STATISTICAL ANALYSIS PLAN

Protocol Title:	**An Open-Label Single Ascending Dose Clinical Study of the Pharmacokinetics, Pharmacodynamics, Tolerability, Safety and Immunogenicity of BCD-131 in Healthy Volunteers as Compared to Mircera® (F. Hoffman-La Roche Ltd., Switzerland) and Aranesp® (Amgen Europe B.V., Netherlands)**
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1. INTRODUCTION

The Statistical Analysis Plan (SAP) provides a detailed analysis plan and steps of study report preparation for the clinical trial BCD-131-1.

2. GOALS AND OBJECTIVES

2.1. Purpose

To evaluate the tolerability, safety, pharmacokinetics, pharmacodynamics and immunogenicity of single ascending doses of BCD-131 in healthy volunteers as compared to Mircera® and Aranesp®.

2.2. Objectives

Study objectives (Stage I):

1. Establish the frequency and severity of adverse events following the administration of single ascending doses of BCD-131 to healthy volunteers.
2. Establish key pharmacokinetic (PK) parameters ($AUC_{0\text{-}1176\ h}$, $AUC_{0\text{-}\infty}$, C_{max}, T_{max}, $T_{½}$, K_{el} and CL) of BCD-131 following the administration of single ascending doses of the drug product to healthy volunteers.
3. Establish pharmacodynamic (PD) parameters ($AUEC_{0\text{–}1176\ h}$, $AC\text{-}E_{max}$) of BCD-131 based on the absolute reticulocyte count and Hb values in the blood of healthy volunteers following the administration of single ascending doses of BCD-131.
4. Study serum levels of binding antibodies (BAbs) in the blood of healthy volunteers following the subcutaneous administration of single ascending doses of BCD-131.
5. Establish main PK ($AUC_{0\text{-}1176\ h}$, $AUC_{0\text{-}\infty}$, C_{max}, T_{max}, $T_{½}$, K_{el} and CL) and PD parameters ($AUEC_{0\text{–}1176\ h}$, $AC\text{-}E_{max}$ based on changes in the absolute reticulocyte count and Hb levels in the blood) as well as immunogenicity of therapeutic doses of Mircera® and Aranesp® given as subcutaneous injections to healthy volunteers.
6. Establish a safe dose of subcutaneous BCD-131 to be used at stage II of the study.

Study objectives (Stage II):

1. Establish key pharmacokinetic parameters ($AUC_{0\text{-}1176\ h}$, $AUC_{0\text{-}\infty}$, C_{max}, T_{max}, $T_{½}$, K_{el} and CL) of BCD-131 given as a single subcutaneous or intravenous injection to healthy volunteers at a dose established at stage I of the study.
2. Establish key pharmacodynamic parameters ($AUEC_{0–1176\ h}$, AC-E_{max} based on changes in the absolute reticulocyte count and Hb levels in the blood) of BCD-131 given as a single subcutaneous or intravenous injection to healthy volunteers at a dose established at stage I of the study.
3. Establish the frequency and severity of adverse events and evaluate the level of BAbs to BCD-131 given as a single subcutaneous or intravenous injection to healthy volunteers at a dose established at stage I of the study.
4. Establish subcutaneous and intravenous doses of BCD-131 to be used in a phase II clinical study.

3. STUDY DESIGN

3.1 Design

Stage I

Stage I of clinical study BCD-131-1 is an open-label, non-randomized clinical study of pharmacokinetics, pharmacodynamics, tolerability, safety and immunogenicity of the novel drug product given to healthy volunteers at ascending doses (Phase 1, a traditional "3+3" design). Also, at stage I the investigators will evaluate the PK and PD parameters of the closest analogues of BCD-131 (Mircera® and Aranesp®) given as subcutaneous injections at therapeutic doses.

Volunteers (no more than 54 subjects at stage I) will be included in the study after they have signed an informed consent form, completed an examination at screening (no more than 14 days) and received the approval of the investigator confirming their eligibility for the study.

