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Statistical Analysis Plan

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

Sponsor:	Acceleron Pharma Inc. 128 Sidney Street Cambridge, MA 02139, USA Tel: PPD Fax: PPD
Investigational Product:	Luspatercept (ACE-536)
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ACCELERON PHARMA SIGNATURE PAGE

The undersigned have approved this Statistical Analysis Plan Version 2 for use in this study.

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1. INTRODUCTION

This statistical analysis plan (SAP) describes the statistical methods to be used for the analysis of Acceleron Protocol A536-06. This SAP should be read in conjunction with the study protocol and electronic case report form (eCRF). This version of the plan has been developed using the protocol amendment 05 dated 12 September 2017 and eCRF v7 dated 14 March 2018. Any further changes to the protocol or eCRF may necessitate updates to the SAP.

The SAP will be signed off before the study database lock. Any deviations from the SAP will be described and justified in the final clinical study report (CSR).

2. STUDY OBJECTIVES

Primary Objective:

- To evaluate the long-term safety and tolerability of ACE-536 in patients with β-thalassemia who were previously enrolled in study A536-04.

Secondary Objectives:

- To evaluate erythroid response defined as the proportion of patients with:
  - Mean hemoglobin increase ≥ 1.5 g/dL over a continuous 12-week interval compared to baseline in non-transfusion dependent (NTD) patients, not influenced by red blood cell (RBC) transfusion, OR
  - Reduction in RBC transfusion burden by ≥ 50% over a continuous 12-week interval compared to the 12 weeks prior to the start of treatment in transfusion dependent (TD) patients.
- To evaluate erythroid response defined as proportion of patients with:
  - Mean hemoglobin increase ≥ 1.5 g/dL over a continuous 8-week interval compared to baseline in NTD patients, not influenced by RBC transfusion, OR
  - Reduction in RBC transfusion burden by ≥ 20% over a continuous 8-week interval compared to the 8 weeks prior to the start of treatment in TD patients.
- To evaluate the time to erythroid response and duration of erythroid response.
- To evaluate the mean change from baseline over an 8- or 12-week period in hemoglobin level in NTD patients not influenced by RBC transfusion
- To evaluate the mean % change from baseline in transfusion burden over an 8- or 12-week period in TD patients.
- To evaluate the mean change in pre-transfusion hemoglobin levels in TD patients
- To evaluate changes in markers of erythropoiesis, hemolysis, iron overload, and iron metabolism
- To examine the pharmacokinetic (PK) profile of ACE-536 in patients with β-thalassemia

Exploratory Objectives:

- To evaluate biomarkers related to the transforming growth factor beta (TGF-β) superfamily
- To evaluate patient self-reported quality of life using tools including but not limited to the Functional Assessment of Cancer Therapy-Anemia Scale (FACT-An) and Short Form (36) Health Survey (SF-36) questionnaires
- To evaluate change in extramedullary hematopoiesis (EMH) mass size by MRI
- To evaluate change in spleen size by MRI
- To evaluate change in bone mineral density (BMD) by DXA
- To evaluate change in leg ulcers
- To evaluate change in the 6-minute walk test (6MWT) distance in NTD patients

3. OVERALL STUDY DESIGN

This open-label extension study will evaluate the effects of up to 60 months of ACE-536 treatment in patients with β-thalassemia previously enrolled and treated with ACE-536 for up to 3 months in study A536-04. The base study (A536-04) is a phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 in patients with β-thalassemia. A total of up to 64 patients may be enrolled in study A536-04 and may be eligible for study A536-06.

3.1. Study Design

Consenting patients that meet the A536-06 eligibility criteria may immediately roll over from A536-04 to study A536-06 following the last ACE-536 dose. These patients may forego the End of Study (EOS) visit in study A536-04 to begin study A536-06. For these patients, Cycle 1 Day 1 (C1D1) of study A536-06 may take place 28 (± 7) days after the last dose administered in study A536-04, which may coincide with the patient's A536-04 End of Treatment (EOT) visit. These patients are considered "patients without treatment interruption".

Patients who complete the EOS visit for study A536-04 and are ≥ 28 days post EOS visit are considered "patients with treatment interruption" and will be re-assessed for eligibility by meeting all eligibility criteria plus additional inclusion criteria, as defined in Section 9.3 of the protocol.

Patients who do not meet the above criteria (e.g., > 35 days after last dose in study A536-04 and < 28 days post EOS visit in study A536-04) may still participate, but should be treated as patients with treatment interruption and should not begin study A536-06 C1D1 until they have reached ≥ 28 days post EOS visit from study A536-04 so that new baseline assessments can be measured.

For patients without treatment interruption, transfusion status (NTD or TD) will carry-over from the base study A536-04. For NTD patients with treatment interruption, transfusion status will be reassessed prior to C1D1 of study A536-06. NTD patients with treatment interruption are defined as patients who require transfusion of < 4 units of RBCs over the 8 weeks prior to Cycle

1 Day 1 of study A536-06. For TD patients with treatment interruption, transfusion status will carry-over from the base study A536-04.

A patient without treatment interruption may continue to be dosed with ACE-536 at the same dose level (rounded to nearest A536-06 starting dose level) administered at their last dose in study A536-04 (unless a dose reduction is required based upon patient dose modification rules from study A536-06). All patients with treatment interruption will be initially treated with ACE-536 at a starting dose level of 0.8 mg/kg which has been determined to be safe and well tolerated by the Safety Review Team (SRT) based on data from study A536-04. Examples of possible starting dose levels with dose modifications (reductions and titrations) are below for reference.

Table 1: Examples of Possible Starting Dose with Dose Modifications (Reductions and Titrations)

3rd Dose Reduction	2nd Dose Reduction	1st Dose Reduction	Starting Dose Level	1st Dose Titration	2nd Dose Titration
0.2 mg/kg	0.4 mg/kg	0.6 mg/kg	**0.8 mg/kg**	1.0 mg/kg	1.25 mg/kg
0.4 mg/kg	0.6 mg/kg	0.8 mg/kg	**1.0 mg/kg**	1.25 mg/kg	
0.6 mg/kg	0.8 mg/kg	1.0 mg/kg	**1.25 mg/kg**		

- Starting dose level for TD and NTD patients to be determined based on data from study A536-04 (range: 0.2-1.25 mg/kg).
- Starting dose level for patients without treatment interruption will be last dose level (rounded to nearest A536-06 starting dose level) administered in study A536-04 unless a dose reduction is required based upon patient dose modification rules from study A536-06.
- Patients may be titrated up and down dose levels as required per protocol to meet dose modification and titration rules.
- Patients who require more than 2 dose reductions due to an AE should be discontinued from treatment and complete the EOT and EOS visits.
- The maximum dose titration will not exceed the maximum dose level tested in base study A536-04.
- Each TD patient will have a defined "pre-transfusion hemoglobin threshold" which will be calculated based on transfusion history and will be used for determining when to transfuse during the study. The baseline pre-transfusion hemoglobin threshold will be the mean of all documented pre-transfusion hemoglobin values during the 12 weeks prior to C1D1 of base study A536-04 (for patients without treatment interruption) or study A536-06 (for patients with treatment interruption). During treatment, if the pre-transfusion hemoglobin level is increased by $\geq$ 1 g/dL compared to the baseline pre-transfusion hemoglobin threshold for that patient, transfusion should be delayed by a minimum of 7 days and/or the number of units transfused should be reduced by 1 or more RBC units. Patients may be transfused at the investigator's discretion for symptoms related to anemia or other requirements (e.g., infection).

Patients will participate in the extension study A536-06 for up to 97 months, including a 28-day (1 month) screening period, a 60-month treatment period and a 3-year (36 months) follow-up period.

3.2. Treatment Discontinuation

Patients were informed that they have the right to withdraw from the study at any time for any reason without prejudice to their medical care.

A patient may be discontinued from treatment for any of the following reasons:

- Patient's request
- Patient's unwillingness or inability to comply with the protocol
- Pregnancy
- Use of prohibited medication (e.g., hydroxyurea)
- Medical reason or adverse event, at the discretion of the investigator and/or the medical monitor
- Hypersensitivity reaction to study drug
- At the discretion of the sponsor (e.g., termination of the study or a dose level)

A patient may be withdrawn from the study for any of the following reasons:

- Patient's request
- Patient's unwillingness or inability to comply with the protocol
- Death
- Loss to follow-up
- At the discretion of the sponsor (e.g., termination of the study)

Patients who discontinue treatment early should complete the end of treatment (EOT) follow-up visit at the time of discontinuation and then complete the end of study (EOS) follow-up visit approximately 28 days later.

3.3. Sample Size

There is no formal sample size calculation for the study although up to 64 patients may participate from the A536-04 study.

