CLINICAL STUDY PROTOCOL	
Protocol Title:	**A Double-blind, Randomized, Controlled Clinical Study of the Pharmacokinetics, Pharmacodynamics, Tolerability, and Safety of Multiple Intravenous Injections of BCD-066 (JSC BIOCAD) and Aranesp® (Amgen Europe B.V., the Netherlands) in Healthy Volunteers)**
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SYNOPSIS	
Protocol ID	BCD-066-3, NCT03693950
Study Title	A Double-blind, Randomized, Controlled Clinical Study of the Pharmacokinetics, Pharmacodynamics, Tolerability, and Safety of Multiple Intravenous Injections of BCD-066 (JSC BIOCAD) and Aranesp® (Amgen Europe B.V., the Netherlands) in Healthy Volunteers
Phase	Phase I
Study Sponsor	JSC BIOCAD, Russia **Postal address:** Petrovo Dalneye, Krasnogorskiy District, Moscow Region, Russian Federation, 143422 **Legal address:** 34 A, Ul. Svyazi, Strelna, Petrodvortsoviy District, Strelna, St. Petersburg, 198515
Test Drug	BCD-066 (INN: darbepoetin alfa, JSC BIOCAD, Russia), solution for injection
Reference Drug	Aranesp® (INN: darbepoetin alfa, Amgen Europe B.V., the Netherlands), solution for injection
Study Purpose and Objectives	**The purpose of the study** is to confirm the equivalent pharmacokinetics, pharmacodynamics, safety, and tolerability of multiple IV injections of BCD-066 and Aranesp® in healthy volunteers. **Objectives:** 1. To evaluate and compare the key pharmacokinetic parameters of darbepoetin alfa in the serum after repeated-dose intravenous injections of 1 µg/kg BCD-066 (JSC BIOCAD, Russia) and 1 µg/kg Aranesp® (Amgen Europe B.V., the Netherlands) in healthy volunteers. 2. To evaluate and compare the key hemoglobin-based pharmacodynamic parameters after repeated-dose intravenous injections of 1 µg/kg BCD-066 (JSC BIOCAD, Russia) and 1 µg/kg Aranesp® (Amgen Europe B.V., the Netherlands) in healthy volunteers. 3. To evaluate and compare the safety and tolerability characteristics after repeated-dose intravenous injections of 1 µg/kg BCD-066 (JSC BIOCAD, Russia) and 1 µg/kg Aranesp® (Amgen Europe B.V., the Netherlands) in healthy volunteers.

Study Design	Clinical study BCD-066-3 is a double-blind controlled, randomized, parallel-group study of the pharmacokinetics, pharmacodynamics, tolerability, and safety of repeated-dose intravenous BCD-066 and Aranesp®. The study is planned to include 62 healthy male volunteers (56 active participants and 6 back up subjects to replace dropouts). Before inclusion in the study, all potential participants are given full information about the study. This information is presented in the Participant Information Sheet. If the potential participant gives his consent to be in the study, he has to sign the Informed Consent Form for participation in a clinical study and then undergo a 14-day screening examination to confirm that he is eligible for the study. After passing the screening and being considered eligible for the study, subjects are centrally randomized at a 1:1 ratio to one of two treatment groups (#1 and #2). Subjects in Group #1 will receive IV injections of 1.0 µg/kg BCD-066 on Day 1, Day 8, Day 15, and Day 22. Subjects in Group #2 will receive IV injections 1 µg/kg Aranesp® on Day 1, Day 8, Day 15, and Day 22. This is a double-blind study, which means that neither the investigator nor the subject will know what drug is used in each group. Blood samples for evaluation of darbepoetin alfa concentration will be collected 30 min, 20 min, and 10 min before the injection, immediately before the injection (not more than 5 min before drug administration), and then 5 min, 10 min, 15 min, 20 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h, 48 h, and 72 h after the first and the fourth IV injection. There will be 4 blood collections before and 14 blood collections after the first and the fourth IV injection of darbepoetin alfa. The serum concentrations will be used to calculate the key PK parameters of darbepoetin alfa. Before the second and the third injection, blood samples will be collected only once. During the entire study, there will be 38 blood collections for PK evaluation. Blood samples for evaluation of hemoglobin (the primary PD endpoint) and secondary PD parameters (reticulocytes, RBC, hematocrit) will be collected before each injection of the test drug/reference drug, then 1 day, 3 days, and 5 days after the first, second, and third injection, and 1 day, 3 days, and 7 days after the fourth injection. Two more blood collections will be performed for immunogenicity assessment (at screening and on Day 29). This study includes a screening examination (not more than 14 days) and 16 study visits. After the first and fourth injection of the test/reference drug, all volunteers will be required to stay in the clinic for at least 24 h for monitoring purposes. The duration of the study from the first drug injection to the final visit is 29 days. <u>Special requirements to volunteers</u>

