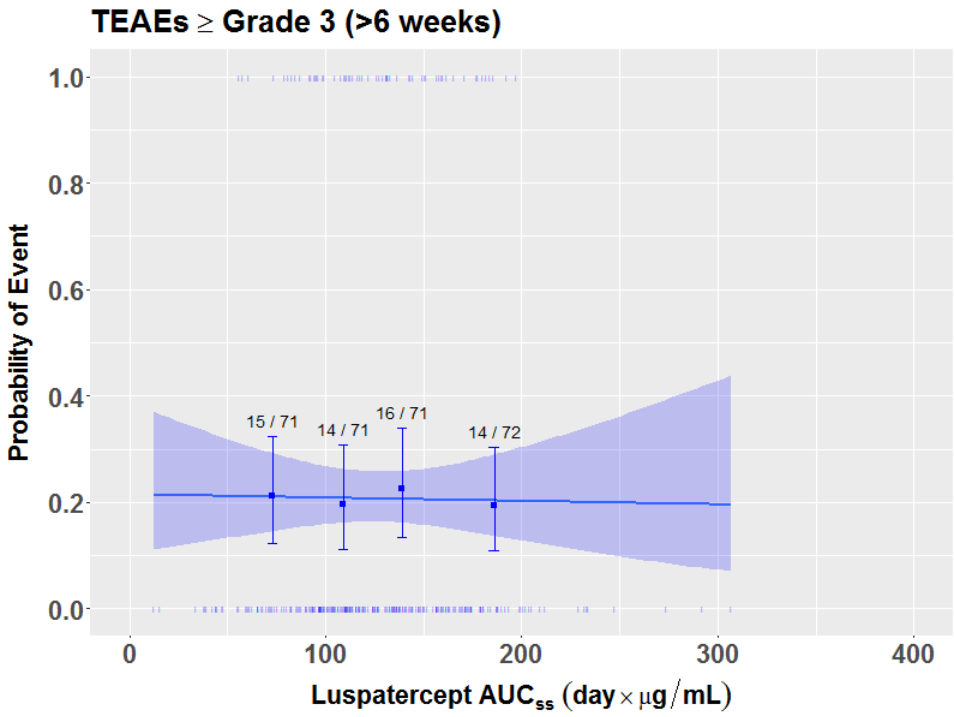

Source: Reviewer's analysis.

**Figure 19-10 Relationship between Luspatercept Serum Exposure and Probability of Experiencing TEAEs ≥ Grade 3 before Dose Escalation at Weeks 0 to 6**




Source: Reviewer's analysis.

**Figure 19-11 Relationship between Luspatercept Serum Exposure and Probability of Experiencing TEAEs ≥ Grade 3 during Dose Escalation after Week 6.**




Source: Reviewer's analysis.

| BLA 761136 Original 1 REBLOZYL (luspatercept -- aamt) | | | | |
|---|---|---|---|---|
| **Signature** | | | | |
| **DISCIPLINE** | **REVIEWER** | **OFFICE/DIVISION** | **SECTIONS AUTHORED/ APPROVED** | **AUTHORED/ APPROVED** |
| Nonclinical Reviewer | Michael Manning | OHOP/DHOT | Sections: 5 | **Select:**<br>☑ Authored<br>☐ Approved |
| | **Signature:** Michael L. Manning -S | | | Digitally signed by Michael L. Manning -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001207192, cn=Michael L. Manning -S<br>Date: 2019.11.04 18:52:19 -05'00' |
| Nonclinical Team Leader/Deputy Division Director (NME Only) | Haleh Saber | OHOP/DHOT | Sections: 5 | **Select:**<br>☐ Authored<br>☑ Approved |
| | **Signature:** Haleh Saber -S | | | Digitally signed by Haleh Saber -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Haleh Saber -S, 0.9.2342.19200300.100.1.1=1300212858<br>Date: 2019.11.05 09:34:45 -05'00' |
| Clinical Pharmacology Reviewer | Lili Pan | OCP/DPV | Sections: 6, 19.5 | **Select:**<br>☑ Authored<br>☐ Approved |
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| Clinical Pharmacology Team Leader | Guoxiang (George) Shen | OCP/DPV | Sections: 6, 19.5 | **Select:**<br>☑ Authored<br>☑ Approved |
| | **Signature:** Guoxiang Shen -S | | | Digitally signed by Guoxiang Shen -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Guoxiang Shen -S, 0.9.2342.19200300.100.1.1=2001813955<br>Date: 2019.11.05 09:41:48 -05'00' |
| Clinical Pharmacology Division Director (NME only) | Nam Atiqur Rahman | OCP/DPV | Sections: 6, 19.5 | **Select:**<br>☐ Authored<br>☑ Approved |
| | **Signature:** Nam A. Rahman -S | | | Digitally signed by Nam A. Rahman -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Nam A. Rahman -S, 0.9.2342.19200300.100.1.1=1300072597<br>Date: 2019.11.06 10:43:04 -05'00' |
| Pharmacometrics Reviewer | Liang Li | OCP/DPM | Sections: 6, 19.5 | **Select:**<br>☑ Authored<br>☐ Approved |
| | **Signature:** Liang Li -S | | | Digitally signed by Liang Li -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Liang Li -S, 0.9.2342.19200300.100.1.1=2001459144<br>Date: 2019.11.04 15:30:59 -05'00' |
| Pharmacometrics Team Leader | Lian Ma | OCP/DPM | Sections: 6, 19.5 | **Select:**<br>☑ Authored<br>☑ Approved |
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| Statistics Reviewer | Weishi (Vivian) Yuan | OB/DBV | Sections: 7,8 | **Select:**<br>☑ Authored<br>☐ Approved |
| | **Signature:** Weishi Yuan -S | | | Digitally signed by Weishi Yuan -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Weishi Yuan -S, 0.9.2342.19200300.100.1.1=1300234839<br>Date: 2019.11.05 02:48:57 -05'00' |
| | | | | **Select:** |