4. ANALYSIS POPULATIONS

4.1. The Intent-To-Treat (ITT) Population

The intent-to-treat (ITT) population consists of all patients enrolled in the study who received at least one dose of ACE-536 in the A536-06 study. This population will be used for all efficacy analysis.

For patients who directly rolled over from A536-04 study, both A536-04 study and A536-06 study data will be included for the analysis.

For interrupted patients, only A536-06 study data will be included for the analysis.

4.2. Safety Population

Same as ITT population.

4.3. Pharmacokinetics (PK) Population

The pharmacokinetics population will include all patients who received at least 1 dose of ACE-536 during Study A536-06 and have sufficient serum ACE-536 values for PK analysis.

5. STATISTICAL METHODOLOGY

5.1. Definitions

Transfusion Status

For patients without treatment interruption, transfusion status (NTD or TD) will carry-over from the base study A536-04.

For NTD patients with treatment interruption, transfusion status will be reassessed prior to C1D1 of study A536-06. NTD patients with treatment interruption are defined as patients who require transfusion of < 4 units of RBCs over the 8 weeks prior to Cycle 1 Day 1 of study A536-06.

For TD patients with treatment interruption, transfusion status will carry-over from the base study A536-04.

Transfusion Dependent (TD) Defined in Base Study A536-04

Based on the protocol, TD patients are defined as those who required ≥ 4 units of RBCs every 8 weeks (confirmed over 6 months prior to study A536-04 Cycle 1 Day 1). For statistical programming purposes, a patient is defined as TD if the total RBCs is ≥ 11 units during 26 weeks on or prior to study A536-04 Cycle 1 Day 1.

Non-transfusion Dependent (NTD) Defined in Base Study A536-04

NTD patients are those who do not meet the above definition for TD. For statistical programming purposes, NTD patients are defined as those who received < 11 units of RBCs during 26 weeks on or prior to study A536-04 Cycle 1 Day 1.

Baseline

For patients who roll over to Study A536-06 without interruption, the baseline for Study A536-04 will be used as the baseline for Study A536-06 for all measurements, as further defined below. For patients who enter Study A536-06 with interruption, the baseline for Study A536-06 will be used, as further defined below. For assessments that are not scheduled to be done or with an inadequate data collection window at the beginning of Study A536-06, the baseline for Study A536-04 will be used as the baseline for Study A536-06.

Baseline Hemoglobin

Baseline hemoglobin will be the average of hemoglobin measurements within 28 days of Cycle 1 Day 1, excluding measurements within 14 days following RBC transfusion.

Baseline Transfusion Burden

Baseline transfusion burden will be calculated as the total amount of RBC transfusions during the 12 weeks on or prior to Cycle 1 day 1.

Baseline for Other Parameters

Baseline erythropoietin (EPO) is defined as the maximum test value within 28 days prior to Cycle 1 Day 1. For all other parameters, baseline is defined as the last observation on or prior to Cycle 1 Day 1.

Transfusion Unit Conversion

A transfusion amount reported in "mL" will be converted to "units" according to medical review.

End of Treatment (EOT)

Procedures and evaluations for the end of treatment visit should be performed 28 days (±7 days) after the last dose of ACE-536.

Post-Treatment Follow-Up (PTFU)

The PTFU visit should occur 2 months (± 7 days) after the last dose of ACE-536. If a patient has a positive ADA result at the last visit, the patient may be asked to return for additional ADA testing every three months, until a negative result is obtained or the result is considered to be stabilized.

Long-Term Follow-Up (LTFU)

LTFU visits should occur beginning every 6 months (± 14 days) after the last dose of ACE-536 for 3 years after the last dose of ACE-536.

End of Study (EOS)

Procedures and evaluations for the end of study visit should be performed approximately 3 years after the last dose of ACE-536.

5.2. General Considerations

Unless otherwise noted, continuous data will be summarized with the following descriptive statistics: number of observations (n), mean, standard deviation (STD), minimum, median, and maximum. Categorical data will be summarized with frequencies (n) and percentages (%). In cases where missing data cause percentages not to sum to 100, a missing data row will be provided. Percentages will use column totals as the denominator unless otherwise indicated. For time to event variables, the Kaplan-Meier curves will be presented if the number of patients is more than 5.

All study data will be included in study data listings. Missing data will generally be treated as missing, not imputed, unless otherwise stated.

Data summaries may also be presented for TD and NTD patients separately as specified. All summaries will be descriptive. No formal hypothesis testing is planned.

5.3. Disposition of Patients

The number and percentage of patients receiving study treatment who completed the treatment period and study period along with the associated reasons for discontinuation from treatment and/or withdrawal from study will be presented.

5.4. Demographic, Baseline Characteristics, and Disease History

The following baseline and demographic characteristics will be summarized by descriptive statistics for the ITT population as well as for TD and NTD patients separately:

- Race, ethnicity, age, sex, height, weight
- Splenectomy (yes or no), iron chelation therapy (yes or no), prior β-thalassemia treatment (yes or no)
- Baseline hemoglobin for NTD patients only and baseline transfusion burden for TD patients only.
- Baseline LIC and serum ferritin
- Baseline EPO and time from diagnosis in years
- Baseline iron intake

Demographic and baseline data, medical history, and disease history data will be listed for each patient. Medical History data will be summarized using descriptive statistics.

5.5. Study Drug Exposure

Study drug exposure will be descriptively summarized for safety population and will present the duration of exposure, the number of treatment cycles, the total dose administered, the number of patients with dose delay and reduction, and the number of patients with dose increase (titration).

The duration of exposure will be calculated as (last dose date – first dose date) + 21.

The total number of cycles will be summarized by presenting the number and percentage of patients in each category.

The total dose administered is the total amount of study drug in mg a patient received during the treatment period.

Study drug administration details will be listed for each patient.

5.6. Prior and Concomitant Medication and Procedures

5.6.1. Prior and Concomitant Medication

The prior and concomitant medications are coded with WHO dictionary. The medications will be summarized for the safety population.

Medications will be assigned as prior or concomitant based on the following rules:

- If both the start and stop date exist and are before the first dose date of study drug, the medication will be counted as prior.
- If the start date is on or after the first dose date of study drug, the medication will be counted as concomitant.
- If the start date is before the first dose date of study drug and the stop date is after the first dose date of study drug or the medication is ongoing, the medication will be counted as prior and concomitant.
- If the start date is missing and the stop date is before the first dose of study drug, the medication will be counted as prior.
- If the start date is missing and the stop date is after the first dose of study drug or the medication is ongoing, the medication will be counted as concomitant.
- If the start and stop dates are missing, the medication will be counted as concomitant.

All prior and concomitant medications will be listed for each patient.

5.6.2. RBC Transfusions

The RBC transfusion records prior to and during treatment will be listed for each patient. The hemoglobin values prior to transfusion will also be listed.

5.6.3. Non-Medication Procedures

Non-medication procedures will be coded using MedDRA Version 20.0. All non-medication procedures will be listed for each patient.

5.7. Efficacy Analyses

No formal hypothesis testing is planned. All efficacy endpoint will be performed using the ITT population.

In general, the below rules apply to the derivations of efficacy endpoints which utilize hemoglobin and RBC transfusion data unless specified otherwise:

- Hemoglobin measurements within 14 days following RBC transfusion will be excluded from the efficacy analysis.
- For each patient, all efficacy endpoints will be derived based on an analysis cutoff day, defined as the last dose + 56 days or the last date from transfusion record data, whichever is earlier.

5.7.1. Primary Efficacy Endpoint

Since the primary objective for this study is to evaluate the long-term safety and tolerability of ACE-536 in patients with β-thalassemia who were previously enrolled in study A536-04, there is no primary efficacy endpoint specified for this study.

5.7.2. Secondary Efficacy Endpoints

5.7.2.1. Erythroid Response

Erythroid response rates will be defined as below for NTD and TD patients.

