Official Protocol Title:	A phase 2, open-label, ascending dose study of ACE-536 for the treatment of anemia in patients with low or intermediate-1 risk myelodysplastic syndromes (MDS).
NCT number:	NCT01749514
Document Date:	19-Feb-2019

Statistical Analysis Plan

A Phase 2, Open-Label, Ascending Dose Study of ACE-536 for the Treatment of Anemia in Patients with Low or Intermediate-1 Risk Myelodysplastic Syndromes (MDS)

Sponsor:	Acceleron Pharma Inc. 128 Sidney Street Cambridge, MA 02139 USA Tel: PPD Fax: PPD
Investigational Product:	Luspatercept (ACE-536)
Protocol Number:	A536-03
EudraCT Number:	2012-002523-14
Version Number:	2 (Final)
Date:	19 February 2019

ACCELERON PHARMA SIGNATURE PAGE

The undersigned have approved this Statistical Analysis Plan 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 A536-03. 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 06 dated 05 July 2017.

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 patients who have a modified erythroid response (mHI-E), defined as a hemoglobin increase of ≥ 1.5 g/dL from baseline for ≥ 14 days (in the absence of red blood cell [RBC] transfusions) in low transfusion burden patients, or, a reduction of either ≥ 4 units or ≥ 50% of units of RBCs transfused compared to pre-treatment in high transfusion burden patients.

Secondary Objectives:

- To evaluate safety and tolerability of luspatercept (ACE-536)
- To examine rates of erythroid, neutrophil and platelet (HI-E, HI-N and HI-P) responses (International Working Group [IWG] 2006 criteria[1]. See Appendix 3)
- To evaluate time to HI-E response and duration of HI-E response
- To evaluate frequency of RBC transfusions in high transfusion burden patients
- To examine the pharmacokinetic (PK) profile of luspatercept
- To examine other pharmacodynamic (PD) effects (e.g., iron metabolism, erythropoietin, reticulocytes, and bone biomarkers)

Exploratory Objectives:

- To evaluate biomarkers related to the TGF-β superfamily
- Evaluation of self-reported quality of life in the expansion cohort using tools including but not limited to the Functional Assessment of Cancer Therapy-Anemia Scale (FACT-An) questionnaire

3. OVERALL STUDY DESIGN

This is a phase 2, open-label, ascending dose study to evaluate the effects of luspatercept (ACE-536) on anemia in patients with low or intermediate-1 risk MDS who are not currently receiving treatment with an erythropoiesis-stimulating agent (ESA).

3.1. Study Design

Patients who meet the study eligibility criteria will be enrolled within 28 days of screening. Patients in all cohorts will receive luspatercept, administered subcutaneously (SC), every 3 weeks for up to 5 cycles. Dose delay(s) and dose reduction(s) may be required for individual patients as outlined in protocol Section 10.8.1, Patient Dose Modification Rules.

Each dose escalation cohort will consist of a minimum of 3 patients. The dose level of luspatercept for the first cohort will be 0.125 mg/kg and the dose level(s) for subsequent cohort(s) will follow a modified Fibonacci dose escalation scheme with a maximum dose level of 0.25 mg/kg for cohort 2, 0.50 mg/kg for cohort 3, 0.75 mg/kg for cohort 4, 1.0 mg/kg for cohort 5, 1.33 mg/kg for cohort 6, 1.75 mg/kg for cohort 7, and a maximum dose level not to exceed 1.75 mg/kg. Once a minimum of 3 patients in a cohort have completed Study Day 29, the Safety Review Team (SRT) will review preliminary safety and hematologic response data and make recommendations to the Sponsor regarding whether or not to enroll an additional 3 patients in that cohort, enroll a new cohort at a higher or lower dose, or proceed to the expansion cohort.

Expansion cohort 1 (n= approximately 30) will be treated with ACE-536 at a starting dose level of 1.0 mg/kg. Expansion cohort 1 will consist of a minimum of 10 patients who are high transfusion burden (HTB) patients and 10 patients who are low transfusion burden (LTB) patients, if feasible.