	Study subjects are required not to drink any alcohol for 24 h before and 72 h after each injection of BCD-066/Aranesp®; not to drink more than 10 units of alcohol per week (1 unit of alcohol is equivalent to 0.5 L of beer, 200 mL of wine, or 50 mL of a strong alcohol beverage); and not to smoke for 1 h before and 3 h after each injection of BCD-066/Aranesp® and for 1 h before each blood pressure measurement.
Study Population	Healthy male volunteers from 18 to 45 years old (inclusive).
Planned Sample Size	Sixty-two healthy male volunteers (56 volunteers in the main sample plus 6 backup volunteers to replace the dropouts).
Inclusion Criteria	1. Signed informed consent form 2. Men from 18 to 45 years old (inclusive) 3. BMI within the normal limits (18.5 to 30 kg/m^2) 4. Hemoglobin from 120 g/L to 150 g/L and hematocrit 41% to 49% at screening (before the first injection) 5. Serum transferring 2.15 g/L to 3.6 g/L; serum ferritin from 20 µg/L to 250 µg/L 6. Vitamin B12 from 187 pcg/mL to 883 pcg/mL; folic acid from 3.1 ng/mL to 20.5 ng/mL 7. Endogenous serum erythropoietin < 30 mIU/mL at screening 8. The subject is verified as "Healthy" according to results of standard clinical, laboratory and instrumental tests 9. Subject's ability (in the investigator's opinion) to follow the protocol procedures 10. The subject and his sexual partner with retained childbearing potential consent to implement reliable contraceptive methods starting 2 weeks before inclusion in the study and up to 2 weeks after the last dose of the test/reference drug. This requirement does not apply to surgically sterile subjects. Reliable contraception methods mean one barrier method in combination with one of the following: spermicides, intrauterine device and/or oral contraceptives used by the subject's partner. 11. The subject agrees not to drink alcohol for 24 h prior to each injection of the test/reference drug and for 72 h after the injection.
Exclusion Criteria	1. Psychiatric disorders or other conditions that can affect the ability of the subject to follow the study protocol 2. Acute infections within 4 weeks before the study start 3. Results of laboratory and/or instrumental tests are outside the normal range

	4. Chronic cardiovascular, bronchial and/or pulmonary, neuroendocrine GI, liver, kidney, and blood diseases, including CAD, arterial hypertension, peripheral vascular and/or cerebral vascular disorders, and thrombocytosis 5. A history of chronic hemorrhages 6. Any malignancy (or a prior history of malignancy) 7. Prior treatment with any erythropoietin/darbepoetin product or other products promoting erythropoiesis received at any time before enrollment 8. Intravenous iron therapy within two years prior to enrollment 9. Treatment with any drugs (including over-the-counter drugs, herbal drugs, or nutritional supplements) within 14 days prior to the first administration of the investigational product. The restriction does not apply to the use of paracetamol (less than 3 g a day) or ibuprofen (less than 1 g a day) 10. Epileptic seizures within six months prior to the first administration of the investigational product 11. Extensive surgery within one month before enrollment 12. Impossibility to insert an intravenous catheter for blood sampling (e.g., because of a skin condition at the venepuncture site) 13. Hypersensitivity to any components of BCD-066 (JSC BIOCAD), Aranesp® (Amgen Europe B.V., the Netherlands) or drug products of the same therapeutic category; intolerance to erythropoietins and/or other recombinant human proteins; intolerance to iron (III) products 14. Episodes of thrombosis and/or thromboembolism in the past (myocardial infarction, stroke, transient ischemic attacks, deep vein thrombosis, pulmonary embolism within 6 months before enrollment); increased risk of deep vein thrombosis 15. Known severe allergies (anaphylaxis or multiple drug allergy) 16. Antibodies to darbepoetin alfa at screening 17. Smoking of more than 10 cigarettes a day 18. The subject consumes more than 10 units of alcohol per week (1 unit equals to 0.5 L of beer, 200 mL of wine or 50 mL of a strong alcohol beverage) or has a history of alcohol, recreational drug or medication abuse

