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Statistical Analysis Plan

A Phase 2, Open-Label, Ascending Dose Study to Evaluate the Effects of ACE-536 in Patients with β-Thalassemia

Sponsor:	Acceleron Pharma Inc. 128 Sidney Street Cambridge, MA 02139 Tel: PPD
Investigational Product:	Luspatercept (ACE-536)
Protocol Number:	A536-04
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Date:	04 August 2017

ACCELERON PHARMA SIGNATURE PAGE

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

Signature: PPD **Date:** 04 AUG 2017
DD/MMM/YYYY

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Signature: PPD **Date:** 04 AUG 2017
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1. INTRODUCTION

This statistical analysis plan (SAP) describes the statistical methods to be used for the analysis of Acceleron Protocol A536-04. 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 04 dated 07NOV2014 and eCRF dated 04 April 2014. 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 proportion of β-thalassemia patients who have an erythroid response, defined as: 1) a hemoglobin increase of ≥ 1.5 g/dL from baseline for ≥ 14 days (in the absence of red blood cell [RBC] transfusions) in non-transfusion dependent patients, or 2) ≥ 20% reduction in RBC transfusion burden compared to pretreatment in transfusion dependent patients

Secondary Objectives:

- To evaluate safety and tolerability of ACE-536
- To evaluate the proportion of transfusion dependent patients who have ≥ 50% reduction of RBC transfusion burden compared to pretreatment
- To evaluate the time to erythroid response and duration of erythroid response
- To evaluate the change in hemoglobin level in non-transfusion dependent patients
- To evaluate the change in pre-transfusion hemoglobin levels in transfusion dependent patients
- To evaluate changes in biomarkers of erythropoiesis, hemolysis, iron metabolism and bone metabolism
- To examine the pharmacokinetic (PK) profile of ACE-536 in patients with β-thalassemia

Exploratory Objectives:

- To evaluate biomarkers related to the TGF-β superfamily
- To evaluate self-reported quality of life using the FACT-An and SF-36 questionnaires (expansion cohort only)
- To evaluate change in spleen size by MRI
- To evaluate change in bone mineral density (BMD) by DXA
- To evaluate change in extramedullary hematopoiesis (EMH) mass size by MRI
- To evaluate change in leg ulcer size
- To evaluate change in the 6-minute walk test (6MWT) distance in NTD patients

3. OVERALL STUDY DESIGN

This is a phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 in patients with β-thalassemia.

3.1. Study Design

Patients who meet the study eligibility criteria were enrolled within 28 days of screening. Patients in all cohorts received ACE 536, administered subcutaneously (SC), every 3 weeks for up to 5 cycles. ACE-536 could not be administered after study day 85 (± 2 days). Dose delay(s) and dose reduction(s) might be required for individual patients as outlined in the protocol.

Each dose escalation cohort consisted of up to 6 patients. The dose level of ACE-536 for the first cohort was 0.2 mg/kg and the dose level(s) for subsequent cohort(s) followed a modified Fibonacci dose escalation scheme (i.e., maximum dose of 0.4 mg/kg for cohort 2, 0.6 mg/kg for cohort 3, 0.8 mg/kg for cohort 4, 1.0 mg/kg for cohort 5, etc.). Once a minimum of 3 patients in a cohort had completed Study Day 29, a Safety Review Team (SRT) reviewed preliminary safety and hematologic response data and made recommendations to the Sponsor regarding whether or not to enroll an additional cohort at a higher or lower dose, complete enrollment of 6 patients at the current dose level, or proceed to the expansion cohort.

The expansion cohort (n=30) was treated with ACE-536 at a starting dose level not to exceed the maximum dose level for the previous cohorts. The expansion cohort included a minimum of 12 patients who are non-transfusion dependent (NTD) and a minimum of 12 patients who are transfusion dependent (TD) (including a minimum of 6 thalassemia major patients with onset of regular transfusions before age 4 years, and a minimum of 6 patients with onset of regular transfusions onset after age 4 years). For subsequent cycles in the expansion cohort, a 26

patient's dose level might be titrated from the previous dose administered for that patient. The maximum dose level given to a patient did not exceed the maximum dose level evaluated in the dose escalation cohorts. Patients were treated with up to 5 doses of ACE 536.