Expansion cohort 2 will be treated with ACE-536 at a starting dose level of 1.0 mg/kg. Expansion cohort 2 will be divided into two groups designated expansion cohort 2A and 2B. The targeted accrual for each group will be 25 eligible and evaluable patients but permitted to range from 22 to 28 for administrative reasons. The maximum number of patients treated will be 56.

- Expansion cohort 2A: LTB patients with ≥ 15% ring sideroblasts in the bone marrow (RS+), less than 4 weeks of exposure to erythropoietin stimulating agents (ESAs), and serum erythropoietin (EPO) level ≤ 200 U/L at screening.
- Expansion cohort 2B: Patients with < 15% ring sideroblasts in the bone marrow (RS-) and ≤ 6 RBC units in 8 weeks prior to cycle 1 day 1 (C1D1). This group will consist of a minimum of 10 patients who have less than 4 weeks of exposure to ESAs and 5 patients who have received ≥ 4 weeks of treatment with ESAs.

Expansion cohort 3 will be treated with ACE-536 at a starting dose level of 1.0 mg/kg. Expansion cohort 3 will consist of patients who are: RS-, have baseline EPO levels of ≤ 500 U/L at screening, are transfused with ≤ 6 RBC units in 8 weeks prior to C1D1, and have no prior ESA treatment. It was planned in the protocol to treat approximately 25 patients in this cohort. At the end of the study, 9 patients were treated.

In the expansion cohorts, a patient's dose level may be titrated based on criteria listed in the protocol Section 10.8.2, Expansion Cohorts Patient Dose Titration. The maximum dose level given to a patient will not exceed the maximum dose level evaluated in the dose escalation

cohorts. Patients in the expansion cohorts will be treated with up to 5 doses of luspatercept administered once every 3 weeks.

Table 1: Dose Escalation

Cohort[a]	Maximum ACE-536 Dose Level[b] (mg/kg)	Number of Patients
1	0.125	3-6
2	0.25	3-6
3	0.50	3-6
4	0.75	3-6
5	1.0	3-6
6	1.33	3-6
7	1.75	3-6
Expansion 1	1.0	30
Expansion 2	1.0	up to 56
Expansion 3	1.0	25[c]
Planned Total:		**up to 153**

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

[b] The ACE-536 dose level for cohort 1 is 0.125 mg/kg. The dose level indicated for all subsequent cohorts is the maximum dose level that can be recommended by the SRT for escalation per the modified Fibonacci dose escalation scheme. Dose escalation will not exceed 1.75 mg/kg.

[c] There were 9 patients treated in Expansion 3 by the end of the study.

The total duration of participation for a patient is approximately 28 weeks (4 week screening period, 12 weeks of treatment period and 12 weeks of follow-up period). If a patient has a positive anti-drug antibody (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.

3.2. Treatment Discontinuation

Patients will be informed that they have the right to discontinue treatment and/or 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., ESA)
- Medical reason or adverse event, at the discretion of the investigator and/or the medical monitor
- Lack of effect (e.g., worsening anemia for NTD patients as evidenced by sustained reduction in Hgb by ≥ 2 g/dL over 8 weeks or transfusion dependence), to be discussed with the medical monitor
- Disease progression (as per IWG criteria[1] for altering natural history of MDS. See Appendix 3):

  - For patients with 5-10% blasts, a second bone marrow sample should be collected within 4 weeks for clinical assessment (e.g., cytomorphology, cytogenetics) to confirm progression before discontinuing patients from treatment.
- Persistent increase in white blood cell (WBC) count as per Patient Dose Modifications (protocol Section 10.8)
- Presence of ≥ 1% blasts in peripheral blood as per Patient Dose Modifications in protocol Section 10.8
- 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
- Lost to follow-up
- At the discretion of the sponsor (e.g., termination of the study)

The reasons for study withdrawal and/or treatment discontinuation must be recorded in the patient's CRF. The investigator must notify the sponsor, the medical monitor and the contract research organization (CRO) immediately when a patient has been discontinued/withdrawn due to an AE. Patients who discontinue treatment early should complete the end of treatment (EOT) follow-up visit at the time of discontinuation and then complete the post-treatment follow-up (PTFU) and end of study (EOS) follow-up visits approximately 28 and 56 days later, respectively.