Evaluation of pharmacokinetics, pharmacodynamics and safety of ascending doses of BCD-131

The evaluation of pharmacokinetics, safety and tolerability of BCD-131 will be conducted in 8 cohorts:

1. Cohort 01 will include no more than 6 volunteers who will receive a single subcutaneous injection of BCD-131 at a dose of 0.05 µg/kg body weight.
2. Cohort 02 will include no more than 6 volunteers who will receive a single subcutaneous injection of BCD-131 at a dose of 0.15 µg/kg body weight.
3. Cohort 03 will include no more than 6 volunteers who will receive a single subcutaneous injection of BCD-131 at a dose of 0.40 µg/kg body weight.
4. Cohort 04 will include no more than 6 volunteers who will receive a single subcutaneous injection of BCD-131 at a dose of 1.05 µg/kg body weight.
5. Cohort 05 will include no more than 6 volunteers who will receive a single subcutaneous injection of BCD-131 at a dose of 1.70 µg/kg body weight.
6. Cohort 06 will include no more than 6 volunteers who will receive a single subcutaneous injection of BCD-131 at a dose of 2.75 µg/kg body weight.
7. Cohort 07 will include no more than 6 volunteers who will receive a single subcutaneous injection of BCD-131 at a dose of 4.45 µg/kg body weight.

Volunteers will be included in the study in a consecutive order. First, three volunteers will be included in Cohort 01 to receive a single subcutaneous injection of BCD-131 at a calculated starting safe dose of 0.05 µg/kg. If during 14 days after the injection these volunteers do not develop any treatment-emergent Grade 3–4 adverse events, the next three volunteers will be included in the study (Cohort 02). The further inclusion algorithm is as follows:

If during 14 days after the injection of BCD-131 the subjects do not develop any treatment-emergent Grade 3-4 AEs (DLT), the next cohort consisting of three volunteers will be included in the study to receive an escalated dose of BCD-131 (Cohort 03, Cohort 04, Cohort 05 etc.).

If during 14 days after the injection of BCD-131 only one volunteer (of three) develops a treatment-emergent Grade 3-4 AE (DLT), three more healthy volunteers will be included in this cohort to receive BCD-131.

If two subjects develop treatment-emergent Grade 3-4 AEs (DLT), the dose of BCD-131 which resulted in these events is considered to be the maximum tolerated dose (MTD). No further dose escalating is performed.

Thus, the dose-limiting toxicity is assessed as close as possible to the time of drug administration during the first 14 days after the injection. However, follow-up of volunteers does not stop; it will continue up to Day 50 from the moment of injection and include the monitoring of volunteers' health as well as safety and PK parameters of BCD-131.

Evaluation of pharmacokinetics, pharmacodynamics and safety of subcutaneous therapeutic doses of Mircera® and Aranesp®

Simultaneously with the evaluation of pharmacokinetics, pharmacodynamics and safety of ascending doses of BCD-131, the investigators will evaluate the pharmacokinetics, pharmacodynamics and safety of subcutaneous therapeutic doses of Mircera® and Aranesp®. For this purpose, 12 more volunteers will be included in the study.

After having been randomized in a 1:1 ratio, the volunteers will be assigned to 2 groups. Six volunteers in Group 1 will receive a single subcutaneous injection of Mircera® at a dose of 1.20 µg/kg (Day 1). Six volunteers in Group 2 will receive a single subcutaneous injection of Aranesp® at a dose of 0.45 µg/kg (Day 1).

After the last volunteer enrolled in stage I of the study has completed all the procedures specified in the Study Protocol, an interim analysis of the data obtained will be performed. Based on the comparison of pharmacokinetics, pharmacodynamics and safety of different doses of the test drug and active comparators, the investigators will establish a dose of BCD-131 which ensures PD effects similar to those of the closest analogues (Mircera®, Aranesp®) given as subcutaneous injections at therapeutic doses. The established dose of BCD-131 will be used at stage II of the study.