Each TD patient in the expansion cohort had a defined "pre-transfusion hemoglobin threshold" which was calculated based on transfusion history and was used for determining when to transfuse during the study. The baseline pre transfusion hemoglobin threshold was the mean of all documented pre-transfusion hemoglobin values during the 12 weeks prior to C1D1. During treatment, if the pre-transfusion hemoglobin value was 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).

Cohort[a]	ACE-536 Dose Level[b] (mg/kg)	Number of Patients
1	0.2	6
2	0.4	6
3	0.6	6
4	0.8	6
5	1.0	6
6	1.25	5
Expansion	0.8	30[c]
Total (All Cohorts): 64		

[a] Cohort escalation was based on SRT review and recommendation to enroll additional cohorts and/or the expansion cohort.

[b] The ACE-536 dose level for cohort 1 was 0.2 mg/kg. The dose level indicated in the table for all subsequent cohorts was the maximum dose level that could be recommended by the SRT for escalation per the modified Fibonacci dose escalation scheme.

[c] The expansion cohort consisted of 30 patients, including a minimum of 12 patients who were non-transfusion dependent and a minimum of 12 patients who were transfusion dependent (including a minimum of 6 thalassemia major patients with onset of regular transfusions before age 4 years, and a minimum of 6 patients with onset of regular transfusions after age 4 years).

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 discontinued treatment early completed the end of treatment (EOT) follow-up visit at the time of discontinuation and then completed the end of study (EOS) follow-up visit approximately 28 days later.

3.3. Rollover to Extension Study

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.

Patients who complete the EOS visit for study A536-04 and are ≥ 28 days post EOS visit may also roll over to study A536-06. These patients will be re-assessed for eligibility by meeting all eligibility criteria plus additional inclusion criteria for study A536-06.

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 not begin study A536-06 C1D1 until they have reached ≥ 28 days po st EOS visit from study A536-04.

3.4. Sample Size

There was no formal sample size calculation for the dose escalation portion of the study. A sample size of 30 evaluable patients in the expansion cohort will provide approximately 87% power with 1-sided significance level of 0.05 to differentiate an erythroid response rate of 30% from a minimal erythroid response rate of 10%.

4. ANALYSIS POPULATIONS

For all analysis populations, patients will be analyzed according to assigned cohort.

4.1. Intent-to-Treat (ITT) Population

The intent-to-treat (ITT) population will consist of all enrolled patients. This population will be used for all efficacy analysis.

4.2. Safety Population

Safety population will include all patients who received at least 1 dose of ACE-536. This population will be used for all safety analysis.

4.3. Pharmacokinetics (PK) Population

Pharmacokinetics population will include all patients who have received at least 1 dose of ACE-536 and have sufficient pharmacokinetic samples collected and assayed for PK analysis

5. STATISTICAL METHODOLOGY

5.1. Definitions

Transfusion Dependent (TD)

Based on protocol, TD patients are defined as those who required ≥ 4 units of RBCs every 8 weeks (confirmed over 6 months prior to Cycle 1 Day 1).

For programming implementation, a patient is defined as TD if the total RBCs is ≥ 11 units during 26 weeks on or prior to Cycle 1 Day 1.

Non-Transfusion dependent (NTD)

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

Baseline Hemoglobin

Baseline hemoglobin will be an 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 of 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

The transfusion amount measured in unit "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.

End of Study (EOS)

Procedures and evaluations for the end of study visit should be performed 28 days (±7 days) after the Day 113/EOT visit (56 days after the last dose of ACE-536).

Combined Dose Groups

Low dose group includes patients on 0.2mg/kg and 0.4 mg/kg dose cohorts. High dose group include patients with dose level 0.6 mg/kg and above. Suitable summaries may be presented by low/high dose as specified.

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-Meir 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.