For NTD patients:

- The proportion of patients with a mean increase of ≥ 1.0 g/dL from pretreatment hemoglobin during any rolling 8 weeks
- The proportion of patients with a mean increase of ≥ 1.5 g/dL from pretreatment hemoglobin during any rolling 8 weeks
- The proportion of patients with a mean increase of ≥ 1.0 g/dL from pretreatment hemoglobin during any rolling 12 weeks
- The proportion of patients with a mean increase of ≥ 1.5 g/dL from pretreatment hemoglobin during any rolling 12 weeks
- The proportion of patients with a mean increase of ≥ 1.0 g/dL from pretreatment hemoglobin during any rolling 24 weeks
- The proportion of patients with a mean increase of ≥ 1.5 g/dL from pretreatment hemoglobin during any rolling 24 weeks
- The proportion of patients with a mean increase of ≥ 1.0 g/dL from pretreatment hemoglobin during weeks 13-24
- The proportion of patients with a mean increase of ≥ 1.5 g/dL from pretreatment hemoglobin during weeks 13-24
- The proportion of patients with a mean increase of ≥ 1.0 g/dL from pretreatment hemoglobin during weeks 37-48

- The proportion of patients with a mean increase of ≥ 1.5 g/dL from pretreatment hemoglobin during weeks 37-48

For TD patients:

- The proportion of patients who have ≥ 20% reduction in RBC transfusion burden compared to pretreatment using rolling 8-week intervals
- The proportion of patients who have ≥ 33% reduction in RBC transfusion burden compared to pretreatment using rolling 8-week intervals
- The proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using rolling 8-week intervals
- The proportion of patients who have ≥ 20% reduction in RBC transfusion burden compared to pretreatment using rolling 12-week intervals
- The proportion of patients who have ≥ 33% reduction in RBC transfusion burden compared to pretreatment using rolling 12-week intervals
- The proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using rolling 12-week intervals
- The proportion of patients who have ≥ 33% reduction in RBC transfusion burden compared to pretreatment during weeks 13-24
- The proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment during weeks 13-24
- The proportion of patients who have ≥ 33% reduction in RBC transfusion burden compared to pretreatment using rolling 24-week intervals
- The proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using rolling 24-week intervals
- The proportion of patients who have ≥ 33% reduction in RBC transfusion burden compared to pretreatment during weeks 37-48
- The proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment during weeks 37-48
- The proportion of patients who have no RBC transfusions ≥ 8 weeks

For each of the above defined erythroid response rates, a point estimate will be presented along with the exact 95% confidence interval based on binomial distribution.

5.7.2.2. Time to and Duration of Erythroid Response

Time to and duration of erythroid response will be analyzed for erythroid responders defined as below:

- For NTD patients, mean increase of ≥ 1.0 g/dL from pretreatment hemoglobin during any rolling 12 weeks

- For NTD patients, mean increase of ≥ 1.5 g/dL from pretreatment hemoglobin during any rolling 12 weeks
- For NTD patients, mean increase of ≥ 1.0 g/dL from pretreatment hemoglobin during any rolling 24 weeks
- For NTD patients, mean increase of ≥ 1.5 g/dL from pretreatment hemoglobin during any rolling 24 weeks
- For TD patients, ≥ 33% reduction in RBC transfusion burden compared to pretreatment using 12-week intervals
- For TD patients, ≥ 50% reduction in RBC transfusion burden compared to pretreatment using 12-week intervals
- For TD patients, ≥ 33% reduction in RBC transfusion burden compared to pretreatment using rolling 24-week intervals
- For TD patients, ≥ 50% reduction in RBC transfusion burden compared to pretreatment using rolling 24-week intervals

Time to erythroid response will be defined as the time from the first dose date to the first date of any rolling 12- or 24-week interval achieving above erythroid response.

Duration of erythroid response will be defined as the time from the starting date of the first rolling 12- or 24-week interval achieving response to the last date of the last consecutive rolling 12- or 24-week interval achieving response. When there are multiple disjointed intervals with response, the longest interval will be used. Patients with response ongoing by the analysis cutoff day will be censored.

Both time to and duration of erythroid response will be analyzed as continuous variables and summarized by descriptive statistics. In addition, duration of erythroid response will also be analyzed as a time to event endpoint.

The derivations of time to and duration of erythroid response will be listed for each patient.

Erythroid responders may experience multiple periods of response (≥ 8 weeks, ≥ 12 weeks, or ≥ 24 weeks). For these responders, the number of episodes and cumulative duration will be summarized by NTD and TD separately. As an exploratory analysis, the cumulative duration of the multiple responses will be analyzed using Kaplan-Meier method.

5.7.2.3. Change of Hemoglobin Level and Transfusion Burden

The mean change in hemoglobin level from baseline over 8- or 12-week period in NTD patients and the mean % change from baseline in transfusion burden over an 8- or 12-week period in TD patients will be summarized. In addition, the mean change in pre-transfusion hemoglobin levels in TD patients (including analysis of patient subset with < 20% change in transfusion burden) will also be analyzed. Summary statistics will be presented for these parameters.

5.7.2.4. Iron-Related Parameter Analysis

Selected iron metabolism parameters will be taken at Day 1 of Cycles 1, 5, 9, 13, 17, 21, Day 1 of every 4 subsequent cycles until cycle 85, EOT, and EOS visits. Descriptive statistics of actual

values, absolute and percentage change from baseline values at each post-baseline time point will be presented. A shift table will be produced for ferritin from baseline to post-baseline.

Plots of iron parameters (serum iron, TIBC, transferrin, calculated transferrin saturation, ferritin, NTBI) for both mean observed values and mean absolute change from baseline over time will be presented.

5.7.2.5. Erythropoiesis Parameters

Erythropoiesis parameters include serum EPO levels, reticulocytes, nucleated RBCs, soluble transferrin receptor, hemoglobin electrophoresis, and folate.

EPO will be taken at Day 1 of Cycle 1, Days 1 and 8 of Cycles 5, 9, 13, 17, Day 1 of every 4 subsequent cycles until cycle 85, EOT, and EOS. Reticulocytes and nucleated RBCs will be taken at Screening, Days 1 and 8 of Cycles 1, 3,5,7,9,11,13,15,17, Day 1 of Cycles, 2,4,8,10,12,14,16 and Cycles 18 to 87, EOT and EOS visits. Soluble transferrin receptor will be taken at Day 1 of Cycles 1, 5, 9, 13, 17, Day 1 of every 4 subsequent cycles until Cycle 85, EOT, and EOS. Hemoglobin electrophoresis will be taken at Day 1 of Cycles 1, 5, 13, 21, 29, 37, 45, 53, 61, 69, 77, EOT, and EOS. Serum folate will be taken at screening for patients with treatment interruption to confirm eligibility, and required at Day 1 of Cycles 1, 5, 13, 21, 29 37, 45, 53, 61, 69, 77, and EOT.

Descriptive statistics of observed values, absolute and percentage change from baseline values at each post-baseline visit will be presented for all patients. Plots of erythropoiesis parameters for mean observed values, mean change, and mean percentage change from baseline over time will be presented.

5.7.2.6. Hemolysis Parameters

Hemolysis parameters include total bilirubin, indirect bilirubin, and lactate dehydrogenase (LDH). Measurements will be done at Screening, Day 1 of Cycles 1, 3, 5, 9, 13, 27, 2, 25, 29, 33, 37, 41, 45, 49, 53, 57, 61, 65, 69, 73, 77, 81, EOT, and EOS visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each post-baseline visit will be presented. Plots of hemolysis parameters for mean observed values, mean change, and mean percentage change from baseline over time will be presented.

5.7.2.7. Liver Iron Content (LIC)

MRI for liver iron content (LIC) will be performed. Descriptive statistics of actual values, absolute and percentage change from baseline at post-baseline visits will be presented. The summary will be done by NTD/TD, baseline LIC ≥ 3 and < 3 mg/g dw subgroups and baseline iron chelation therapy (ICT) use (yes vs. no) respectively.

LIC responses will be defined as LIC reduction ≥ 1 mg/g dw at post-baseline visits compared with baseline. LIC response rates along with the exact 95% confidence interval will be estimated for NTD and TD patients respectively, as well as by baseline LIC (< or > = 3 mg/g dw) and baseline ICT use (yes vs. no). As an exploratory analysis, LIC change from baseline will be summarized by TD responders and non-responders, where TD responder is defined as 33% reduction in rolling 24 week and 50% reduction in rolling 12 weeks, respectively.

5.7.3. Exploratory Endpoints

5.7.3.1. Biomarkers Related to TGF-β

Biomarkers hepcidin, GDF8, GDF11, and GDF15 will be presented in the listing.

5.7.3.2. Quality of Life (QoL) Questionnaire

QoL questionnaires including FACT-An and SF-36 will be completed at Day 1 of Cycles 1, 5, 9, 13, 17, 25, 33, 41, 49, 57, 65, 73, 81, and EOT. The scoring algorithms for FACT-An and SF-36 are included in Appendix 1.

Individual subscale scores and total scores will be summarized by visit for FACT-An, FACIT-F, and SF-36. FACIT-F scores will also be summarized by visit for TD and NTD groups respectively. NTD-PRO score will be listed and summarized by visit for NTD group only. All derived subscale and total scores along with all individual responses will be listed for each patient.

Pairwise correlations among 6MWT, FACIT-F, SF-36, and hemoglobin will be evaluated at Week 16 and 48. In addition, correlation between NTD-PRO Tiredness and Weakness domain score and FACIT-F score will be evaluated for the during of study.