3.3. Sample Size

There is no formal sample size calculation for the dose escalation portion of the study. A standard dose escalation design will be implemented, and up to 42 patients will be enrolled at up to 7 different dose levels to further evaluate erythroid response.

A sample size of 30 evaluable patients in the expansion cohort will provide approximately 90% 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% based on Fisher exact test.

A sample size of 25 evaluable patients in each group of expansion cohorts 2 and 3 will provide approximately 80% 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 % based on Fisher exact test.

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 patients who have received at least 1 dose of ACE-536. This population will be used for all efficacy analyses.

4.2. Safety Population

Safety population will consist of 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

Low Transfusion Burden (LTB)

LTB patients are defined as those who received < 4 units of RBCs within 8 weeks prior to Cycle 1 Day 1.

High Transfusion Burden (HTB)

HTB patients are defined as those who required ≥ 4 units of RBCs within 8 weeks 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 7 days following RBC transfusion.

Baseline Transfusion Burden

Baseline transfusion burden will be calculated as the total amount of RBC transfusion during the 8 weeks 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.

End of Treatment (EOT)

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

Post Treatment Follow-Up (PTFU)

Procedures and evaluations for the post treatment follow-up visit should be performed on Day 141 (±7 days) (28 days after EOT visit).

End of Study (EOS)

Procedures and evaluations for the end of study visit should be performed on Day 169 (±7 days) (56 days after the Day 113/EOT visit).

Patients who discontinue treatment early should complete the end of treatment visit at the time of discontinuation and complete the post-treatment follow-up (PTFU) and EOS follow-up visits 28 days (± 7 days) and 56 days (± 7 days) after the EOT visit.

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 data in the database will be used in the analysis, with the exception of exclusions defined in section 5.7 for efficacy analyses. All study data will be included in study data listings. Missing data will generally not be imputed, unless otherwise stated.

All summaries will be presented by dose cohort, and may also be presented for LTB and HTB 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 summarized by descriptive statistics by dose cohort for ITT population:

- Race, ethnicity, age, sex, height, weight
- Prior MDS Therapies, time from diagnosis; Prior erythropoiesis stimulating agents (ESA); IPSS and IPSS-R risk group; WHO Subtypes
- Baseline ECOG performance status
- Baseline transfusion status (LTB or HTB)
- Baseline hemoglobin for LTB patients only and baseline transfusion burden for HTB patients only.
- Baseline transfusion burden for patients with 2 units or more baseline transfusion.
- Baseline erythropoietin (EPO) and baseline serum ferritin
- RS status and SF3B1 mutation status; any splicing factor (yes or no)
- Baseline bone marrow blasts
- Baseline iron chelation therapy

Demographic and baseline data, medical history, and disease history data will also 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 cohorts 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 dictionary. The medications will be presented 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 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 20.0 or higher. All non-medication procedures will be listed for each patient.

5.7. Efficacy Analyses

The analyses for all efficacy endpoints will be performed using the ITT population.

All efficacy summaries will be presented by dose cohort for LTB and HTB patients separately. No formal hypothesis testing is planned. In addition, summaries will also be provided for the following group of patients:

- All patients in the ITT population
- Lower dose group includes patients on 0.125, 0.25 and 0.5 mg/kg dose cohorts
- Higher dose group includes patients on 0.75 mg/kg and above

- Patients in the expansion cohorts 1, 2A, 2B, and 3
- Patients in 0.125 to 0.50 mg/kg dose cohorts, and
- Patients in 0.75 to 1.75 mg/kg dose cohorts.

Efficacy Results will also be summarized with the following groups:

- All RS+ patients
- All RS- patients
- RS+ patients in the high dose (≥ 0.75 mg/kg) cohorts, and
- RS- patients in the high dose (≥ 0.75 mg/kg) cohorts.

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

- Hemoglobin measurements within 7 days following RBC transfusion will be excluded from efficacy analysis
- For each patient, all efficacy endpoints will be derived based on 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 modified erythroid response (mHI-E), defined as below:

- For low transfusion burden (LTB) patients, a hemoglobin increase of ≥ 1.5 g/dL from baseline for ≥ 14 days (in the absence of RBC transfusions). Hemoglobin measurements within 7 days following RBC transfusion will be excluded from analysis.
- For high transfusion burden (HTB) patients, a ≥ 4 units or ≥ 50% reduction in RBC transfusion burden during any rolling 8-week window on treatment compared to baseline. The rolling 8-week window starts on Day 1 and increments by 1 day, e.g., from day 1 to day 56, day 2 to day 57, day 3 to day 58, etc.