All summaries will be presented by dose cohort, and may also be presented by low/high dose groups as specified. The 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 by dose cohort.

5.4. Demographic, Baseline Characteristics, and Disease History

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

- Race, ethnicity, age, gender, height, weight
- Splenectomy (yes or no), iron chelation therapy (yes or no), prior β-thalassemia treatment (yes or no)
- Baseline haemoglobin for NTD patients only and baseline transfusion burden for TD patients only.
- Baseline LIC and serum Ferritin

Demographic and baseline data, medical history, and disease history data will be listed for each patient.

5.5. Study Drug Exposure

Study drug exposure will be descriptively summarized by dose cohort 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 (for expansion cohort only).

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 doses a patient received during the treatment period.

The number of patients experiencing dose delay and reduction will be summarized by dose cohort. For the expansion cohort, the number of patients with dose increase will also be summarized.

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- DDB2E. The medications will be summarized by dose group for the ITT 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 complete 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 15.1 or higher. All non-medication procedures will be listed for each patient.

5.7. Efficacy Analyses

All efficacy endpoint will be performed using the ITT population.

All efficacy summaries will be presented by dose cohort and for NTD and TD patients separately. In addition, the dose cohorts will be combined and presented as low dose group and high dose group. Low dose group includes patients on 0.2 and 0.4 mg/kg dose cohorts. High dose group includes patients on 0.6 mg/kg and above. No formal hypothesis testing is planned.

In general, 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 efficacy analysis
- For each patient, all efficacy endpoints will be derived based on an analysis cut-off 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

The primary efficacy endpoint is the proportion of β-thalassemia patients who have an erythroid response, defined as below:

- For non-transfusion dependent (NTD) patients, a hemoglobin increase of ≥ 1.5 g/dL from baseline sustained for ≥ 14 days (in the absence of RBC transfusions). Hemoglobin measurements within 14 days following RBC transfusion or after 56 days of last dose will be excluded from analysis.
- For transfusion dependent (TD) patients, a ≥ 20% reduction in RBC transfusion burden during any rolling 12-week window on treatment compared to baseline. The rolling 12-week window starts on Day 1 and increments by 1 day, e.g., from day 1 to day 84, day 2 to day 85, day 3 to day 86, etc. The search window ends on last dose day + 56 days or last day of hemoglobin measurement, whichever is earlier.

A point estimate of the proportion of patients having a response will be presented along with its associated exact 95% confidence interval based on binomial distribution for NTD and TD patients separately.

The complete hemoglobin measurements and RBC transfusion records will be listed for each patient.

5.7.2. Secondary Efficacy Endpoints

5.7.2.1. Modified Erythroid Response

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

For non-transfusion dependent (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

For transfusion dependent (TD) patients:

- The proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using 12-week intervals.
- The proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using 8-week intervals.
- The proportion of patients who have no RBC transfusions ≥ 8 weeks.

For each of the above defined modified erythroid response rate, 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 TD patients, ≥ 50% reduction in RBC transfusion burden compared to pretreatment using 12-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-week window achieving above erythroid response.

Duration of erythroid response will be defined as the time from the starting date of the first rolling 12-week window achieving response to the last date of the consecutive rolling 12-week window achieving response. When there are multiple disjoint 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.

5.7.2.3. Iron Parameter Related Analysis

Iron metabolism parameters including serum iron, total iron binding capacity (TIBC), transferrin, soluble transferrin receptor, ferritin, non-transferrin bound iron (NTBI), and hepcidin will be taken at Screening, Day 1, 8, and 15 of Cycles 1, Day 1 of Cycle 2, 3, 4, 5, EOT and EOS visits. Descriptive statistics of actual values, absolute and percentage change from baseline values at each postbaseline time point will be presented by dose cohort. The summary will also be provided by NTD and TD.

MRI for Liver Iron Content (LIC) will be performed at baseline and EOT. Descriptive statistics of actual values, absolute and percentage change from baseline at EOT visit will be presented by dose cohort. The summary will be done by NTD/TD and baseline LIC ≥ 3 and < 3 mg/g dw subgroups separately.