5.7.3.3. Other Analysis.

The following additional exploratory endpoints will be presented in listings only:

- Extramedullary hematopoiesis (EMH) mass size by MRI
- Spleen size by MRI (in subset of patients with no prior-splenectomy)
- %change and absolute change of bone mineral density (BMD) raw score and z-score by DXA
- Leg ulcer size (area)
- Change and %change in 6-minute-walk test (6MWT) distance in NTD patients
- Hormone parameters (including estradiol, free testosterone, and total testosterone)

5.7.4. Subgroup Analysis

Subgroup analyses of erythroid response will be performed with the following baseline factors, for NTD and TD patients separately:

- Age (< 32 and ≥ 32 years)
- Sex (male and female)
- Prior iron chelation therapy (yes and no)
- Baseline LIC (< 3, 3-5, ≥ 5 mg/g dw)
- Baseline EPO (< 200 and ≥ 200 IU/L)
- Splenectomy (yes and no)
- Baseline iron intake (< 0.4 mg Fe/kg/day and ≥ 0.4 mg Fe/kg/day)

- Baseline transfusion burden (RBC transfusion > 10 units per 12 weeks vs < =10 units per 12 weeks)

5.8. Safety Analysis

The safety endpoints will be summarized using the Safety Population. The safety endpoints include treatment emergent adverse events, changes in laboratory tests, vital signs and ECG's.

Severity of AEs will be coded using National Cancer Institute Common Toxicity Criteria for Adverse Events version 4.0 (NCI-CTCAE v4.0).

5.8.1. Adverse Events

For patients with treatment interruption, all non-serious AEs occurring after signing of the ICF until a patient is dosed on C1D1 are to be documented on the medical history CRF. All AEs and SAEs occurring after the Cycle 1 Day 1 dose through 56 days after the last study drug administration are to be reported and documented on the AE CRF. All AEs collected in this study are treatment emergent adverse events (TEAE). A drug-related TEAE is defined as any TEAE related to the study medication as assessed by the investigator or with missing assessment of the causal relationship.

The following summaries will be presented for all dosed:

- Number and percentage of patients reporting each AE, categorized by System Organ Class (SOC) and Preferred Term (PT)
- Number and percentage of patients reporting each AE experienced by ≥ 5% and ≥ 10% of patients in all patients by PT
- Number and percentage of patients reporting SAE, categorized by SOC and PT
- Number and percentage of patients reporting Grade ≥ 3 AE, categorized by SOC and PT
- Number and percentage of patients reporting related AE, categorized by SOC and PT
- Number and percentage of patients reporting AE leading to drug withdrawal, categorized by SOC and PT
- Number and percentage of AE of interests by cycle

Note that counting will be by patient, not event, and patients are only counted once within each SOC or PT. If a patient experiences the same AE at more than one severity, or with more than one relationship to study drug, the most severe rating or the stronger causal relationship to study drug will be given precedence. Any missing severity, causality, or outcome will not be imputed and classed as unknown.

All AEs will be listed. The following listings will also be provided: 1) patients with SAEs; 2) patients with Grade ≥ 3 AEs; and 3) patients with AEs leading to study drug discontinuation; 4) death.

Concomitant medication use, severity, time to the worst CTCAE grade and recovery will be listed and summarized.

5.8.2. Laboratory Evaluations

The following laboratory parameters will be measured over time:

Hematology:

RBC, white blood cell (WBC) with differential, hemoglobin, hematocrit, haptoglobin (optional), reticulocyte count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), and nucleated RBCs (nRBCs).

Chemistry:

Sodium, potassium, chloride, carbon dioxide/bicarbonate (optional), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin, indirect bilirubin, alkaline phosphatase, blood urea nitrogen (BUN)/urea, creatinine, gamma-glutamyl transpeptidase (GGT), calcium, phosphorus, glucose, amylase, lipase, total protein, albumin, and uric acid.

Urine Analysis:

Local lab dipstick analysis will be done for specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if performed.

Urine Chemistry:

Microalbumin and creatinine will be performed by the central lab; albumin/creatinine ratio will be provided.

Categorical and numeric variables will be presented separately. Actual values and changes in hematology and biochemistry laboratory values from baseline will be summarized by time point. Shift tables for the hematology and biochemistry laboratory parameters comparing values above, within and below the normal reference range at baseline to the end of treatment visit will be presented using standard reference ranges.

Actual urine laboratory values will be listed.

Peripheral blood smears data will be listed only. All laboratory values will be listed for all patients.

5.8.3. Vital Signs

Vital signs parameters include weight (kg), heart rate, systolic and diastolic blood pressure, respiratory rate, and temperature (°C). For each parameter at each time point, the change from baseline to post baseline will be summarized.

Plots of vital sign parameters for both mean observed and mean change from baseline will be presented for each parameter. Vital signs will also be listed for all patients.

5.8.4. Echocardiogram (ECG) Results

The quantitative ECG assessments (ventricular rate, QRS width, PR interval, and QTc interval) will be summarized at each time point.

ECG overall interpretation (normal, abnormal not clinically significant and abnormal clinically significant) will be presented for actual values and changes from baseline (Screening observation) to each post baseline visit [expressed as Improvement, No Change, and Deterioration].

Note:

- Improvement = Abnormal Clinically Significant (CS) to Abnormal Not Clinically Significant (NCS)/Normal, Abnormal NCS to Normal
- Deterioration = Normal to Abnormal NCS/CS, Abnormal NCS to Abnormal CS
- No change = Normal to Normal, Abnormal NCS to Abnormal NCS, Abnormal CS to Abnormal CS

If either result is missing for any patient, then an 'Unknown' category will be presented.

ECG results will be listed for all patients.

5.8.5. Physical Examination

Physical exam details will be listed only.

5.8.6. Post-treatment Follow-up and Long-term Follow-up

A listing of post-treatment follow-up and Long-term follow-up data including the visit date, malignancy status, and survival status will be provided.

5.9. Pharmacokinetics Analysis

5.9.1. Pharmacokinetic Sampling Schedule

Blood samples will be collected from all patients at the following schedule:

Visit	Study Day
Cycle 1 Day 1	1
Cycle 1 Day 8	8
Cycle 2 Day 1	22
Cycle 9 Day 1	169
Cycle 9 Day 8	176
Cycle 10 Day 1	190
Cycle 17 Day 1	337
Cycle 17 Day 8	344

5.9.2. Data Handling

Concentrations that are below the limit of quantitation (BLQ) prior to the first dose will be assigned a numerical value of zero. Post-treatment concentrations that are BLQ will be treated as missing.

Concentrations assigned a value of missing will be omitted from the descriptive statistics. A concentration value of zero will be excluded from the computation of the geometric mean (geometric CV%). If any patients are found to be noncompliant with respect to dosing, have incomplete data, or encounter other circumstances that would affect the evaluation of pharmacokinetics, a decision will be made on a case-by-case basis as to their inclusion in the pharmacokinetic analysis. Data excluded from pharmacokinetic analysis will be included in the data listings, but not in the summaries.

In tables and listings for the derived pharmacokinetic data, there should be four decimal places for numerical values below 1, three decimal places for numeric values below 10 but above 1, and two decimal places for numeric values above 10. However, the listings of raw data should not have more decimal places than the actual data.

5.9.3. Pharmacokinetic Analysis

All ACE-536 serum concentrations will be listed by patient and scheduled time (visit and study). Actual dosing/sampling time, sample time relative to first dosing time, visit, and concentration as done for the 04 study will be presented in the listing.

The ACE-536 serum concentrations will be summarized by scheduled time, including N (number of observations), arithmetic mean, arithmetic standard deviation (SD), arithmetic coefficient of variation (CV%), geometric mean, geometric CV%, minimum, median, and maximum. Mean (SD) serum concentration-time profiles will be presented on linear scales.

5.10. Interim Analysis

There are no planned interim analyses. However, safety and erythroid response data will be reviewed periodically throughout the study.

5.11. Protocol Deviations

Protocol deviations will be listed for the following categories:

- Not meeting inclusion/Exclusion criteria
- Withdraw criteria met, but patient not discontinued:
- Failure to perform key procedures
- Study treatment deviation
- Prohibited concomitant medication
- GCP related deviation

5.12. Data Handling

5.12.1. Analysis Visit Window

As specified in Section 5.1, for patients who rollover to Study 06 without interruption, the baseline for Study 04 will be used as the baseline for Study 06; for patients who rollover to Study 06 with interruption, the last value before the first dose of Study 06 will be considered as the baseline. As Study 04 and Study 06 have different schedules of events (Appendix 3 and

Appendix 4), analysis window will be derived for summary of endpoints by time points. Analysis window determination will be data driven by each parameter. Detailed rules will be specified in the programming specifications.

5.12.2. Handling of Missing Data

As a general principle, no imputation of missing data for other variables will be done. Exceptions are the start and stop dates of AEs and concomitant medication with the rules listed below. The imputed dates will be used to allocate the medication as prior or concomitant medications and to determine whether an AE is/is not treatment emergent. Listings of the AEs and concomitant medications will present the actual partial dates; imputed dates will not be shown.