	19. Acute hemorrhage or blood/plasma donation or blood transfusion within 2 months before enrollment 20. Participation in other clinical studies within 30 calendar days before enrollment in this study 21. Prior participation in this study.
Study Therapy	The study subjects will receive 1 µg/kg darbepoetin alfa (BCD-066 or Aranesp®) as weekly IV injections on Day 1, Day 8, Day 15, and Day 22. Blood samples will be collected for investigation of the PK and PD.
Study Procedures	In this study, blood samples will be collected according to the schedule to measure the concentration of darbepoetin alfa, evaluate the hemoglobin-based PD parameters, and to evaluate additional characteristics of the red blood (reticulocytes, RBC, hematocrit). The study also involves procedures to monitor the safety. Blood samples for darbepoetin alfa concentration will be collected before and after each IV injection of the test/reference drug. Collections will be performed as follows: • Visit 1 (Day 1 – Day 2): 30 min, 20 min, 10 min and immediately before the first injection of darbepoetin alfa and then 5 min, 10 min, 15 min, 20 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 16 h, and 24 h after the first injection. • Visit 2 (Day 3): 48 h after the first injection. • Visit 3 (Day 4): 72 h after the first injection. • Visit 5 (Day 8): immediately before the second injection. • Visit 9 (Day 15): immediately before the third injection. • Visit 13, (Day 22 – Day 23): 30 min, 20 min, 10 min and immediately before the fourth injection of darbepoetin alfa and then 5 min, 10 min, 15 min, 20 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 16 h, and 24 h after the fourth injection. • Visit 14 (Day 24): 48 h after the fourth injection. • Visit 15 (Day 25): 72 h after the fourth injection. Blood samples for PD study will be collected as follows: • Visit 1 (Day 1 – Day 2): before the injection and 24 h (one day) after the first injection. • Visit 3 (Day 4): 72 h (3 days) after the first injection. • Visit 4 (Day 6): 120 h (5 days) after the first injection. • Visit 5 (Day 8): 168 h after the first injection (immediately before the second injection). • Visit 6 (Day 9): 192 h after the first injection (24 h after the second injection).

	• Visit 7 (Day 11): 240 h after the first injection (3 days after the second injection). • Visit 8 (Day 13): 288 h after the first injection (5 days after the second injection). • Visit 9 (Day 15): 336 h after the first injection (immediately before the third injection). • Visit 10 (Day 16): 360 h after the first injection (one day after the third injection). • Visit 11 (Day 18): 408 h after the first injection (3 days after the third injection). • Visit 12 (Day 20): 432 h after the first injection (5 days after the third injection). • Visit 13 (Day 22 – Day 23): 504 h after the first injection (immediately before the fourth injection); 528 h after the first injection (one day after the fourth injection). • Visit 15 (Day 25): 576 h after the first injection (3 days after the fourth injection). • Visit 16 (Day 29): 672 h after the first injection (7 days after the fourth injection). <u>Safety monitoring procedures:</u> For safety evaluation, the study team will monitor vital signs (BP, pulse, respiration rate, and body temperature), perform regular physical examinations including assessment of the injection site, and run hematology and chemistry tests, blood tests for anti-darbepoetin alfa binding and neutralizing antibodies, urinalysis, and ECG.
Total Study Duration	Expected study duration is 2 years including up to 18 months of enrollment and 6 months of data collection and statistical processing. Each study subject is expected to be in the study for 64 days, which includes the screening (14 days), administration of the test/reference drug and investigation of the PK and PD (29 days), and the follow-up period (Day 30 to Day 50).
PK Endpoints	• **Endpoints**: – The area under the *Time vs. Concentration* curve for darbepoetin alfa from injection to 72 h ($AUC_{(0-72)}$) after the first and the fourth IV injection of BCD-066 or Aranesp® – The maximum concentration of darbepoetin alfa in the serum after the first and the fourth IV injection of BCD-066 or Aranesp® (C_{max})

	– The elimination half-life (T½) – The total area under the *Concentration vs. Time* curve from 0 to infinity (AUC_{0-168}) – The time to maximum drug concentration in the serum (T_{max}) – The residual area – The elimination constant (k_{el}) – The total clearance (CL). **Methods used for PK assessment:** The PK analysis will include all subjects who have received all doses of the test/reference drug as per protocol. Not more than 2 PK blood collections after the first injection and not more than 2 PK blood collections after the fourth injection can be missed. Subjects who have two consecutive blood samples missed will be not included in the analysis. The PK parameters will be analyzed based on darbepoetin alfa concentrations in the subject's serum.
PD Endpoints	**Endpoints:** • **Primary endpoint:** — The area under the baseline-adjusted *Effect vs. Time* curve from injection to Day 29 after the repeated-dose IV administration of the test/reference drug ($AUEC_{(1-29)}$). • **Secondary endpoints:** — The maximum increase in hemoglobin from baseline, estimated from injection to Day 29 after the repeated-dose IV administration of the test/reference drug ($AC\text{-}E_{max}$). **Supplementary endpoints:** — The time to the absolute maximum increase in hemoglobin from injection to Day 29 after the repeated-dose IV administration of the test/reference drug (T_{max}). In addition, PD parameters (AUEC, $AC\text{-}E_{max}$, and T_{max}) will be estimated based on the reticulocyte and RBC counts and on hematocrit values. **Methods used for pharmacodynamic assessment:**