LIC response will be defined as LIC reduction ≥ 1 mg/g dw at EOT compared with baseline, for patients with baseline LIC ≥ 3 mg/g dw only. LIC response rate along with the exact 95% confidence interval will be estimated for NTD and TD patients respectively, as well as by baseline ICT use (yes vs no).

Shift analyses will also be performed to summarize serum ferritin EOT change from baseline. Serum ferritin will be grouped into three categories: < 300, ≥ 300 and ≤ 1000, ≥ 1000 (μg/L).

Plots of iron parameters for both mean observed values and mean change from baseline over time will be presented by dose cohort.

5.7.2.4. Erythropoiesis Parameters

Blood samples relating to erythropoiesis parameters include serum erythropoietin (EPO) levels, hemoglobin electrophoresis, globin chain RNA, reticulocytes and nucleated RBCs. EPO will be taken at Screening, Day 1 of Cycles 1, 2, 4, EOT, and EOS. Hemoglobin electrophoresis and globin chain RNA will be taken at C1D1, C4D1 and EOT. Reticulocytes

and nucleated RBCs will be taken at Screening, Day 1, 8, and 15 of Cycles 1, 2, 3, 4, 5, EOT and EOS visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each postbaseline visit will be presented by dose cohort and for TD and NTD separately. Plots of erythropoiesis parameters for both mean observed values and mean percentage change from baseline over time will be presented by dose cohort.

5.7.2.5. Bone Metabolism Parameters

Bone metabolism parameters including bone specific alkaline phosphatase (BSAP) and C-telopeptide of type I collagen (CTX) will be taken at Cycle 1 Day 1, Cycle 4 Day 1, and EOT visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each postbaseline visit will be presented by dose cohort.

5.7.2.6. Hemolysis Parameters

Hemolysis parameters include haptoglobin, indirect bilirubin, and lactate dehydrogenase (LDH). Haptoglobin will be taken at Screening, Day 1, 8, and 15 of Cycles 1, 2, 3, 4, 5, EOT and EOS visits. Indirect bilirubin and LDH will be taken at Screening, Day 1 and 15 of Cycle 1, Day 1 of Cycles 2, 3, 4, 5, EOT and EOS visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each postbaseline visit will be presented by dose cohort. Plots of hemolysis parameters for both mean observed values and mean percentage change from baseline over time will be presented by dose cohort and for TD and NTD separately.

5.7.3. Exploratory Endpoints

5.7.3.1. Biomarkers Related to TGF-β

For biomarkers GDF8, GDF11, and GDF15, descriptive statistics of observed values at baseline, observed values, changes and percentage changes from baseline at each postbaseline visit will be presented by dose cohort.

5.7.3.2. QoL Questionnaire

QoL questionnaires including FACT-An and SF-36 will be completed at Screening, Cycle 5 Day 1 or End of Treatment (for patients who withdraw early) visits for the Expansion Cohort only. The scoring algorithms for FACT-An and SF-36 are included in the appendix.

Individual subscale scores and total scores will be summarised by visit. All derived subscale and total scores along with all individual responses will be listed for each patient.

5.7.3.3. Other Analysis

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

- Change in spleen size by MRI (in subset of patients with no prior-splenectomy)
- Change in bone mineral density (BMD) by DXA
- Change in extramedullary hematopoiesis (EMH) mass size by MRI
- Change in leg ulcer size (area)
- Change in the 6-minute walk test (6MWT) distance in NTD patients
- Hormone parameters (including estradiol, free testosterone, and total testosterone)

For the following continuous variables, the descriptive statistics including mean, median and range will be presented by dose cohort:

- Change from pretreatment in transfusion burden in transfusion dependent patients
- Change in hemoglobin level in non-transfusion dependent patients
- Change in pre-transfusion hemoglobin levels in transfusion dependent patients

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)
- Gender (male and female)
- Iron chelation therapy (yes and no)
- Baseline LIC (< 3, 3-5, ≥ 5)
- Baseline EPO (< 200 and ≥ 200), (< 200, 200-500, and ≥ 500), (< 500, and ≥ 500)
- Splenectomy (yes and no)

5.8. Safety Analysis

The safety endpoints will be summarized by dose cohort using the Safety Population. The safety endpoints include the incidence of dose-limiting toxicities (DLTs), treatment emergent adverse events, changes in laboratory tests, vital signs and ECG's.