A point estimate of the proportion of patients achieving a modified erythroid response will be presented along with its associated exact 95% confidence interval (CI) based on binomial distribution for LTB and HTB 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. RBC Transfusion Independence

For patients with ≥ 2 units of RBC transfusion at baseline, RBC transfusion independence (RBC-TI) response is defined as not requiring RBC transfusion for more than 8 weeks while on treatment.

The proportion of RBC-TI responders will be estimated by a point estimate along with the exact 95% confidence interval based on binomial distribution. The denominator is the number of patients with ≥ 2 units of RBC transfusion at baseline.

Time to RBC-TI will be defined the time from the first dose date to the starting date of the first consecutive 8-week window not requiring RBC transfusion. Duration of RBC-TI will be

calculated as the longest period of time not requiring RBC transfusion during treatment and will be censored at the last record date for ongoing responses.

Both time to and duration of RBC-TI will be analyzed as continuous variables and summarized by descriptive statistics for RBC-TI responders only. Duration of RBC-TI will also be analyzed as a time to event variable using Kaplan-Meier method.

The derivations of RBC-TI response, time to and duration of RBC-TI will be listed for each patient.

5.7.2.2. HI-E Response Rate

Erythroid (HI-E) response will be defined following IWG 2006 criteria.

- For low transfusion burden (LTB) patients, a hemoglobin increase of ≥ 1.5 g/dL from baseline during any rolling 8-week in the absence of transfusion. The rolling 8-week starts and ends at a hemoglobin measurement date with duration > = 8 weeks.
- For high transfusion burden (HTB) patients, a reduction by ≥ 4 units of RBC transfusions during any rolling 8-week window on treatment compared to baseline.

A point estimate of the proportion of HI-E responders will be presented along with its associated exact 95% confidence interval based on binomial distribution for LTB and HTB patients separately. HI-E response will also be listed for each patient.

5.7.2.3. HI-N and HI-P Response Rate

Platelet (HI-P) and neutrophil (HI-N) response will be defined for patients with abnormal baseline values, following IWG 2006 criteria.

HI-P response will be defined for patients with baseline platelet count < 100×10^9/L:

- For patients with baseline ≥ 20×10^9/L, an absolute mean increase of ≥ 30×10^9/L during any rolling 8-week window on treatment;
- For patients with baseline < 20×10^9/L, mean value of > 20×10^9/L and mean percentage increase ≥ 100% during any rolling 8-week window on treatment.

HI-N response will be defined for patients with baseline neutrophil count (ANC) < 1.0×10^9/L:

- Mean percentage increase ≥ 100% and an absolute mean increase > 0.5×10^9/L during any rolling 8-week window on treatment compared with baseline.

The number and percentage of HI-N/HI-P responders will be estimated. The HI-N and HI-P response will also be listed for each patient.

5.7.2.4. Time to and Duration of HI-E Response

Time to and duration of HI-E response will be analyzed for HI-E responders only.

Time to HI-E response will be defined as the time from the first dose date to the first date of any rolling 8-week window achieving HI-E response.

Duration of HI-E response will be defined as below for LTB and HTB patients, respectively:

a. For LTB patients, duration of HI-E response will be calculated as the longest interval during which all hemoglobin measurements having increase of ≥ 1.5 g/dL from baseline.
b. For HTB patients, duration of HI-E response will be calculated as the time from the starting date of the first rolling 8-week window achieving response to the last date of the consecutive rolling 8-week window achieving response. When there are multiple disjoint intervals with response, the longest interval will be used.

Patients with response ongoing at the last record date will be censored. Both time to and duration of HI-E response will be analyzed as continuous variables and summarized by descriptive statistics. In addition, duration of HI-E response will also be analyzed as a time to event endpoint. The survival curves will be estimated using Kaplan-Meier method if the number of patients is more than 5.