Subjects will be asked to stay at the study site for the first 24 h after the injection because multiple blood samples are to be collected over this period.

Regardless of the group/cohort to which a volunteer has been assigned, s/he will be followed-up for 50 days during which 16 visits will be performed.

Stage II

Stage II of the study aims to further evaluate pharmacokinetics, pharmacodynamics and safety of subcutaneous and intravenous injections of BCD-131 at a dose which ensures PD effects similar to those of the closest analogues (Mircera®, Aranesp®) given as subcutaneous injections at therapeutic doses. For this purpose, 12 volunteers will be included in the study after they have signed an informed consent form, completed an examination at screening (no more than 14 days) and received the approval of the investigator confirming their eligibility for the study.

After having been randomized in a 1:1 ratio, the volunteers will be assigned to 2 groups.

- Six volunteers in Group 1 will receive a single intravenous injection of BCD-131 at a dose established at stage I of the study (Day 1).
- Six volunteers in Group 2 will receive a single subcutaneous injection of BCD-131 at a dose established at stage I of the study (Day 1).

Subjects will be asked to stay at the study site for the first 24 h after the injection because multiple blood samples are to be collected over this period. Regardless of the group/cohort to which a volunteer has been assigned, s/he will be followed-up for 50 days during which 16 visits will be performed.

3.2 Time Points for Blood Sampling to Evaluate PK at Stage I of the Study

In the groups of volunteers receiving BCD-131 and Mircera®, blood sampling for the PK evaluation will be performed as follows:

1. PK1-1 — 5 min before the injection of BCD-131/Mircera®
2. PK2-1 — 30 ± 5 min after the injection of BCD-131/Mircera®
3. PK3-1 — 2 h ± 10 min after the injection of BCD-131/Mircera®
4. PK4-1 — 8 h ± 15 min after the injection of BCD-131/Mircera®
5. PK5-1 — 24 h ± 20 min after the injection of BCD-131/Mircera®
6. PK6-1 — 48 h ± 30 min after the injection of BCD-131/Mircera®
7. PK7-1 — 72 h ± 30 min after the injection of BCD-131/Mircera®
8. PK8-1 — 96 h ± 60 min after the injection of BCD-131/Mircera®
9. PK9-1 — 120 h ± 60 min after the injection of BCD-131/Mircera®
10. PK10-1 — 168 h ± 60 min after the injection of BCD-131/Mircera®

11. PK11-1 — 216 h ± 120 min after the injection of BCD-131/Mircera®
12. PK12-1 — 288 h ± 120 min after the injection of BCD-131/Mircera®
13. PK13-1 — 336 h ± 120 min after the injection of BCD-131/Mircera®
14. PK14-1 — 504 h ± 120 min after the injection of BCD-131/Mircera®
15. PK15-1 — 672 h ± 120 min after the injection of BCD-131/Mircera®
16. PK16-1 — 840 h ± 120 min after the injection of BCD-131/Mircera®
17. PK17-1 — 1008 h ± 120 min after the injection of BCD-131/Mircera®
18. PK18-1 — 1176 h ± 120 min after the injection of BCD-131/Mircera®

In the group of volunteers receiving Aranesp®, blood sampling for the PK evaluation will be performed as follows:

1. PK1-1 — 5 min before the injection of Aranesp®
2. PK2-1 — 30 ± 5 min after the injection of Aranesp®
3. PK3-1 — 2 h ± 10 min after the injection of Aranesp®
4. PK3-1 — 8 h ± 15 min after the injection of Aranesp®
5. PK5-1 — 16 h ± 20 min after the injection of Aranesp®
6. PK6-1 — 24 h ± 20 min after the injection of Aranesp®
7. PK7-1 — 36 h ± 30 min after the injection of Aranesp®
8. PK8-1 — 48 h ± 30 min after the injection of Aranesp®
9. PK9-1 — 72 h ± 30 min after the injection of Aranesp®
10. PK10-1 — 96 h ± 60 min after the injection of Aranesp®
11. PK11-1 — 120 h ± 60 min after the injection of Aranesp®
12. PK12-1 — 168 h ± 60 min after the injection of Aranesp®
13. PK13-1 — 216 h ± 120 min after the injection of Aranesp®
14. PK14-1 — 288 h ± 120 min after the injection of Aranesp®
15. PK15-1 — 336 h ± 120 min after the injection of Aranesp®
16. PK16-1 — 504 h ± 120 min after the injection of Aranesp®
17. PK17-1 — 672 h ± 120 min after the injection of Aranesp®
18. PK18-1 — 840 h ± 120 min after the injection of Aranesp®

19. PK19-1 — 1008 h ± 120 min after the injection of Aranesp®
20. PK20-1 — 1176 h ± 120 min after the injection of Aranesp®

3.3 Time Points for Blood Sampling to Evaluate PK at Stage II of the Study

1. PK1-2 — 5 min before the injection of BCD-131
2. PK2-2 — 15 ± 3 min after the injection of BCD-131
3. PK3-2 — 30 ± 5 min after the injection of BCD-131
4. PK4-2 — 60 ± 5 min after the injection of BCD-131
5. PK5-2 — 2 h ± 10 min after the injection of BCD-131
6. PK6-2 — 4 h ± 15 min after the injection of BCD-131
7. PK7-2 — 8 h ± 15 min after the injection of BCD-131
8. PK8-2 — 24 h ± 20 min after the injection of BCD-131
9. PK9-2 — 48 h ± 30 min after the injection of BCD-131
10. PK10-2 — 72 h ± 30 min after the injection of BCD-131
11. PK11-2 — 96 h ± 60 min after the injection of BCD-131
12. PK12-2 — 120 h ± 60 min after the injection of BCD-131
13. PK13-2 — 168 h ± 60 min after the injection of BCD-131
14. PK14-2 — 216 h ± 120 min after the injection of BCD-131
15. PK15-2 — 288 h ± 120 min after the injection of BCD-131
16. PK16-2 — 336 h ± 120 min after the injection of BCD-131
17. PK17-2 — 504 h ± 120 min after the injection of BCD-131
18. PK18-2 — 672 h ± 120 min after the injection of BCD-131
19. PK19-2 — 840 h ± 120 min after the injection of BCD-131
20. PK20-2 — 1008 h ± 120 min after the injection of BCD-131
21. PK21-2 — 1176 h ± 120 min after the injection of BCD-131

3.4. Selection of Study Population

3.4.1. Planned Sample Size

Up to 66 healthy volunteers.

3.4.2. Inclusion criteria

1. Signing of the Informed Consent Form;
2. Male sex;
3. Age of 18 to 45 years, inclusive;
4. BMI within normal limits (18.5–24.9 kg/m2);
5. Healthy patients, which is proved by their medical history, physical examination and laboratory findings:
   - No clinically significant abnormalities of circulatory, respiratory, nervous, hematopoietic, endocrine and digestive systems, liver and kidneys in the past medical history and at screening;
   - No history of cardiovascular disorders or thyroid disorders;
   - No history of hematologic disorders, including but not limited to any type of anemia, myelodysplastic syndrome, blood cancers, hemolytic syndrome, hemoglobinopathies, coagulopathies;
   - CBC results within normal limits, including:
     - Hemoglobin within 132–173 g/L;
     - Hematocrit (based on CBC results) within 39–49%;
     - Platelet count within 150–400*10^9/L;
     - Absolute reticulocyte count within 30.4–93.5 * 10^9/L;
   - Blood biochemistry and urinalysis results within normal limits;
   - Serum ferritin within 20–250 µg/L;
   - Serum endogenous erythropoietin within 4.3–29.0 MIU/mL;
   - Hemodynamic parameters within normal limits: systolic blood pressure within 100–139 mmHg; diastolic blood pressure within 60–90 mmHg; heart rate within 50–90 bpm;
   - No history of chronic infections (tuberculosis) or chronic inflammation;
   - No hepatitis B or C, HIV, or syphilis;
   - No acute infections within 4 weeks prior to inclusion in the study;
   - No psychiatric disorders and other conditions (including depression) that can interfere with the volunteer's ability to follow the study protocol;