| | | | | |
|---|---|---|---|---|
| Statistics Team Leader | Yeh-Fong Chen | OB/DBV | Sections: 7,8 | ☑ Authored<br>☑ Approved |
| | **Signature:** Yehfong Chen -S<br>Digitally signed by Yehfong Chen -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yehfong Chen -S, 0.9.2342.19200300.100.1.1=1300157970<br>Date: 2019.11.05 11:02:18 -05'00' | | | |
| Statistical Deputy Director (NME only) | Thomas Gwise | OB/DBV | Sections: 7,8 | **Select:**<br>☐ Authored<br>☑ Approved |
| | **Signature:** Thomas E. Gwise -S<br>Digitally signed by Thomas E. Gwise -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300369224, cn=Thomas E. Gwise -S<br>Date: 2019.11.05 09:47:04 -05'00' | | | |
| Associate Director for Labeling | Virginia Kwitkowski | OHOP/DHP | Sections: 11 | **Select:**<br>☑ Authored<br>☐ Approved |
| | **Signature:** Virginia E. Kwitkowski -S<br>Digitally signed by Virginia E. Kwitkowski -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300395223, cn=Virginia E. Kwitkowski -S<br>Date: 2019.11.04 16:27:57 -05'00' | | | |
| Cross-Disciplinary Team Leader (CDTL) | Tanya Wroblewksi | OHOP/DHP | Sections: All | **Select:**<br>☑ Authored<br>☑ Approved |
| | **Signature:** Tanya M. Wroblewski -S3<br>Digitally signed by Tanya M. Wroblewski -S3<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0011605845, cn=Tanya M. Wroblewski -S3<br>Date: 2019.11.05 13:14:07 -05'00' | | | |
| Supervisory Associate Division Director (NME only) | Albert Deisseroth | OHOP/DHP | Sections: All | **Select:**<br>☐ Authored<br>☑ Approved |
| | **Signature:** Albert B. Deisseroth -S<br>Digitally signed by Albert B. Deisseroth -S<br>DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000589069, cn=Albert B. Deisseroth -S<br>Date: 2019.11.05 12:52:07 -05'00' | | | |
| Office Director or signatory (NME only) | Richard Pazdur | OHOP | Sections: All | **Select:**<br>☐ Authored<br>☑ Approved |
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ROSA J LEE-ALONZO
11/08/2019 10:07:04 AM

TANYA M WROBLEWSKI
11/08/2019 10:16:35 AM

RICHARD PAZDUR
11/08/2019 10:20:04 AM

# Summary Review for Regulatory Action

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|---|---|
| **Date** | November 3, 2019 |
| **From** | Albert Deisseroth MD, PhD, Supervisory Associate Division Director |
| **Subject** | Division Director Summary Review |
| **BLA #** | BLA 761136 |
| **Applicant** | Celgene |
| **Date of Submission** | April 4, 2019 |
| **Date Received** | April 4, 2019 |
| **Proper Name** | Luspatercept-aamt |
| **Proprietary Name** | Reblozyl |
| **Recommendation** | Approval |
| **Indications** | For the treatment of adult patients with beta-thalassemia associated anemia who require red blood cell (RBC) transfusions. |

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|---|---|
| Material Reviewed/Consulted | |
| CDTL Review | Dr. Tanya Wroblewski |
| Reviewers | Drs. Laurel Menapace and Vivian Yuan |

# Signatory Authority Review

(This Section depend in part on the reviews of the CDTL (Dr. Tanya Wroblewski) and Drs. Laurel Menapace and Dr. Weishi (Vivian) Yuan, PhD.)