In addition, partial disease diagnosis dates are imputed to calculate time since disease diagnosis. The listing will present the actual partial dates.

5.12.3. Missing Dates for Adverse Event

a. Imputing partial AE start dates: If the year is unknown, the date will not be imputed and will be assigned a missing value.
b. If the month is unknown, then:
   - If the year matches the first dose date, then impute the month and day of the first dose date.
   - Otherwise, assign January.
c. If the day is unknown, then:
   - If the month and year match the first AE stop month and year and AE stop day is not missing, then impute the start day as the stop day.
   - Otherwise impute start day using the last day of the start month.

Imputing partial AE stop dates:

a. If the year is unknown, the date will not be imputed and will be assigned a missing value.
b. If the month is unknown, then assign December.
c. If the day is unknown, then assign the last day of the month.

5.12.4. Missing Dates for Concomitant Medication

If start date is missing or partial:

a. if month is missing, use January
b. if day is missing, use the first day of the month under consideration
c. if year is missing, use year of the informed consent date
d. if entire date is missing, use informed consent date

If stop date is missing or partial:

a. if month is missing, use December
b. if day is missing, use the last day of the month under consideration

c. if year or the entire date is missing, set to 31 December 2099

If the imputed start date is after the stop date, then the imputed start date will be one day prior to the stop date.

5.12.5. Missing Dates for Disease Diagnosis Date

For disease diagnosis dates, the imputation rules are

a. if day is missing, use 15th of the month
b. if both day and month are missing, impute as January 1st
c. if month is missing, impute as January
d. if year is missing, set to missing

5.12.6. Changes in Conduct or Planned Analyses from the Protocol

Major changes between SAP and the planned analysis in Protocol Amendment 4 are described below:

Changes	Rationale
Definition of baseline hemoglobin	Baseline hemoglobin for NTD patients will be the average of two or more measurements performed during the screening period for base study A536-04 (for patients without treatment interruption) or study A536-06 (for patients with treatment interruption). Hemoglobin measurements within 2 weeks following RBC transfusion will be excluded. This definition is revised in this SAP to reflect the derivation rule implemented for this study.
Remove Efficacy Evaluable (EE) population	ITT provides more conservative estimate of treatment effect. Since there is no ITT population, the word mITT was change to ITT to be consistent with study 04 analysis population.

6. REFERENCES

National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0 (NCI-CTCAE v4.0)

7. APPENDICES

7.1. Appendix 1 - FACT-An and SF-36 Scoring Algorithm

The Fact-An questionnaire contains 47 questions which are divided into the following sub scales:

- Physical Well-Being (PWB) (7 questions: Item Score range 0 - 28)
- Social/Family Well-Being (SWB) (7 questions: Item Score range 0 - 28)
- Emotional Well-Being (EWB) (6 questions: Item Score range 0 - 24)
- Functional Well-Being (FWB) (7 questions: Item Score range 0 - 28)
- Anemia Subscale (AnS) (20 questions: Item Score range 0 - 80)
- Fatigue Subscale (FACIT-F) (13 questions: Item Score range 0 – 52)
- Fatigue Experience (FE) (5 questions: Item Score range 0 – 20)
- Fatigue Impact (FI) (8 questions: Item Score range 0 – 32)

Patients give individual responses to each question on a scale of 0 to 4 (0=Not at all; 1=A little bit; 2=Somewhat; 3=Quite a bit; 4=Very much). Item scores in the PWB and EWB sub scales will be derived by subtracting the response value from 4. Similarly, all Item Scores in the AnS subscale except Item Codes An5, An7, BL4 and An13[3] will be derived by subtracting the response value from 4. Thus, a higher Item Scores indicates a better quality of life.

Subscale totals will be derived as follows:

$$\frac{\text{Sum of Item Scores x Number of Items in Subscale}}{\text{Number of Items Answered}}$$

For example, if 6 questions are answered in the Physical Well-Being subscale and the Item scores sum to a total score 18 the subscale score will be (18 x 7)/6 = 21.

If 50% or more of responses in any subscale are missing the subscale total will be set to missing.

The following total scores will also be derived:

FACT-An Total Outcome Index (TOI) derived as: PWB + FWB + AnS (Score range 0 – 136)

FACT-G Total Score (FACT-G) derived as: PWB + SWB + EWB + FWB (Score range 0 – 108)

FACT-An Total Score (FACT-An) derived as: PWB + SWB + EWB + FWB + AnS (Score range 0 – 188)

If 20% or more of the responses that contribute to the FACT-G score are missing the FACT-G score will be set to missing (i.e. at least 22 of the 27 items contributing to FACT-G must be present). Furthermore, FACT-An TOI, FACT-G and FACT-An scores should only be calculated if all component subscales have valid scores. If any subscale total is missing the respective total scores to which the subscale contributes will also be set to missing. A higher FACT total score indicates a better quality of life.

SF-36 Scoring Algorithm

The 36 individual items scores will be used to compute the 8 domain scores (Physical Functioning, Role-Physical, Bodily Pain, General Health, Vitality, Social Functioning, Role-Emotional, and Mental Health). The 8 domains scores will be transformed to a scale from 0 to 100. The transformed scores will be used in the analysis. The Physical Component Scores (PCS) and Mental Component Scores (MCS) will be computed from the transformed domain scores.

The scoring of the domain and component scores will follow the following steps:

- Reverse code 10 of the items (Items 1, 6, 7, 8, 9a, 9d, 9e, 9h, 11b and 11d). Algorithm for reverse coding for those 10 items is as follows:
  1. If (Item 1 = 1 or 4 or 5) then Item 1 = 6 – Item1,

     Else if Item 1 = 2 then Item 1 = 4.4,

     Else if Item 1 = 3 then Item 1 = 3.4
  2. Item 6 = 6 – Item 6
  3. If (Item 7 = 1 or 6) then Item 7 = 7 – Item 7,

     Else if Item 7 = 2 then Item 7 = 5.4,

     Else if Item 7 = 3 then Item 7 = 4.2,

     Else if Item 7 = 4 then Item 7 = 3.1,

     Else if Item 7 = 5 then Item 7 = 2.2
  4. Reverse scoring of Item 8 depends on whether Item 7 is answered.

     If Item 7 is answered, then recode Item 8 as follows:

     If pre-coded Item 7 = 1 and Item 8 = 1 then Item 8 = 6, else Item 8 = 6 – Item 8.

     If Item 7 is not answered, then recode Item 8 as follows:

     If Item 8 = 1 then Item 8 = 6,

     Else if Item 8 = 2 then Item 8 = 4.75,

     Else if Item 8 = 3 then Item 8 = 3.5,

     Else if Item 8 = 4 then Item 8 = 2.25,

     Else if Item 8 = 5 then Item 8 = 1
  5. Item 9a = 6 – Item 9a
  6. Item 9d = 6 – Item 9d
  7. Item 9e = 6 – Item 9e
  8. Item 9h = 6 – Item 9h
  9. Item 11b = 6 – Item 11b
  10. Item 11d = 6 – Item 11d

- Compute raw scores of each domain by summing up the items in each domain as specified by Table 2. A domain score will be computed only if at least half of the items in the domain are non-missing. If at least half of the items in the domain are non-missing, missing score in the same domain will be replaced by the average value of the non-missing scores. Recoded scores will be used in the summation and the imputation. The raw domain scores will then be transformed into a scale of 0 to 100 based on their lowest and highest value in Table 2, using the following formula:

$$\text{Transformed Score} = ((\text{Actual} - \text{Lowest})/(\text{Highest} - \text{Lowest})) \times 100$$

Table 2: SF-36 Domain Score Items

Domain	Items	Lowest	Highest
Physical Functioning (PF)	Items 3a, 3b, 3c, 3d, 3e, 3f, 3g, 3h, 3i, 3j	10	30
Role-Physical (RP)	Items 4a, 4b, 4c, 4d	4	20
Bodily Pain (BP)	Items 7 and 8	2	12
General Health (GH)	Items 1, 11a, 11b, 11c, 11d	5	25
Vitality (VT)	Items 9a, 9e, 9g, 9i	4	20
Social Functioning (SF)	Items 6 and 10	2	10
Role-Emotional (RE)	Items 5a, 5b, 5c	3	15
Mental Health (MH)	Items 9b, 9c, 9d, 9f, 9h	5	25

Transformed score will be standardized to z scores based on the following formulas:

PF_Z = (PF – 83.29094) / 23.75883

RP_Z = (RP – 82.50964) / 25.52028

BP_Z = (BP – 71.32527) / 23.66224

GH_Z = (GH – 70.84570) / 20.97821

VT_Z = (VT – 58.31411) / 20.01923

SF_Z = (SF – 84.30250) / 22.91921

RE_Z = (RE – 87.39733) / 21.43778

MH_Z = (MH – 74.98685) / 17.75604

Transform z scores to T scores based on the following formulas:

PF_T=50+PF_Z*10

RP_T=50+RP_Z*10

BP_T=50+BP_Z*10

GH_T=50+GH_Z*10

VT_T=50+VT_Z*10

SF_T=50+SF_Z*10

RE_T=50+RE_Z*10

MH_T=50+MH_Z *10

The aggregate physical (AGG_PHYS) and mental (AGG_MENT) component scores are computed as:

AGG_PHYS = (PF_Z * .42402) + (RP_Z * .35119) + (BP_Z * .31754) + (GH_Z * .24954) + (VT_Z * .02877) + (SF_Z * -.00753) + (RE_Z * -.19206) + (MH_Z * -.22069)

AGG_MENT = (PF_Z * -.22999) + (RP_Z * -.12329) + (BP_Z * -.09731) + (GH_Z * -.01571) + (VT_Z * .23534) + (SF_Z * .26876) + (RE_Z * .43407) + (MH_Z * .48581)

Physical Component Scores (PCS) and Mental Component Scores (MCS) are computed as:

PCS = 50 + (AGG_PHYS * 10)

MCS = 50 + (AGG_MENT * 10)

7.2. Appendix 2 - List of Abbreviations

Abbreviation	Definition
6MWT	Six Minute Walk Test
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BSAP	Bone specific alkaline phosphatase
BP	Blood pressure
BUN	Blood urea nitrogen
CI	Confidence interval
C_{max}	Maximum concentration
CREAT	Creatinine
CRF	Case report form
CRO	Contract research organization
CTX	C-telopeptide of type I collagen
DLT	Dose-limiting toxicity
ECHO	Echocardiogram
ECG	Electrocardiogram
EE	Efficacy Evaluable
EOS	End of Study
EPO	Erythropoietin
EOT	End of Treatment
FACIT-F	Functional Assessment of Chronic Illness Therapy - Fatigue
FACT-An	Functional Assessment of Cancer Therapy-Anemia Scale
HbA	Adult hemoglobin
HbF	Fetal hemoglobin
HTB	High Transfusion Burden
ICF	Informed consent form
IEC	Independent ethics committee

Abbreviation	Definition
ITT	Intent-to-Treat
IB	Investigator's brochure
LDH	Lactate dehydrogenase
LIC	Liver Iron Content
LTB	Low Transfusion Burden
LTFU	Long-term follow-up
MedDRA	Medical Dictionary for Regulatory Activities
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MRI	Magnetic resonance imaging
MUGA	Multi gated acquisition scan
NCI-CTCAE	National Cancer Institute-Common terminology criteria for adverse events
nRBCs	Nucleated red blood cells
NTBI	Non-transferrin bound iron
PD	Pharmacodynamic
PK	Pharmacokinetic
PTFU	Post-treatment follow-up
QoL	Quality of life
RBC	Red blood cell
RDW	Red blood cell distribution width
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SD	Standard deviation
SF-36	Short Form (36-item) Health Survey
SRT	Safety review team
SUSAR	Suspected unexpected serious adverse reaction
$T_{1/2}$	Elimination half-life
TGF-β	Transforming growth factor beta

Abbreviation	Definition
TIBC	Total iron binding capacity
T_{max}	Time to maximum concentration
ULN	Upper limit of normal
WBC	White blood cell

7.3. Appendix 3 - Schedule of Events for Study A536-04

	Screen	Treatment Period																Follow up period	
		Cycle 1				Cycle 2			Cycle 3			Cycle 4			Cycle 5			EOT[12]	EOS[13]
		C1D1[2]	C1D8	C1D11	C1D15	C2D1[2,15]	C2D8	C2D15	C3D1[2,15]	C3D8	C3D15	C4D1[2,15]	C4D8	C4D15	C5D1[2,15]	C5D8	C5D15	Day 113 (± 7d)	Day 141 (± 7d)
	Day -28 to -1	Day 1	Day 8 (± 1d)	Day 11 (± 1d)	Day 15 (± 1d)	Day[14] 22 (± 2d)	Day 29 (± 2d)	Day 36 (± 2d)	Day[14] 43 (± 2d)	Day 50 (± 2d)	Day 57 (± 2d)	Day[14] 64 (± 2d)	Day 71 (± 2d)	Day 78 (± 2d)	Day[14] 85 (± 2d)	Day 92 (± 2d)	Day 99 (± 2d)		
Informed consent	X																		
Inclusion/Exclusion	X	X																	
Medical history	X																		
QoL Questionnaires[19]	X														X			X[20]	X
Physical examination	X	X[23]				X			X			X			X			X[23]	X[23]
Vital signs[1]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECG (12 lead)	X					X												X	
MRI (Liver Iron Content)[17]	X[3]																	X	
MRI for EMH masses and spleen[24]	X[3]																	X	
DXA for BMD of total body, lumbar spine, and total hip[24]	X[3]																	X	
Leg Ulcer Assessment[25]	X	X				X			X			X			X			X	X
Abdominal Ultrasound[21]	X[3]																	X	
ECHO, MUGA or cardiac MRI	X[3]																		
6MWT[24]	X																	X	
Serum iron studies[4]	X	X	X		X	X			X			X			X			X	X
Serum folate and B_{12}	X					X			X			X			X			X	X
Erythropoietin levels	X	X				X						X						X	X
Hematology[5]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Peripheral blood smear	X	X				X			X			X			X			X	
Serum chemistry[6]	X	X			X	X			X			X			X			X	X
Urinalysis and Urine Chemistry[7]	X	X				X						X						X	X
Anti-drug antibody[16]		X										X						X	X[16]
PK collection		X	X	X	X	X	X					X	X	X	X	X	X	X	X
PD Biomarkers[8]		X				X			X			X			X			X	X
Hemoglobin electrophoresis		X										X						X	
Globin mRNA sample		X										X						X	
Bone Biomarkers[9]		X										X						X	
Pregnancy test/menstrual history[10]	X					X			X			X			X			X	X[22]
Evaluate transfusion frequency/volume[11]	X	X				X			X			X			X			X	X
Concomitant medications and AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer ACE-536[18]		X				X[14]			X[14]			X[14]			X[14]				

[1] **Vital signs:** Weight, heart rate, systolic and diastolic blood pressure, respiration rate, and temperature (measured in degrees Celsius). Height is measured only at Screening. If at any visit the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg, and/or the absolute increase in either measure from baseline is ≥ 20 mmHg, perform one repeat of the blood pressure assessment after a minimum of 15 minutes.

[2] **Study procedures** must be done prior to administration of study drug.

[3] Screening MRI for Liver Iron Content, MRI of the chest and abdomen for EMH masses and spleen (expansion cohort only), BMD by DXA (expansion cohort only), abdominal ultrasound of the spleen (if no prior splenectomy; dose escalation cohorts only) and ECHO, MUGA or cardiac MRI can be performed up to 56 days prior to C1D1. If performed as part of standard of care, it does not need to be repeated.

[4] **Iron Studies:** Serum iron, TIBC, transferrin, soluble transferrin receptor, ferritin, and NTBI.

[5] **Hematology:** RBC, WBC with differential, hemoglobin, hematocrit, haptoglobin (optional), reticulocyte count, platelet count, MCV, MCH, MCHC, RDW, and nRBCs. On dosing days, hematology values are to be drawn and resulted (up to 1 day) prior to C1D1 (see Protocol Section 10.8.1 Patient Dose Modification Rules). Historical hemoglobin values will be collected for 12 weeks prior to C1D1. Historical transfusion history will be collected for 12 months prior to Cycle 1 Day 1. Baseline hemoglobin will be the average of two measurements; one measure performed within one day prior to Cycle 1 Day 1 and the other performed during the screening period (Day -28 to Day -1). Note: For any RBC transfusions received during the study, collect hemoglobin value just prior to transfusion.

[6] **Chemistry**: Sodium, potassium, chloride, carbon dioxide/bicarbonate (optional), AST, ALT, lactate dehydrogenase (LDH), total bilirubin, indirect bilirubin, alkaline phosphatase, blood urea nitrogen (BUN)/urea, creatinine, GGT, calcium, phosphorus, glucose, amylase, lipase, total protein, albumin, and uric acid.

[7] **Urinalysis by dipstick analysis:** pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if indicated. Microalbumin and creatinine will be performed by the central lab.

[8] **PD Biomarkers:** Hepcidin, GDF15 and others to be determined.

[9] **Bone Biomarkers:** BSAP and CTX.

[10] **Pregnancy** test (urine or serum) and menstrual history is required prior to C1D1 for female patients of child bearing potential only.