- Well-being (in the volunteer's opinion) within 30 days prior to inclusion in the study;

6. No history of or current (at baseline) alcohol or drug abuse;
7. Ability of the volunteer, in the investigator's opinion, to follow the study protocol procedures;
8. Willingness of volunteers and their sexual partners with preserved reproductive potential to use reliable contraception within 2 weeks before inclusion in the study and up to 7 weeks after the injection of the test product. This criterion is not applicable to subjects who underwent surgical sterilization. Reliable methods of contraception include one barrier method in combination with one of the following methods: spermicides, intrauterine device/oral contraceptives (for sexual partners).
9. Willingness of volunteers to avoid alcohol intake within 24 hours before and 8 days after each injection of the test drug;

3.4.3. Exclusion criteria

1. History of treatment with erythropoietins or any other ESAs;
2. Acute bleeding, blood/plasma donation or blood transfusion within 2 months before inclusion in the study;
3. History of chronic bleeding;
4. Standard laboratory and instrumental findings outside normal limits at screening;
5. History of allergies (anaphylactic shock or multiple drug allergy syndrome);
6. Known allergy or intolerance to any components of the investigational product;
7. Major surgery within 30 days prior to screening, or surgery being scheduled for any time during the study;
8. Impossibility to install a venous catheter for blood sampling (e.g. because of skin disorders at the sites of venipuncture);
9. Diseases or other conditions that can interfere with the pharmacokinetics of the investigational drug (e.g. chronic liver, kidney, blood, circulatory system, lung or neuroendocrine diseases, including diabetes mellitus and others);
10. History of fever of 40 °C or more;
11. History of elevated hepatic transaminases (above 2.5xULN);

12. Episodes of thrombosis and/or thromboembolia in past medical history (myocardial infarction, stroke, transient ischemic attacks, deep vein thrombosis, pulmonary embolism within 6 months prior to inclusion in the study) as well as an increased risk of deep vein thrombosis;
13. History of epileptic attacks or seizures;
14. History or current (at screening) depression, suicidal thoughts/ attempts;
15. Regular oral or parenteral use of any medications including over-the-counter drugs, vitamins and nutritional additives within 2 weeks before a scheduled injection of the test drug;
16. Use of drugs, including OTC products, that significantly affect the hemodynamics, hepatic function, etc. (barbiturates, omeprazole, cimetidine, etc.) within 30 days before a scheduled injection of the test drug;
17. Vaccination within 4 weeks before a scheduled injection of the test drug;
18. Smoking more than 10 cigarettes per day;
19. Consumption of more than 10 portions of alcohol per week (one portion equals to 0.5 L of beer, 200 mL of wine or 50 mL of ethanol) or a history of alcohol, drug or medication abuse;
20. Participation in other clinical studies within 1 month before screening or simultaneous participation in another clinical study;
21. Previous participation in this study.

4. EVALUATION CRITERIA

4.1 Endpoints to Evaluate Single-Dose Pharmacokinetics

Primary endpoint

- $AUC_{0–1176\ h}$ (area under the concentration vs. time curve from the moment of injection to 1176 h [Day 50 of follow-up]) and $AUC_{0-\infty}$ (to infinity).

Secondary endpoints

- C_{max} (maximum serum concentration),
- T_{max} (time to C_{max}),
- T½ (half-life),

- k_{el} (elimination constant),
- CL (total clearance).