Adverse events will be coded using MedDRA Version 15.1 and above. 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. Dose Limiting Toxicities

A Dose-Limiting Toxicity (DLT) is defined as any of the following toxicities, at any dose level occurring within 28 days of the first administered dose:

- Treatment related serious adverse event (SAE) of Grade ≥ 3
- Treatment related non-hematologic adverse event (AE) of Grade ≥ 3
- Treatment related hematologic AE of Grade ≥ 4

Incidence rates of DLTs will be presented by dose cohort. Details of DLTs will be presented in listings.

5.8.2. Adverse Events

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 by dose cohort and by low/high dose for all dosed patients as well as for NTD and TD patients separately:

- Number and percentage of patients reporting each AE, categorized by System Organ Class (SOC) and Preferred Terms (PT)
- Number and percentage of patients reporting each AE experienced by ≥ 5% of patients in all patients by PT
- Number and percentage of patients reporting SAE, categorized by System Organ Class (SOC) and Preferred Terms (PT)
- Number and percentage of patients reporting Grade ≥ 3 AE, categorized by System Organ Class (SOC) and Preferred Terms (PT)
- Number and percentage of patients reporting related AE, categorized by System Organ Class (SOC) and Preferred Terms (PT)
- Number and percentage of patients reporting AE leading to drug withdrawal, categorized by System Organ Class (SOC) and Preferred Terms (PT)
- Number and percentage of patients reporting each AE categorized by SOC, preferred term, and maximum severity grade group

Note that counting will be by subject, not event, and subjects are only counted once within each SOC or preferred term. If a subject 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 discontinuation

5.8.3. Laboratory Evaluations

Results from the following laboratory parameters, recorded at their respective time points will be summarised by dose cohort and time point:

Haematology: (Screening, Days 1, 8 and 15 of each cycle, End of Treatment, Post Treatment Follow Up and End of Study).

RBC, WBC with differential, hemoglobin, hematocrit, haptoglobin (optional), reticulocyte count, platelet count, MCV, MCH, MCHC, RDW, and nRBCs

Chemistry: (Screening, Days 1 and 15 of Cycle 1, and Days 1 of each subsequent cycle, End of Treatment and End of Study).

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

Urine: (Day 1 of Cycles 1, 2 and 4, End of Treatment and End of Study).

PH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if indicated; Microalbumin and creatinine

Urine Chemistry: (Day 1 of Cycles 1, 2 and 4, End of Treatment and End of Study).

Microalbumin and creatinine will be performed by the central lab**.**

Categorical and numeric variables will be presented separately.

Actual values and changes in haematology and biochemistry laboratory values from baseline will be summarised by dose cohort and by time point.

Shift tables for the haematology 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 summarised by dose cohort.

Peripheral blood smears are taken at Screening, Day 1 of Cycles 1, 2, and 5 and at the end of treatment visit. These data will be listed only.

All laboratory values will be listed for all patients.

5.8.4. Vital Signs

Vital signs parameters include weight (kg), heart rate, systolic and diastolic blood pressure, respiratory rate, and temperature (˚C). Vital signs are recorded at Screening and on Days 1, 8 and 15 of each cycle and EOT. For each parameter at each time point, the change from baseline to post baseline will be summarised by dose cohort. The baseline assessment is defined as the last measurement on or before Cycle 1 Day 1.

Plots of vital sign parameters for both mean observed and mean change from baseline will be presented by dose cohort for each parameter.

Vital signs will also be listed for all patients.