[11] Transfusion history will be collected for 12 months prior to C1D1.

[12] **End of Treatment (EOT):** Should be performed 28 days (± 7 days) after the last dose of ACE-536. Patients who discontinue treatment early should complete the end of treatment visit at the time of discontinuation and complete the Day 141 follow up visit 28 days (± 7 days) after the EOT visit.

[13] **End of Study (EOS):** Should be performed 28 days (± 7 days) after the Day 113/EOT visit (56 days after the last dose of ACE-536).

[14] **Day 85** ± 2 days is the last possible study day that ACE-536 may be administered, regardless of the cycle.

[15] If a **dose delay** is required per the dose modification rules the patient will not be dosed. The patient will return weekly to assess hematology results and adverse events until the patient is eligible to administer the next dose of ACE-536.

[16] If the patient has a positive ADA result at their last assessment, the patient may be asked to return approximately every three months for additional testing until a negative result is obtained or the result is considered stabilized.

[17] MRI for liver iron content will be performed at selected sites.

[18] For the first dose of ACE-536, dosing should occur after a minimum of 7 days post-transfusion; subsequent doses should not be given within 24 hours of transfusion or planned transfusion.

[19] Administration of quality of life questionnaires, including but not limited to FACT-An and SF-36 is required for patients in the expansion cohort only.

[20] QoL questionnaires including but not limited to FACT-An and SF-36 should be completed at the EOT visit only for expansion cohort patients who discontinue treatment early.

[21] Abdominal ultrasound of the spleen is required for patients with no prior splenectomy in the dose escalation cohorts only. Patients in these cohorts with clinical signs of a change in spleen size or abnormality should have an abdominal ultrasound as needed throughout study.

[22] Pregnancy test is not required at Day 141/EOS.

[23] Physical exam should include an optional evaluation of gonadal size in males at C1D1, EOT, and EOS visits only.

[24] MRI of the chest and abdomen for EMH masses and spleen, DXA for BMD and 6MWT for NTD patients will be performed in the expansion cohort patients only, at selected sites.

[25] Patients with leg ulcers should have regular assessment of the leg ulcer(s) throughout the study. Photographs of the leg ulcer(s) should be obtained to document any changes in leg ulcer(s) size.

7.4. Appendix 4 - Schedule of Events for Study A536-06

	Screening Day -28 to Day -1[1]	Cycle 1		Cycle 2	Cycle 3		Cycle 4	Cycle 5		Cycles 6-17: Repeat Cycles 2 through 5 three times[2,29]
		C1D1[1,2,13]	C1D8	C2D1[2,13]	C3D1[2,13]	C3D8	C4D1[2,13]	C5D1[2,13]	C5D8	
		Day 1	Day 8 (± 2d)	Day 22 (± 5d)	Day 43 (± 5d)	Day 50 (± 2d)	Day 64 (± 5d)	Day 85 (± 5d)	Day 92 (± 2d)	
Informed consent	X[1]									Cycles 6-17: Day 106 (± 2d) through 357 (± 2d)
Inclusion/Exclusion	X	X[2]								
Medical history[3]	X									
QoL Questionnaires[4]		X						X		
Physical examination[5]	X	X						(X)[5]		
Vital signs[6]	X	X		X	X		X	X		
ECG (12 lead)		X								
MRI for liver iron content[2, 7] (optional)		X						(X)[7]		
MRI for EMH masses[2, 8]		X						(X)[8]		
MRI for spleen size[2, 9]		X						(X)[9]		
DXA for BMD of total body, lumbar spine and total hip[29]		X						(X)[29]		
6MWT for NTD patients[30]		X						X		
Leg Ulcer Assessment[10]	Collected at each visit as applicable									
ECHO/MUGA (interruption patients only)	X[1]									
Serum iron studies[11]		X						X		
Serum folate[12]	X	X						(X)[12]		
Erythropoietin levels		X						X	X	
Hematology[13]	X	X	X	X	X	X	X	X	X	
Peripheral blood smear[14]		X						X		
Serum chemistry[15]	X	X			(X)[15]			X		
Urinalysis/urine chemistry[16]	X	X						X		
Anti-drug antibody[17]		X						X		
PK collection[18]		X	X	(X)[18]				(X)[18]	(X)[18]	
PD biomarkers[19]		X						X		
Hemoglobin Electrophoresis[20]		X						(X)[20]		
Pregnancy test[21]	X	X		X	X		X	X		
Menstrual History[22]	X	X		X	X		X	X		
Evaluate transfusion frequency/volume[23]	X	X		X	X		X	X		
Administer ACE-536		X		X	X		X	X		
Concomitant medications and AEs[24]	Collected Continuously									

	Cycle 18	Cycle 19	Cycle 20	Cycle 21	Cycle 22	Cycle 23	Cycles 24-87: Repeat Cycles 20 through 23 sixteen times[2,30]	EOT[25]	PTFU[26]	LTFU[27]	EOS[28]
	C18D1[2,1,3]	C19D1[2,1,3]	C20D1[2,1,3]	C21D1[2,1,3]	C22D1[2,1,3]	C23D1[2,1,3]		28 days (± 7 days) after the last dose of ACE-536 or at the time of discontinuation	2 months (± 7 days) after the last dose of ACE-536	Every 6 months (± 14 days) after the last dose of ACE-536	3 years after the last dose of ACE-536
	Day 358 (± 5d)	Day 379 (± 5d)	Day 400 (± 5d)	Day 421 (± 5d)	Day 442 (± 5d)	Day 463 (± 5d)					
QoL Questionnaires[4]				(X)[4]			Cycles 24-87: Day 484 (± 5d) through 1807 (± 5d)	X			
Physical examination[5]					(X)[5]			X			
Vital signs[6]	X	X	X	X	X	X		X			
MRI for liver iron content (optional)[7]				(X)[7]				X			
MRI for EMH masses[8]				(X)[8]				X			
MRI for spleen size[9]				(X)[9]				X			
DXA for BMD of total body, lumbar spine and total hip[31]				(X)[29]				X			
6MWT for NTD patients[32]				(X)[30]				X			
Leg Ulcer Assessment[10]	Collected at each visit as applicable							X			
Serum iron studies[11]				X				X	X		
Serum folate[12]				(X)[12]				X			
Erythropoietin levels				X				X	X		
Hematology[13]	X	X	X	X	X	X		X	X		
Peripheral blood smear[14]				X				X			
Serum chemistry[15]				X				X			
Urinalysis/urine chemistry[16]				X				X			
Anti-drug antibody[17]				X				X[17]			
PD biomarkers[19]				X				X			
Hemoglobin Electrophoresis[20]				(X)[20]				X			
Pregnancy test[21]	X	X	X	X	X	X		X			
Menstrual History[22]				X							
Evaluate transfusion frequency/volume[23]	X	X	X	X	X	X		X	X		
Administer ACE-536	X	X	X	X	X	X					
Concomitant medications and AEs[24]	Collected Continuously										
Malignancy and Pre-Malignancy Monitoring										X	X

[1] **Screening procedures:** Other than informed consent, procedures listed as part of the 28-day screening period are only applicable to patients with treatment interruption. ECHO or MUGA (only required for patients with treatment interruption) can be performed up to 56 days prior to C1D1. If performed as part of standard of care, ECHO/MUGA does not need to be repeated. Patients with treatment interruption will need to qualify per the additional inclusion criteria listed in Section 9.3.2. For patients without treatment interruption, C1D1 may coincide with EOT visit of the base study A536-04. Procedures that are required to confirm eligibility for patients without treatment interruption can be performed at the base study A536-04 EOT visit and used to confirm eligibility prior to dosing on C1D1 of study A536-06.

[2] **Study procedures** must be done prior to administration of study drug. Note that all windows on visits should be determined relative to the date of the previous dose of ACE-536. For patients without treatment interruption, C1D1 procedures shaded grey may be conducted as part of the EOT visit for study A536-04 and may not need to be repeated for study A536-06. All patients must be assessed for eligibility prior to dosing on C1D1. C1D1 MRI for LIC (at selected sites) and EMH masses (unless site does not have feasibility to perform the assessment) and MRI of the spleen (if no prior splenectomy) can be performed up to 56 days prior to C1D1. If performed as part of standard of care, MRI for LIC, MRI for EMH and spleen size do not need to be repeated. All screening and C1D1 procedure results required to confirm eligibility must be obtained and reviewed prior to study drug administration in A536-06. **On dosing days:** Note that the patient dose must be calculated based on the patient's weight on the day of dosing. Starting dose level for C1D1, dose modification rules and titration rules must be reviewed and implemented prior to dosing as required per protocol (see Section 10.7, Section 10.8 and Section 10.9). If a dose delay is required per the dose modification rules (Section 10.8), the patient will not be dosed. The patient will return weekly for assessment of hematology results and AEs until the patient is eligible to receive the next dose of ACE-536 and start the next cycle. The patient should resume the study at the planned dosing cycle (e.g. if the patient missed a dose at C4D1, then they would resume dosing at C4D1 and not skip to C5D1).