**Background:** On April 4, 2019, Celgene submitted BLA 761136, in which approval was requested of luspatercept-aamt (Reblozyl) for the treatment of "adult patients with beta-thalassemia associated anemia who require red blood cell (RBC) transfusions." Luspatercept-aamt is a fusion protein comprised of a sub-unit of the Activin IIB receptor (ActRIIB) linked with the human IgG1 Fc-domain which binds to and therefore sequesters the natural ligand(s) for this receptor (GDF8, GDf11, BMP6, and activin B) from binding to the Activin IIB receptor. Since prevention of binding of the natural ligand(s) to the Activin IIB receptor results in improvement of hematological parameters in a mouse model of ineffective erythropoiesis, one can predict that luspatercept-aamt will have a similar effect on improving the hematological profile of ineffective erythropoiesis in patients with transfusion dependent beta-thalassemia.

The request for approval of the Sponsor's proposed indication relied upon Study ACE-536-B-Thal-001 (the Believe trial) which was a Phase 3, double blind trial which randomized 336 patients with beta-thalassemia between best supportive care with placebo (n=112) vs best supportive care with luspatercept-aamt (n=224). These are patients who required transfusion of 6-20 units of blood in the 24 weeks prior to the randomization between best supportive care with placebo vs best supportive care with luspatercept-aamt. Best supportive care (BSC) includes iron-chelating agents, anti-infective agents, nutritional support, and RBC transfusions as prespecified in the SAP. The primary endpoint was the proportion of patients on each arm exhibiting a ≥33% reduction from baseline of the RBC transfusion burden as well as a reduction of at least 2 units of RBCs administered from week 13 to week 24.

**Efficacy Results:** The Ace-536-B-Thal-001 (Believe) trial met it pre-specified primary endpoint: a statistically significant increase in the proportion of patients (21.4% or 48/224) on the experimental arm which exhibited a ≥33% reduction from baseline in the RBC transfusion burden

with a reduction of at least 2 units from week 13 to week 24 on the luspatercept-aamt + BSC arm, as compared to the percentage (4.5% or 5/112) of patients in the placebo + BSC arm ($p<0.0001$). This response profile was durable and stable over the period of 48 weeks.

**Safety Results**: Possible AML on luspatercept arm: One death occurred on the luspatercept-aamt arm in a 26 year old male treated with induction chemotherapy for AML (7+3) for a diagnosis of M6 erythroleukemia which developed during luspatercept-aamt treatment. The patient presented with febrile neutropenia and hypotension following which the patient was admitted in shock to the ICU. Because of the discovery of 33% blasts in the peripheral blood, the patient was given 1 course of induction chemotherapy (7 days of cytarabine with 3 days daunomycin) for M6 AML. The patient improved sufficiently to be discharged from the unit but later the leukemia recurred and the patient then deteriorated and died. The AML was never completely documented by cytogenetic or molecular analysis of the bone marrow cells. Nonetheless this is worrisome given the proposed mechanism of action of luspatercept-aamt, and the fact that the treatment plan for responders is to administer continuous therapy long term.

Safety findings on the luspatercept-aamt arm included thromboembolic events in 3.6% of patients (8/223) and hypertension in 10.7% (61/671). Grade 3-4 hypertension over 4 trials was 1.8%-8.6%. Four of the 284 patients treated on the luspatercept aamt arm and evaluable for the presence of antibodies were positive for anti-drug antibodies of whom 2 (0.7%) had neutralizing antibodies. There were no severe systemic hypersensitivity reactions reported. Although the safety profile was favorable, the follow up is short and the therapy is proposed to be given life-long.

**Benefit-Risk Discussion:** The benefits are potentially of significance and the toxicity was manageable but the follow-up is short.

**Regulatory Recommendation**: Approval with PMRs for long term safety follow-up.

APPEARS THIS WAY ON ORIGINAL

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ALBERT B DEISSEROTH
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