5.8.5. ECG Results

12 lead ECG results are recorded at Screening, Cycle 2 Day 1, and at the end of treatment visit. The quantitative ECG assessments (Ventricular rate, QRS width, PR interval, and QTc interval) will be summarised at each time point by dose cohort.

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] by dose cohort.

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 subject, then an ‘Unknown’ category will be presented.

ECG results will be listed for all patients.

5.8.6. Physical Examination

A physical examination is conducted at Screening and on Day 1 of each cycle and at the end of treatment and end of study visits. Physical exam details will be listed only.

5.9. Pharmacokinetics Analysis

5.9.1. Pharmacokinetic Sampling Schedule

Blood samples for determination of ACE-536 serum concentrations are collected from all subjects at the following schedule:

Treatment/Follow Up	Visit	Study Day	PK Sample
Dose 1	Cycle 1 Day 1	1	X
	Cycle 1 Day 8	8	X
	Cycle 1 Day 11	11	X
	Cycle 1 Day 15	15	X
Dose 2	Cycle 2 Day 1	22	X
	Cycle 2 Day 8	29	X
Dose 4	Cycle 4 Day 1	64	X
	Cycle 4 Day 8	71	X
	Cycle 4 Day 15	78	X
Dose 5 (Final Dose)	Cycle 5 Day 1	85	X
	Cycle 5 Day 8	92	X
	Cycle 5 Day 15	99	X
Follow up Period	End of Treatment	113	X
	Post-Treatment Follow Up	141	X
	End of Study	169	X

Acceptable time windows for pharmacokinetic blood draws are ± 1 day in Cycle 1, ± 2 days in Cycles 2 to 5, and ± 7 days in the follow-up period.

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 subjects 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 pharmaockinetic 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. Noncompartmental Pharmacokinetic Analysis

Pharmacokinetic parameters of ACE-536 will be derived using noncompartmental analysis method with the software program Phoenix WinNonlin (version 6.2 or higher). The ACE-536 serum concentrations after the first dose (Cycle 1 Day 1 to Cycle 2 Day 1) will be used to estimate the drug serum exposure parameters within the first treatment cycle, and the ACE-536 serum concentrations after the final dose (Cycle 5 Day 1 to end of study), if sufficient, will be used to estimate the terminal elimination half-life in serum. Actual sampling and dosing times will be used in this analysis. The following noncompartmental pharmacokinetic parameters will be estimated:

T_{max}	Time to maximum observed serum concentration, obtained directly from the observed concentration-time data (for the first dose only)
C_{max}	Maximum observed serum concentration, obtained directly from the observed concentration-time data (for the first dose only)
AUC_{21d}	Area under the serum concentration-time curve from Day 1 to Day 21, calculated by the linear trapezoidal method (for the first dose only)
λz	Apparent terminal rate constant, calculated by linear regression of the terminal portion of the log-concentration-time curve in serum. Visual assessment may be used to identify the terminal linear phase of the log concentration-time profile. A minimum of 3 data points will be used for the calculation. The λz will not be estimated if the terminal phase of the log-concentration-time profile does not exhibit a linear decline phase, or if the regression coefficient (Rsq) is less than 0.8 (after the final dose)
$t_{1/2}$	Terminal elimination half-life in serum, calculated as $[(\ln 2)/\lambda z]$. The terminal elimination half-life will only be calculated when a reliable estimate for λz can be obtained (after the final dose)

The following pharmacokinetic parameters will be calculated for diagnostic purposes and listed, but they will not be summarized:

λz lower: lower limit of time (days) included in the calculation of λz

λz upper: upper limit of time (days) included in the calculation of λz

λz N: number of data points used in the calculation of λz

Rsq: regression coefficient for calculation of λz

Additional noncompartmental pharmacokinetic parameters may be determined when appropriate.

5.9.4. Compartmental Pharmacokinetic Analysis

Pharmacokinetic parameters of ACE-536 will also be derived using a one-compartment model with first order absorption and elimination (Phoenix WinNonlin). All ACE-536 serum concentration values obtained from Cycle 1 Day 1 to the end of study will be included in this analysis. Actual sampling and dosing times will be used in this analysis.