4.2. Pharmacodynamics Endpoints

Primary endpoint

- AUEC0-1176 h (total area under the effect vs. time curve from the moment of injection to 1176 h [Day 50 of follow-up]) based on the change in the absolute reticulocyte count after a single-dose injection of the drug product.
- AC-Emax (absolute maximum increase in the test parameter vs. baseline) based on the change in the absolute reticulocyte count in the blood after a single-dose injection of the drug product.

Secondary endpoints:

- AUEC0-1176 h (total area under the effect vs. time curve from the moment of injection to 1176 h [Day 50 of follow-up]) based on the change in the Hb level in the blood after a single-dose injection of the drug product.
- AC-Emax (absolute maximum increase in the test parameter vs. baseline) based on the change in the Hb level in the blood after a single-dose injection of the drug product.

4.3. Endpoint for Pain Assessment

- The average VAS score evaluating the pain during the injection.

4.4. Safety assessment

- Total SAEs.
- Total AEs.
- Incidence of administration site reactions.
- Incidence of Grade 3-4 AEs and SAEs.
- Frequency of early withdrawals due to AEs and SAEs.

4.5. Immunogenicity Endpoints

- Incidence of BAbs to the drug product on Day 50 from the moment of a single-dose injection of the drug.

5. THE PLANNED ANALYSIS

The study report will be made after the study completion (after all included patients will finish the study).

6. SAMPLE SIZE CALCULATION

The sample size for the study was calculated based on the "3+3" design used in the study of doses and additional test parameters:

Cohort	Dose, µg/kg	Number of volunteers to be enrolled (obligatory)	Number of volunteers who can be enrolled if DLT signs develop
01	0.05	3	3
02	0.15	3	3
03	0.40	3	3
04	1.05	3	3
05	1.70	3	3
06	2.75	3	3
07	4.45	3	3

To assess the PK and PD of the closest analogues of BCD-131, 6 more volunteers will be included in the study to receive Mircera® and 6 more volunteers will be enrolled to receive Aranesp®.

To assess the PK and PD of BCD-131 given as a subcutaneous or intravenous injection at a dose established at stage I of the study, 6 more volunteers will be included in the study to receive BCD-131 as an intravenous injection and 6 more volunteers will be enrolled to receive BCD-131 as a subcutaneous injection.

7. STATISTICAL ANALYSIS OF POPULATION

Pharmacokinetics analysis

The pharmacokinetics (PK) analysis will include data of all volunteers who have received one dose of BCD-131 or reference drugs. If a subject has had more than two PK serum samples missed / lost / spoilt and skipped blood sampling time points more than twice, his/her data will not be included in the PK analysis.

Pharmacodynamics analysis

The pharmacodynamics (PD) analysis will include data of all volunteers who have received one dose of BCD-131 or reference drugs. If a subject has had more than two PK serum samples missed / lost / spoilt and skipped blood sampling time points more than twice, his/her data will not be included in the PD analysis.

Safety analysis

The safety analysis will include all patients who have received one dose of the test/reference drug.

Immunogenicity analysis

The immunogenicity analysis will include data of all volunteers who have received one dose of BCD-131 or reference drugs. If a subject has had serum samples taken on Day 1 and Day 50 missed / lost / spoilt, his/her data will not be included in the immunogenicity analysis.

8. ANALYSIS PLAN AND STATISTICAL METHODS

8.1. The software

For statistical analysis and to construct the tables and diagrams would be used the software STATISTICA 10 and statistical programming language R.

8.2. Description of the Statistical Methods to be Employed

Quantitative Data

The following quantitative data will be analyzed in the study:

Pharmacokinetics:

- Plasma concentration of drug,
- Area under plasma concentration curve,
- Time (e.g., maximum plasma concentration time, half-life)

- Elimination parametres (e.g. clearance, terminal rate constant)

Pain Assessment:

- VAS score

Safety:

- CBC results,
- Blood chemistry results,
- Vital signs;
- ECG findings,
- Urea analysis results,
- Skin disorders size at the sites of venipuncture,
- Skin disorders lifetime at the sites of venipuncture.