The derivations of time to and duration of mHI-E (or HI-E) response will be listed for each patient.

5.7.2.5. 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, Cycle 1 Day 1, Day 1 and Day 8 of Cycle 2, Day 1 of Cycle 3, 4, and 5, EOT, PTFU and EOS visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each post baseline visit will be presented by dose cohort.

Shift analyses will also be performed to summarize serum ferritin EOT change from baseline. Serum ferritin will be grouped into three categories: < 300, ≥ 300 for males; < 150, >= 150 for females 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.

5.7.2.6. Erythropoietin Parameters

Blood samples relating to erythropoietin parameters including serum erythropoietin levels, reticulocytes and nucleated RBCs will be taken at Screening, Day 1, 8, and 15 of Cycles 1, Day 1 of Cycle 2and 4, EOT, PTFU and EOS visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each post baseline visit will be presented by dose cohort. Plots of erythropoietin parameters for both mean observed values and mean percentage change from baseline over time will be presented by dose cohort.

5.7.2.7. 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 post baseline visit will be presented by dose cohort.

5.7.2.8. Hemolysis Parameters

Hemolysis parameters include direct bilirubin, total bilirubin, and lactate dehydrogenase (LDH). Descriptive statistics of observed values, absolute and percentage change from baseline values at each post baseline 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.

5.7.2.9. Other Analysis

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

- Maximum reduction in transfusion burden from baseline in HTB patients
- Maximum increase in hemoglobin level from baseline in LTB patients
- Maximum mean change of hemoglobin from baseline during any rolling 8-week in LTB patients
- Maximum percentage decrease in serum ferritin
- Maximum change in absolute reticulocytes
- Change in pre-transfusion hemoglobin levels in high transfusion burden patients
- Frequency of RBC transfusions in high transfusion burden patients
- The proportion of patients with an increase of ≥ 1.0 g/dL from baseline hemoglobin maintained ≥ 8 weeks in LTB patients

5.7.3. Exploratory Endpoints

5.7.3.1. Biomarkers Related to TGF-β

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

5.7.3.2. FACT-An Questionnaire

The FACT-An Questionnaire will be completed at Screening and Cycle 5 Day 1 or End of Treatment (for patients who withdraw early) visits for the Expansion Cohort only. 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)

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.

Individual subscale scores and total scores will be summarized by visit. All derived subscale and total scores and all individual responses will be listed.

The association between FACT-An improvement and RBC-TI response will also be explored.

5.7.3.3. Subgroup Analyses

Subgroup analyses of the efficacy endpoints will be performed for the following baseline parameters:

- RS mutation status (yes or no),
- Low (< 0.75 mg/kg) vs High (> = 0.75 mg/kg) Dose Group
- SF3B1 mutation status (yes or no)
- Any splicing factor (yes or no)
- Baseline bone marrow blasts (< 5% or ≥ 5%)
- Baseline erythropoietin (EPO) (≤ 200 or > 200): (< = 500 or > 500)
- IPSS risk group (low, Int-1, Int-2, or high)
- IPSS-R risk group (low, intermediate, high, very high)

5.8. Safety Analyses

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 20.0. Severity of AEs will be coded using National Cancer Institute Common Toxicity Criteria for Adverse Events version 5.0 (NCI-CTCAE v5.0).

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:

a. Treatment related serious adverse event (SAE) of Grade ≥ 3
b. Treatment related non-hematologic adverse event (AE) of Grade ≥ 3
c. 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.

Treatment emergent adverse events (TEAE) are defined as

1. AE starting or worsening after the first date of study drug;
2. AE occurs on the first date of study drug and the onset check box is marked "Onset after first dose of study drug";
3. AE with a missing start date and a non-missing stop date on or after the first dose of study drug;
4. AE with both a missing start and stop date.

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 also be presented:

- 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% and ≥ 10% 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 AE of interests by cycle: AE of interests include new malignancy and premalignant lesions (excluding benign tumors or benign neoplasia)

Note that counting will be by patient, not event, and patients are only counted once within each SOC or preferred term. 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 discontinuation.

5.8.3. Laboratory Evaluations

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

Hematology: (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 and 8 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; individual and mean UACRs over time (for the ITT group) will be computed

Categorical and numeric variables will be presented separately.