	The PD analysis will include all subjects who have received all doses of the test/reference drug as per protocol. Not more than 3 PD blood collections can be missed over the entire study. Subjects who have two consecutive blood samples missed will be not included in the analysis. The PD analysis will be based on the hemoglobin concentrations, reticulocyte and RBC counts, and hematocrit. The limiting hemoglobin level in this study is 180 g/L. If the subject reaches this level before the last injection of darbepoetin alfa, he will be withdrawn from the study. If the subject reaches the limiting Hb concentration after he receives the last (fourth) injection of darbepoetin alfa, he will not be withdrawn from the study.
Safety Assessment	**Endpoints:** • The proportion of subjects who developed AE(s) after receiving at one injection of darbepoetin alfa • The rate of SAEs • The rate of CTCAE grade 3/4 AEs • The rate of withdrawals due to AEs • The proportion of subjects who had injection site reactions • The proportion of subjects who had antibodies to darbepoetin alfa on Day 29. **Methods used for safety assessment:** The safety analysis will include all subjects who have received at least one dose of the test/reference drug. The safety will be analyzed based on the information about: – The reported AEs/SAEs, – Results of physical examinations with an assessment of the injection site, vital signs, and instrumental and laboratory test results (hematology and chemistry, blood testing for binding and neutralizing anti-darbepoetin alfa antibodies, and urinalysis).
Statistical Analysis	**Determination of sample size** The sample size was determined using the variation coefficients for hemoglobin increase (Hb AUEC) obtained by Cheung, 2001[1]. The estimated Type I error was 5% (α=0.05) and Type II error was 20% (β=0.2), with the test power of 80%. The sample size was calculated with the "sampleN.TOST" function of the "PowerTOST" package for R. The calculation used the CV values and the ratios of the mean Hb increases (AUEC). The study

[1] W.Cheung, N. Minton, K.Gunawardena. Pharmacokinetics and pharmacodynamics of epoetin alfa once weekly an three times weekly, 2001

	should include 28 subjects per group and 6 subjects as backups to replace potential early withdrawals (62 volunteers total). **Methods used to analyze PK and PD parameters** Descriptive statistics for the data on drug concentration in the serum, effect values, and protocol-specified PK and PD parameters will be performed using the following: mean values, geometric mean values, standard deviations, medians, upper and lower quartiles, min and max, and the coefficient of variation. The statistical comparison involves the calculation of two-sided parametric 90% confidence intervals (CIs) for the ratios of the $AUEC_{(1-29)}$ means for hemoglobin and darbepoetin alfa following the IV injection of BCD-066 and Aranesp®, and comparison of these CIs with the PD equivalence interval: $AUEC_{(1-29),}$: [0.8; 1.25] (80-125%). The study drugs will be considered equivalent if the estimated 90% CIs for the mean $AUEC_{(1-29)}$ ratio for darbepoetin in BCD-066 and darbepoetin in Aranesp® will fall in the said range. **Methods used to analyze the safety and demographic data** The interval (quantitative) data will be presented as mean values, standard deviations, medians, upper and lower quartiles, min and max. The normality of the interval data will be tested with the Shapiro-Wilk test. The categorical (qualitative) data will be presented as the absolute and relative (proportions or percentages) number of observations. The normally distributed interval data will be compared using the ANOVA and *t*-test for dependent and independent samples. Non-normally distributed interval data will be analyzed using non-parametric tests: Friedman's ANOVA, Kruskal-Wallis H test, Wilcoxon T test, and Mann-Whitney test. Categorical data will be compared using the χ^2 test or the Fisher's exact test. Correction for multiple comparisons will be performed with the Benjamini-Yekutieli procedure. Statistical methods will be chosen based on the type and distribution of raw data. Applicability of certain statistical tests will be evaluated after all the data are collected because it is impossible to predict the distribution pattern, data homogeneity and other data characteristics in advance.