The model will be parameterized in terms of the absorption rate constant (k01), apparent clearance (CL/F), and apparent volume of distribution (V/F). Other parameters will be derived from the established model. The main compartmental pharmacokinetic parameters to be estimated are outlined as following:

k01	Absorption rate constant
CL/F	Apparent clearance from the central compartment
V/F	Apparent volume of distribution of the central compartment
T_{max}	Time to maximum serum concentration, calculated as ln(k01/k10)/(k01-k10), where k10 = (CL/F)/(V/F)
C_{max}	Maximum serum concentration for the starting dose, predicted at t_{max}
AUC	Area under the serum concentration-time from time zero to infinity for the starting dose, calculated as (Starting Dose)/(CL/F).
$t_{1/2}$	Elimination half-life, calculated as (ln2)/k10

Additional compartmental pharmacokinetic parameters may be determined when appropriate.

5.9.5. Statistical Analysis

All ACE-536 serum concentrations will be listed by dose group, subject, and scheduled time (visit and study day). The ACE-536 serum concentrations will be summarized by dose group and 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 both linear and semilogarithmic scales by dose group for the first 4 weeks. Individual concentration plots will be provided by presenting the observed concentration data along with the one-compartment model-predicted concentration-time profile on the linear scale using actual sampling times and multiple dose data.

Pharmacokinetic parameters will be listed by dose group and subject, and they will be summarized descriptively by dose group (N, mean, SD, minimum, median, maximum, CV%, geometric mean, and geometric CV%). Dose proportionality may be assessed using the exposure data (e.g., C_{max}, AUC) after the first dose. The relationship between ACE-536 exposure and response (i.e., safety, efficacy, and biomarkers) may be explored, if appropriate.

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 subject not discontinued: we not have any patients who fall in this category; will be noted as such in CSR
- Failure to perform key procedures
- Study treatment deviation
- Prohibited concomitant medication
- GCP related deviation

5.12. 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.

5.12.1. 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:

    1) If the year matches the first dose date, then impute the month and day of the first dose date.

    2) Otherwise, assign January.

c) If the day is unknown, then:

    1) 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.

    2) 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.

5.12.2. 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 December 31st, 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.

5.12.3. 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

5.13. 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	Protocol definition: Baseline hemoglobin will be the average of at least two measures (not influenced by transfusion within 7 days of measurement); one measure performed within one day prior to Cycle 1 Day 1 and the other performed 7-28 days prior to Cycle 1 Day 1. This definition is revised in this SAP to reflect the derivation rule implemented for this study.
Remove Efficacy Evaluable (EE) population	EE population is very similar to ITT population. ITT provides more conservative estimate of treatment effect.

6. REFERENCES

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

7. APPENDICES

7.1. FACT-An 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)

Subjects 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 (ie 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.

7.2. 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 precoded 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. 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, using the following formula:

Transformed Score = ((Actual – Lowest)/(Highest – Lowest)) x 100

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)

8. LIST OF ABBREVIATIONS

Abbreviation	Definition
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
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
ITT	Intent-to-Treat
IB	Investigator's brochure
LDH	Lactate dehydrogenase
LTB	Low Transfusion Burden
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation	Definition
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
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
SRT	Safety review team
SUSAR	Suspected unexpected serious adverse reaction
$T_{1/2}$	Elimination half-life
TGF-β	Transforming growth factor beta
TIBC	Total iron binding capacity
T_{max}	Time to maximum concentration
ULN	Upper limit of normal
WBC	White blood cell

9. SCHEDULE OF EVENTS

	Screen	Treatment Period																Follow up period	
		Cycle 1				Cycle 2			Cycle 3			Cycle 4			Cycle 5			EOT[12]	EOS[13]
		C1D 1[2]	C1D8	C1D1 1	C1D1 5	C2D1[2, 15]	C2D8	C2D15	C3D1[2,15]	C3D8	C3D15	C4D1[2,15]	C4D8	C4D1 5	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. 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.