Actual values and changes in hematology and biochemistry laboratory values from baseline will be summarize by dose cohort and 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.

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. For each parameter at each time point, the observed values and change from baseline will be summarized by dose cohort. Vital sign data 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, QTcF, interval) will be summarized 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 patient, 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 patients 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
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 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 analyses. 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 excluding C_{max} 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 of determination 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, patient, 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 patient, 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.

5.10. Exposure/Response Analysis

The relationship between ACE-536 exposure and Hb and RBC units will be explored.

The exposure endpoint will be AUC for the starting dose. The erythroid response endpoints will include the following depending on study population.

- Patients who are transfusion-free on treatment:
  - Mean of change from baseline in Hb in Week 1-3 (average of weekly Hb measures from Day 7 to 21). This measure represents the initial rate of Hb increase. Patients who receive at least 1 luspatercept dose will be included.
  - Mean of change from baseline in Hb in Week 1-15 (average of weekly Hb measures from Day 7 to 105). Patients who receive at least 3 luspatercept doses will be included.
- Patients with high transfusion burden (requiring ≥ 4 RBC units in 8 weeks at baseline): Change from baseline in RBC units transfused in Week 1-15. Patients who received at least 3 luspatercept doses will be included.

Change in Hb level or RBC unit will be plotted as a function of luspatercept AUC. Linear or nonlinear function may be used to describe the relationship. The relationship may be stratified by baseline EPO levels.

5.11. Anti-drug Antibody Analysis

The results of anti-drug and neutralizing antibody testing for ACE-536 and human ActRIIB protein versus time will be presented. Exploratory analysis will be performed on the potential effect of anti-drug antibodies on ACE-536 PK and drug exposure if anti-drug antibody tests are deemed positive.

5.12. Interim Analysis

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

5.13. Protocol Violations or Deviations

Protocol violations and deviations will be reviewed and may result in a patient and/or patient visit data being excluded from the statistical summaries. All decisions regarding exclusion of any patient data will be made and recorded during the final data review.

5.13.1. Violation Criteria

Patients who meet any of the following criteria will be listed:

- Non-compliance with inclusion criteria
- Non-compliance with exclusion criteria
- Unauthorized concomitant therapy
- Less than 8 weeks historical hemoglobin data
- Dose not modified according to Dose Modification Criteria
- Study assessment outside of visit window

5.13.2. Protocol Deviations

Deviations from the protocol, as defined in the protocol, will be documented on an ongoing basis by the study monitors and project manager throughout the study period.

At the time of database lock and while the protocol violations are being reviewed, the project manager will forward all relevant documentation highlighting protocol deviations to the study statistician. These deviations will be included in the protocol violation document for agreement and will be listed with the protocol violations in the CSR.

5.14. Handling of Missing Data and Visit Window

5.14.1. 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. 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.

5.14.2. Missing Dates for Adverse Event

Imputing partial AE start 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:
    - 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 dose date, then impute the day of the first dose date.
    - Otherwise, assign ‘01’.

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.

5.14.3. Missing Dates for Concomitant Medication

If start date is missing or partial:

- if month is missing, use January
- if day is missing, use the first day of the month under consideration
- if year is missing, use year of the informed consent date
- if entire date is missing, use informed consent date

If stop date is missing or partial:

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

5.14.4. Visit Windows

The following naming conventions for visit will be presented in the tables, listings and figures. The analysis window will be used for all safety assessments. For each patient, if more than one observation exists within the analysis window, an average value will be included in the analysis.