Pharmacodynamics:

- absolute reticulocyte count.
- Hb level.
- Area under effect curve «absolute reticulocyte count — time».
- Area under effect curve «Hb level — time».
- Time (e.g. the time of the maximum increase of absolute reticulocyte count vs. baseline).

The statistical analysis will include two-tailed hypothesis testing; the chosen significance level is 0.05.

Quantitative variables will be tested for normality using the Shapiro-Wilk test.

Normally distributed quantitative variables will be tested using the two-sample Student's t-test, Welch's t-test, and ANOVA.

Non-normally distributed quantitative variables will be tested using the Mann-Whitney U-test, the Wilcoxon test, the Kruskal-Wallis test, and the Friedman test.

Normally distributed quantitative data will be described using the following descriptive statistics: mean, SD, CV, min, and max. Non-normally distributed quantitative data will be described using the following descriptive statistics: median, quartiles, CV, min, and max.

Categorical Data

The following categorical data will be analyzed in the study:

Safety:

- Skin disorders sort at the sites of venipuncture.
- Rate and severity grades of AEs/SAEs,
- Rate of Grade 3-4 AEs,
- Frequency of local reactions (total and by skin disorder type).
- Frequency of early study termination cases by AEs/SAEs.

Categorical data will be processed using frequency tables, contingency tables, the Fisher's exact test, the test of equal frequencies, Pearson's χ^2 test, and the Cochran-Mantel-Haenszel test. Percentages or proportions will be used to describe categorical data.

The Benjamini-Yekutieli correction for multiple testing will be used.

Statistical methods will be chosen according to the type of initial data and their distribution. Appropriate statistical tests will be established after the data collection has been completed because the type of data distribution, sample homogeneity, etc. are unknown before the study start. For appropriate data processing, the list of statistical methods used may expand during the analysis.

8.3. Accounting for missing, unavailable or doubtful data, outliers

All information specified in the e-CRFs should be supported by relevant data in source documents.

After entering all data into the electronic database, a database specialist checks it for inconsistencies, errors, and missing data points. The Clinical Study Database Manager or Medical Expert of JSC BIOCAD generates queries to correct error data or to request missing data; the queries are site-specific and subject-specific (i.e., individual queries are generated for each subject). The Clinical Study Monitor will send queries to the study site by fax or email. The queries should be resolved by the investigator within five business days from the date they have been submitted to the study site. Copies of responses to queries must be kept at the study site; original responses must be stored at JSC BIOCAD.

When responses to queries are received from investigators, the database specialist checks it for inconsistencies, errors, and missing data points. When all data at all study sites have been collected and entered, the database is closed. Afterwards, statistical processing can be started.

Missing, unused, and spurious data will not be substituted.

Spurious and unevaluable data are revealed during the outlier analysis by examination of Mahalanobis or Cook distance, visual analysis of scatter plots and box plots.

All actions taken to handle missing, unevaluable, spurious data and outliers before/during the statistical analysis will be described in the Clinical Study Report.

8.4. The data conversion

Pharmacokinetic parameters

Based on determined concentrations in blood a number of the pharmacokinetic parameters will be calculated for the study drug:

1. **$AUC_{(0\text{-}1176)}$**– area under the plasma concentration curve from administration to 1176 h:

- At stage I for BCD-131 and Mircera® products:

$$AUC_{(0-1176)} = \sum_{p=2}^{18} \frac{(C_p + C_{p-1}) * (t_p - t_{p-1})}{2},$$

where $C_p -$ plasma concentration at the moment t_p (hours); $t_p \in \{0; 0.5; 2; 8; 24; 48; 72; 96; 120; 168; 216; 288; 336; 504; 672; 840; 1008; 1176\}$; $p=1,\ldots,18$