[3] **Medical history:** Medical history for patients with treatment interruption will include medical events occurring after EOS visit in study A536-04 and prior to C1D1 for study A536-06. Medical history for patients without treatment interruption will be taken from study A536-04.

[4] **Quality of life questionnaires** are required at C1D1, C5D1, C9D1, C13D1 C17D1, C25D1, C33D1, C41D1, C49D1, C57D1, C65D1, C73D1, C81D1 and EOT.

[5] **Physical exam:** Physical exam should also include an optional evaluation of gonadal size of male patients on C1D1, C5D1, C13D1, C22D1, C30D1, C38D1, C46D1, C54D1, C62D1, C70D1, C78D1 EOT and EOS.

[6] **Vital signs** will include weight, heart rate, and SBP and DBP. If at any visit the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg and/or the absolute increase in either measure from baseline is ≥20 mmHg, perform one repeat of the blood pressure assessment after a minimum of 15 minutes.

[7] **MRI for liver iron content** will be performed at selected sites at C1D1, C9D1 C17D1, C25D1, C33D1, C41D1, C49D1, C57D1, C65D1, C73D1, C81D1 and EOT within a +/- 10-day window for each scan. A MRI for liver iron content is not required if < 3 months since the previous MRI for liver iron content, unless clinically indicated.

[8] **MRI of the chest and abdomen for EMH mass measurement** will be performed for at selected sites at C1D1. If the assessment at C1D1 is negative (no masses), additional scans are not required unless clinically indicated. If the assessment at C1D1 is positive (masses present), repeat MRI at C9D1, C17D1, C25D1, C33D1, C49D1, C65D1, C81D1 and EOT within a +/- 10-day window for each scan. A MRI of the chest and abdomen for EMH mass measurement is not required if < 3 months since the previous MRI of the chest and abdomen for EMH mass measurement, unless clinically indicated

[9] **MRI of the spleen:** will be performed at selected sites at C1D1 on patients with no prior splenectomy. If there is no indication of an enlarged spleen on the C1D1 scan, additional scans are not required unless clinically indicated. If there is an enlarged spleen on the C1D1 scan, repeat imaging at C9D1 C17D1, C25D1 C33D1, C49D1, C65D1, C81D1 and EOT within a +/- 10-day window for each scan. Patients with clinical signs of a change in spleen size or abnormality should have an abdominal ultrasound or MRI performed as needed throughout the study. A MRI for the spleen is not required if < 3 months since the previous MRI for the spleen, unless clinically indicated.

[10] **Leg ulcer assessment:** Patients with leg ulcers should have regular assessment of the leg ulcer(s) throughout the study. Photographs of the leg ulcer(s) pre- and post- dose should be provided when available to document any changes in the ulcer(s).

[11] **Serum iron studies:** May include serum iron, TIBC, transferrin, soluble transferrin receptor, calculated transferrin saturation, ferritin, NTBI. The sponsor may perform additional sample analysis for biomarkers for exploratory research purposes only.

[12] **Serum folate:** Required at screening for patients with treatment interruption to confirm eligibility. Required for all patients at C1D1, C5D1, C13D1, C21D1, C29D1 C37D1, C45D1, C53D1, C61D1, C69D1, C77D1, and EOT.

[13] **Hematology:** RBC, nucleated red blood cells (nRBC) (local and central lab), white blood cell (WBC) with differential, hemoglobin, hematocrit, reticulocyte count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW). On dosing days, hemoglobin values are to be drawn and resulted (up to 24 hours) prior to dosing (see Section 10.8, Patient Dose Modification Rules).

[14] **Peripheral blood smear:** Peripheral blood smears will be prepared centrally from blood sample.

[15] **Chemistry**: Sodium, potassium, AST, ALT, LDH, total bilirubin, indirect bilirubin, alkaline phosphatase, blood urea nitrogen (BUN)/urea, creatinine, GGT, calcium, phosphorus, glucose, amylase, lipase, total protein, albumin, and uric acid. Perform at screening, C1D1, C3D1, C5D1, C9D1, C13D1, C17D1, C21D1, C25D1, C29D1, C33D1, C37D1, C41D1, C45D1, C49D1, C53D1, C57D1, C61D1, C65D1, C69D1, C73D1, C77D1, C81D1, EOT and EOS.

[16] **Urinalysis by dipstick analysis (local lab):** pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if indicated. **Urine chemistry (central lab):** urine chemistries include but are not limited to: microalbumin and creatinine.

[17] If the patient has a **positive ADA** result at their last assessment, the patient may be asked to return approximately every three months for additional testing, until a negative result is obtained or the result is considered stabilized.

[18] **PK collection** is only required on C1D1, C1D8, C2D1, C9D1, C9D8, C10D1, C17D1, and C17D8.

[19] **PD biomarkers:** May include hepcidin, GDF15, GDF8, GDF11, activin A and others to be determined. The sponsor may perform additional sample analysis for biomarkers for exploratory research purposes only.

[20] **Hemoglobin electrophoresis:** Samples will be collected for central hemoglobin electrophoresis on C1D1, C5D1, C13D1, C21D1, C29D1, C37D1, C45D1, C53D1, C61D1, C69D1, C77D1, EOT, and EOS.

[21] **Pregnancy test:** (urine or serum) is required for female patients of child bearing potential only.

[22] **Menstrual history:** is required for female patients of child bearing potential only

[23] **Transfusion frequency/volume:** Transfusion history will be collected from the EOS visit in study A536-04 through the C1D1 visit of A536-06 as available; TD patients will have a "pre-transfusion hemoglobin threshold" for requiring transfusion during the study which will be determined based on transfusion history. For TD patients without treatment interruption, baseline pre-transfusion hemoglobin threshold will be the mean of all documented pre-transfusion hemoglobin values during the 12 weeks prior to C1D1 of base study A536-04 or study A536-06 (for TD patients with treatment interruption). During treatment, if the pre-transfusion hemoglobin level is increased by ≥ 1 g/dL compared to the baseline pre-transfusion hemoglobin threshold for that patient, transfusion should be delayed by a minimum of 7 days and/or the number of units transfused should be reduced by 1 or more RBC units. Patients may be transfused at the investigator's discretion for symptoms related to anemia or other requirements (e.g., infection).

[24] **Adverse events:** Patients should be monitored for AEs throughout the study. All AEs and abnormal findings that might require modification of dosing (see Section 10.8) should be reviewed prior to dosing to ensure that the patient is still eligible to receive additional doses of ACE-536.

[25] **End of Treatment (EOT)**: Should be performed 28 days (± 7 days) after the last dose of ACE-536. Patients who discontinue treatment early should complete the EOT visit at the time of discontinuation.

[26] **Post-Treatment Follow-Up (PTFU):** Should be performed 2 months (± 7 days) after the last dose of ACE-536.

[27] **Long-Term Follow-Up (LTFU)**: Should be performed every 6 months (± 14 days) after the last dose of ACE-536 for 3 years after the last dose of ACE-536 to monitor for presence of malignancy and pre-malignancy, as per standard of care.

[28] **End of Study (EOS):** Should be performed 3 years after the last dose of ACE-536.

[29] **Cycles 6 through 17:** Procedures and visits for Cycles 6 through 9, 10 through 13, and 14 through 17 follow the same procedures and visits as scheduled for Cycles 2 through 5, with some exceptions as noted above.

[30] **Cycles 24 through 87:** Patients may not exceed 87 cycles or 1825 days after first dose, whichever occurs first. The last dose of ACE-536 may not be administered after Day 1825, regardless of the number of cycles completed. Procedures and visits for Cycles 24 through 27, 28 through 31, 32 through 35, 36 through 39, 40 through 43, 44 through 47, 48 through 51, 52 through 55, 56 through 59, 60 through 63, 64 through 67, 68 through 71, 72 through 75, 76 through 79, 80 through 83 and 84 through 87 follow the same procedures and visits as scheduled for Cycles 20 through 23, with some exceptions as noted above.

[31] **DXA for BMD** will be performed at selected sites at C1D1, C9D1, C17D1, C25D1, C33D1, C49D1, C65D1, C81D1 and EOT within a +/- 10-day window for each scan. A DXA for BMD is not required if < 3 months since the previous DXA for BMD, unless clinically indicated.

[32] **6MWT for NTD patients** will be performed at selected sites at C1D1, C5D1, C9D1, C13D1, C17D1, C25D1, C33D1, C41D1, C49D1, C57D1, C65D1, C73D1, C81D1 and EOT.