Visit	Abbreviated Visit	Visit window
Screening (Day -28)	Screen	28 Days prior to C1D1
Cycle 1 Day 1	C1 D1	Day 1
Cycle 1 Day 8	C1 D8	Day 8 ± 3 Day
Cycle 1 Day 11[a]	C1 D11	Day 11 ± 3 Day
Cycle 1 Day 15	C1 D15	Day 15 ± 3 Day
Cycle 2 Day 1	C2 D1	Day 22 ± 3 Day
Cycle 2 Day 8	C2 D8	Day 29 ± 3 Day
Cycle 2 Day 15	C2 D15	Day 36 ± 3 Day
Cycle 3 Day 1	C3 D1	Day 43 ± 3 Day
Cycle 3 Day 8	C3 D8	Day 50 ± 3 Day
Cycle 3 Day 15	C3 D15	Day 57 ± 3 Day
Cycle 4 Day 1	C4 D1	Day 64 ± 3 Day
Cycle 4 Day 8	C4 D8	Day 71 ± 3 Day
Cycle 4 Day 15	C4 D15	Day 78 ± 3 Day
Cycle 5 Day 1	C5 D1	Day 85 ± 3 Day
Cycle 5 Day 8	C5 D8	Day 92 ± 3 Day
Cycle 5 Day 15	C5 D15	Day 99 ± 3 Day
End of Treatment	EOT	Day 113 ± 7 Day
Post-Treatment Follow Up	PTFU	Day 141 ± 7 Day
End of Study	EOS	Day 169 ± 7 Day

[a] For PK data only.

5.15. Changes in Conduct or Planned Analyses from the Protocol

Major changes between SAP and the planned analysis in Protocol Amendment 6 are described below:

Item	Difference between Protocol and SAP
NTD/TD	In SAP, the terms LTB/HTB are used in place of NTD/TD in protocol.
Secondary objectives: time to and duration of mHI-E	The secondary endpoints time to and duration of mHI-E will not be analyzed.
Efficacy Evaluable Population (EE)	Analysis on Efficacy Evaluable population will not be performed. All analyses are on ITT population
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.

6. REFERENCES

1. Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108:419-25.
2. National Cancer Institute Common Toxicity Criteria for Adverse Events, version 5.0 (NCI-CTCAE v5.0)

7. APPENDICES

7.1. Appendix 1 - List of Abbreviations

Abbreviation	Definition
ActRIIB	Activin receptor IIB
AE	Adverse Event
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
BSAP	Bone specific alkaline phosphatase
BUN	Blood urea nitrogen
CI	Confidence interval
C_{max}	Maximum concentration
CRF	Case report form
CRO	Contract research organization
CTX	C-telopeptide of type I collagen
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
EE	Efficacy Evaluable
EOS	End of Study
EPO	Erythropoietin
EOT	End of Treatment
FACT-An	Functional Assessment of Cancer Therapy-Anemia Scale
GDF	Growth and differentiation factor
Hgb	Hemoglobin
HTB	High Transfusion Burden
ITT	Intent-to-Treat
IPSS	International Prognostic Scoring System
LDH	Lactate dehydrogenase
LTB	Low Transfusion Burden
mHI-E	Modified erythroid response
MedDRA	Medical Dictionary for Regulatory Activities
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration

Abbreviation	Definition
MCV	Mean corpuscular volume
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
RS	Ringsideroblasts
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SRT	Safety review team
$T_{1/2}$	Elimination half-life
TIBC	Total iron binding capacity
T_{max}	Time to maximum concentration
WBC	White blood cell
WHO	World Health Organization

7.2. Appendix 2 - Schedule of Events

	Screen	Treatment Period																Follow up period		
		Cycle 1				Cycle 2			Cycle 3			Cycle 4			Cycle 5			EOT[12]	Post-Treatment Follow Up	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 -28	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)	Day 113 (± 7d)	Day 141 (± 7d)	Day 169 (± 7d)
Informed consent	X																			
Inclusion/Exclusion	X	X																		
Medical history	X																			
QoLQuestionnaires[18]	X														X[18]			X[19]		X
Physical examination	X	X				X			X			X			X			X		X
Vital signs[1]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
ECOG Status	X								X			X						X		X
ECG (12 lead)	X					X												X		
Bone marrow aspirate/biopsy[3]	X								X[3]									X		
Serum iron studies[4]	X	X				X	X		X			X			X			X	X	X
Serum folate and B_{12}	X																			
Erythropoietin levels	X	X	X		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	X
Peripheral blood smear	X	X				X									X			X		
Serum chemistry[6]	X	X			X	X	X		X	X		X	X		X	X		X		X
Urinalysis and Urine Chemistry[7]		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
PD biomarkers[8]		X				X			X			X			X			X		X
Bone biomarkers[9]		X										X						X		
Pregnancy test[10]	X	X				X			X			X			X			X		X
Evaluate transfusion frequency[11]	X	X				X			X			X[2]			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 [17]		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.