- At stage I for Aranesp® product:

$$AUC_{(0-1176)} = \sum_{p=2}^{20} \frac{(C_p + C_{p-1}) * (t_p - t_{p-1})}{2},$$

where $C_p -$ plasma concentration at the moment t_p (hours); $t_p \in \{0; 0.5; 2; 8; 16; 24; 36; 48; 72; 96; 120; 168; 216; 288; 336; 504; 672; 840; 1008; 1176\}$; $p=1,\ldots,20$

- At stage II:

$$AUC_{(0-1176)} = \sum_{p=2}^{21} \frac{(C_p + C_{p-1}) * (t_p - t_{p-1})}{2},$$

where $C_p -$ plasma concentration at the moment t_p (hours); $t_p \in \{0; 0.25; 0.5; 1; 2; 4; 8; 24; 48;$

72; 96; 120; 168; 216; 288; 336; 504; 672; 840; 1008; 1176}; p=1,…,21

2. **C_{max} -** maximum plasma concentration**.**
3. **T_{max}-** time until **C_{max}** is reached.
4. **Terminal rate constant (k_{el}**, elmination constant**)** will be calculated for the plasma concentration curve during the terminal log-linear phase by regression.
5. **$AUC_{(0-\infty)}$**– area under the plasma concentration curve from administration to infinity:

$$AUC_{(0-\infty)} = AUC_{(0-1176)} + \frac{C_{1176}}{k_{el}},$$

where C_{1176} – plasma concentration at the moment 1176 h; k_{el} – elimination constant.

6. **Plasma concentration half-life ($T_{1/2}$)** will be calculated as: $T_{1/2} = \frac{\ln(2)}{k_{el}}$, where k_{el} – elimination constant.
7. **Total clearence (CL)** will be calculated as:

$$CL = \frac{D}{AUC_{(0-1176)}}, \qquad \text{where } D - \text{single dose of the drug product.}$$

Pharmacodynamic parameters

Based on reticulocyte counts and on hemoglobin values a number of key pharmacodynamic parameters will be calculated:

1. **$AUEC_{(0-1176)}$** – (area under effect curve from the moment of injection to 1176 h [Day 50 of follow-up]) based on the change in the absolute reticulocyte count after a single-dose injection of the drug product;:

$$AUEC_{(0-1176)} = \sum_{p=2}^{15} \frac{(C_p + C_{p-1}) * (t_p - t_{p-1})}{2},$$

where C_p the absolute reticulocyte count at the moment t_p (hours);

$t_p \in \{0; 24; 48; 72; 96; 120; 168; 216; 288; 336; 504; 672; 840; 1008; 1176\}$; p=1,…,15

2. **$AUEC_{(0-1176)}$** – (area under effect curve from the moment of injection to 1176 h [Day 50 of follow-up]) based on the change in the Hb level in the blood after a single-dose injection of the drug product:

$$AUEC_{(0-1176)} = \sum_{p=2}^{15} \frac{(C_p + C_{p-1}) * (t_p - t_{p-1})}{2},$$

where C_p the Hb level at the moment t_p (hours);

$t_p \in \{0; 24; 48; 72; 96; 120; 168; 216; 288; 336; 504; 672; 840; 1008; 1176\}$; p=1,…,15

3. AC-E_{max} (absolute maximum increase in the test parameter vs. baseline) based on the change in the absolute reticulocyte count in the blood after a single-dose injection of the drug product.
4. AC-E_{max} (absolute maximum increase in the test parameter vs. baseline) based on the change in the Hb level in the blood after a single-dose injection of the drug product**.**

8.5. Multivariate Comparison and Multiplicity

Refer to Section 8.2.

8.6. Subgroup analysis, interaction and related variables

Not available.

9. OTHER PLANNED ANALYSES

No additional analyses are planned in this study.

10. DEVIATIONS FROM ANALYSIS METHODS DESCRIBED IN STUDY PROTOCOL

This Statistical Analysis Plan has no deviations from methods described in Study Protocol.