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

[3] **Bone marrow aspirate/biopsy** including cytogenetics at screening must be performed ≤ 3 months prior to C1D1 for evaluation of patient eligibility and other PD biomarkers to be determined (e.g., GDF15, GDF8, and GDF11).

- For all patients, at the end of treatment visit, a bone marrow aspirate must be performed (a bone marrow biopsy is optional).
- Dose escalation cohorts and expansion cohort 1 patients only: a bone marrow aspirate must be performed within 21 days after C3D1 (a bone marrow biopsy is not required). Note: A bone marrow aspirate is not required within 21 days after C3D1 for expansion cohort 2 or 3 patients.
- Dose escalation cohorts and expansion cohort 1 patients only: If hemoglobin increases ≥ 2 g/dL, an optional bone marrow aspirate can be performed within 7 days of the result.

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

[5] **Hematology:** RBC, WBC with differential, hemoglobin, hematocrit, nRBC, reticulocyte count, platelet count, peripheral blasts (on dosing days only: C1D1, C2D1, C3D1, C4D1, C5D1), MCV, MCH, MCHC, and RDW. On dosing days, hemoglobin values are to be drawn and resulted (up to 1 day) prior to dosing (see Section **Error! Reference source not found.**, Patient Dose Modification Rules). Historical hemoglobin data will be collected for 24 weeks, where available, prior to C1D1. During the screening period, hemoglobin will be measured twice; 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. Neither hemoglobin measure should be influenced by transfusion within 7 days of measurement.

[6] **Chemistry**: Sodium, potassium, AST, ALT, lactate dehydrogenase (LDH), total bilirubin, direct 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 (local lab):** pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if indicated. **Urine Chemistry (central lab):** Microalbumin and creatinine.

[8] **PD Biomarkers:** Hepcidin, GDF15, GDF8, GDF11, and others to be determined.

[9] **Bone Biomarkers:** BSAP and CTX. Not required for expansion cohort 2 or 3 patients.

[10] **Pregnancy test:** (urine or serum) is required for female patients of child bearing potential at screening and prior to each dose of ACE-536.

[11] **Transfusion history** will be collected for 24 weeks, where available, 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 post-treatment follow-up (PTFU) and EOS follow-up visits 28 days (± 7 days) and 56 days (± 7 days) after the EOT visit.

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

[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. 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). Note: study days may vary if a dose delay occurred during the treatment period.

[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] For the **first dose of ACE-536**, dosing should occur after a minimum of 7 days post-transfusion and a minimum of 24 hours prior to a scheduled transfusion.

[18] **Expansion cohorts** only, administration of quality of life questionnaires. Quality of life questionnaire not required for expansion cohort 2 or 3 patients at C5D1.

[19] **Quality of life questionnaires** should be completed at the EOT visit for patients that discontinue treatment early and for all expansion cohort 2 and 3 patients.

7.3. Appendix 3: International Working Group (IWG) Criteria - Erythroid Response Evaluation[1]

Hematologic Improvement (HI)[a]	Response Criteria (Responses must last at least 8 weeks)
Erythroid response (Hi-E) (pretreatment Hgb < 11 g/dL)	Hgb increase of ≥ 1.5 g/dL for patients not transfused; or, as defined by having received less than 4 units of RBCs within 8 weeks of Cycle 1 Day 1 Reduction by ≥ 4 units of RBCs transfused (for a Hgb ≤ 9.0 g/dL) during any 8-week period on study, compared with the 8-week period prior to study day 1
Platelet response (HI-P) (pretreatment, < 100 x10^9/L)	Absolute increase of ≥ 30 x 10^9/L for patients starting with > 20 x 10^9/L platelets Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%
Neutrophil response (HI-N) (pretreatment, < 1.0 x10^9/L)	At least 100% increase and an absolute increase > 0.5 x 10^9/L

[a] Pre-treatment counts are averages of at least 2 measurements (not influenced by transfusions) ≥ 1 week apart within 28 days prior to Day 1.
Hgb indicates hemoglobin; RBC: red blood cell; HI: hematologic improvement.