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ORIGINAL RESEARCH

# Comparison of Pharmacokinetic, Pharmacodynamic and Tolerability Profiles of CKD-11101, Darbepoetin Alfa (NESP®) Biosimilar, to Those of NESP® After a Single Subcutaneous or Intravenous Administration to Healthy Subjects



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**Introduction:** Darbepoetin alfa (NESP® and ARANESP®) has a sustained erythropoietic activity with a longer half-life than conventional recombinant human erythropoietin. CKD-11101 is under clinical development as a biosimilar of darbepoetin alfa. The purpose of this study was to compare the pharmacokinetic (PK), pharmacodynamic (PD), and tolerability profiles of CKD-11101 with those of reference drug in healthy subjects.

**Methods:** This study was performed in two parts for healthy subjects. In each period, CKD-11101 and reference, both at 60 μg, were administered via intravenous (IV) or subcutaneous (SC) route of administration.

**Results:** After both IV or SC dose, the geometric mean ratio (GMR) of CKD-11101 to reference drug and its 90% confidence intervals (CIs) for $C_{max}$, $AUC_{0-last}$ and $AUC_{0-\infty}$ were all within 0.8–1.25. No statistically significant differences were noted in the maximum baseline adjusted reticulocyte count or the area under the baseline adjusted reticulocyte count-time between the CKD-11101 and reference drug after IV or SC dose (all *p*-value>0.05). Both CKD-11101 and reference drug were generally well tolerated.

**Discussion:** After a single IV or SC dose, the CKD-11101 was well tolerated and showed comparable PK and PD characteristics with reference drug.

**Keywords:** pharmacokinetics, pharmacodynamics, biosimilar, darbepoetin alfa

## Introduction

Erythropoietin (EPO), a glycoprotein cytokine, is an essential factor for the erythropoiesis from erythropoietic stem cells within bone marrow.[1] It is primarily produced from the kidney in response to hypoxic stimuli, and EPO deficiency is commonly observed in patients with chronic kidney disease (CKD).[1] Recombinant human EPO (rhEPO) therapy has been widely used for the treatment of anemia due to CKD or due to cytotoxic chemotherapy.[2–4] However, due to the short serum half-life (4 to 12 hours) of rhEPO, it has to be administered 3 times weekly in CKD patients.[5]

Darbepoetin alfa is a second-generation of rhEPO, co-developed by Amgen Inc. (ARANESP®, Amgen Inc. CA, USA) and Kyowa Hakko Kirin Co., Ltd.

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(NESP®, Kyowa Hakko Kirin Co., Ltd, Tokyo, Japan). Owing to the 5 N-linked oligosaccharide chains and to the larger molecular weight than rhEPO darbepoetin alfa has an enhanced serum half-life (2 to 3 times longer than rhEPO), which enables once weekly administration in CKD patients.[6,7] It has same mechanism of action with rhEPO and was effective for the treatment of anemia in the CKD patients and patients receiving cytotoxic chemotherapy.[8,9]

A biosimilar is a biological product that has a highly similar quality, safety and efficacy profile to the original biological product which is approved in regulatory agencies.[10,11] According to European Medicines Agency (EMEA) and US Food and Drug Administration guidelines, various evidence from analytical (structural and physicochemical characteristics), nonclinical and clinical studies should be considered collectively to demonstrate the biosimilarity between the two biological products.[10,11]

CKD-11101 is a darbepoetin alfa developed by Chong Kun Dang Pharmaceutical Corp. (Seoul, Republic of Korea) is developed as a biosimilar of darbepoetin alfa which used as a test drug in this study. CKD-11101 is produced by recombinant DNA technology in modified Chinese hamster ovary cells and it showed high structural similarity with reference drug in terms of amino acid sequence characteristics, peptide mapping characteristics, disulfide bond characteristics. The CKD-11101 also showed highly similar physicochemical and immunological characteristics with reference drug in various in-vitro tests. Although the CKD-11101 showed some minor difference with reference drug in the relative contents of neutral sugar, in-vitro and in-vivo biological activity of CKD-11101 was similar to reference drug. Therefore, CKD-11101 (Nesbell, Chong Kun Dang Pharmaceutical Corp., Seoul, Republic of Korea and Mylan EPD G. K., Tokyo, Japan) is approved by Korea's Ministry of Food and Drug Safety (MFDS) in 2018 and by Japan's Pharmaceuticals and Medical Devices Agency (PMDA) in 2019.

Based on these quality and nonclinical studies results, these clinical studies aimed to compare the pharmacokinetic (PK), pharmacodynamic (PD) and tolerability profiles of CKD-11101 with that of reference drug, after a single intravenous (IV) or after a subcutaneous (SC) administration in healthy subjects.

# Materials and Methods

## Study Design and Subjects

This study (ClinicalTrials.gov identifier: NCT01684605 and NCT01685671) was approved by Ministry of Food and Drug Safety of Republic of Korea and the Institutional Review Board of Seoul National University Hospital (Seoul, Republic of Korea). The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice and the ethical principles of the Declaration of Helsinki. All of the subjects provided written informed consent prior to the participation of this study.

The study was performed using randomized, double-blind, single-dose, two-way, two-period, two-sequence, crossover design which was referenced from various comparative PK studies.[12–14] The study consisted of two parts, one with IV dose (Part 1, Figure 1) and the other with SC dose (Part 2, Figure 1). Male subjects aged 20 and 55 years weighing at least 55 kg and below 90 kg, and with a body mass index (BMI) between 18 and 27 kg/m$^2$ were eligible for these studies if they were healthy, assessed by medical histories, physical examinations, vital sign measurements, 12-lead electrocardiograms (ECGs) and clinical laboratory tests within 4 weeks prior to administration of the study drug. Subjects with anemia or with a history of drug abuse or with a positive urine drug screening test result were excluded.

Eligible subjects were randomly allocated to one of the treatment sequences and received a test drug (CKD-11101 60 μg prefilled syringe, Chong Kun Dang Pharmaceutical Corp., Seoul, Republic of Korea) in one period and a reference drug (60 μg prefilled syringe, Kyowa Hakko Kirin Co., Ltd, Tokyo, Japan) in the other period. To maintain double-blind, unblinded staffs were designated for drug administration and those staffs did not participate in any other study-related procedures. Three weeks of washout were set between each dose considering the half-life of darbepoetin alfa and the time for recovery of reticulocyte (PD endpoint) to the baseline.[15–18]

## Bioanalytical Methods

The serum concentrations of darbepoetin alfa were determined using a validated enzyme-linked immunosorbent assay (ELISA) method. Human erythropoietin ELISA Kit (STEMCELL Technologies, Vancouver, Canada) was used to capture darbepoetin alfa. Serum PK samples were diluted and then added to the microplate with quality

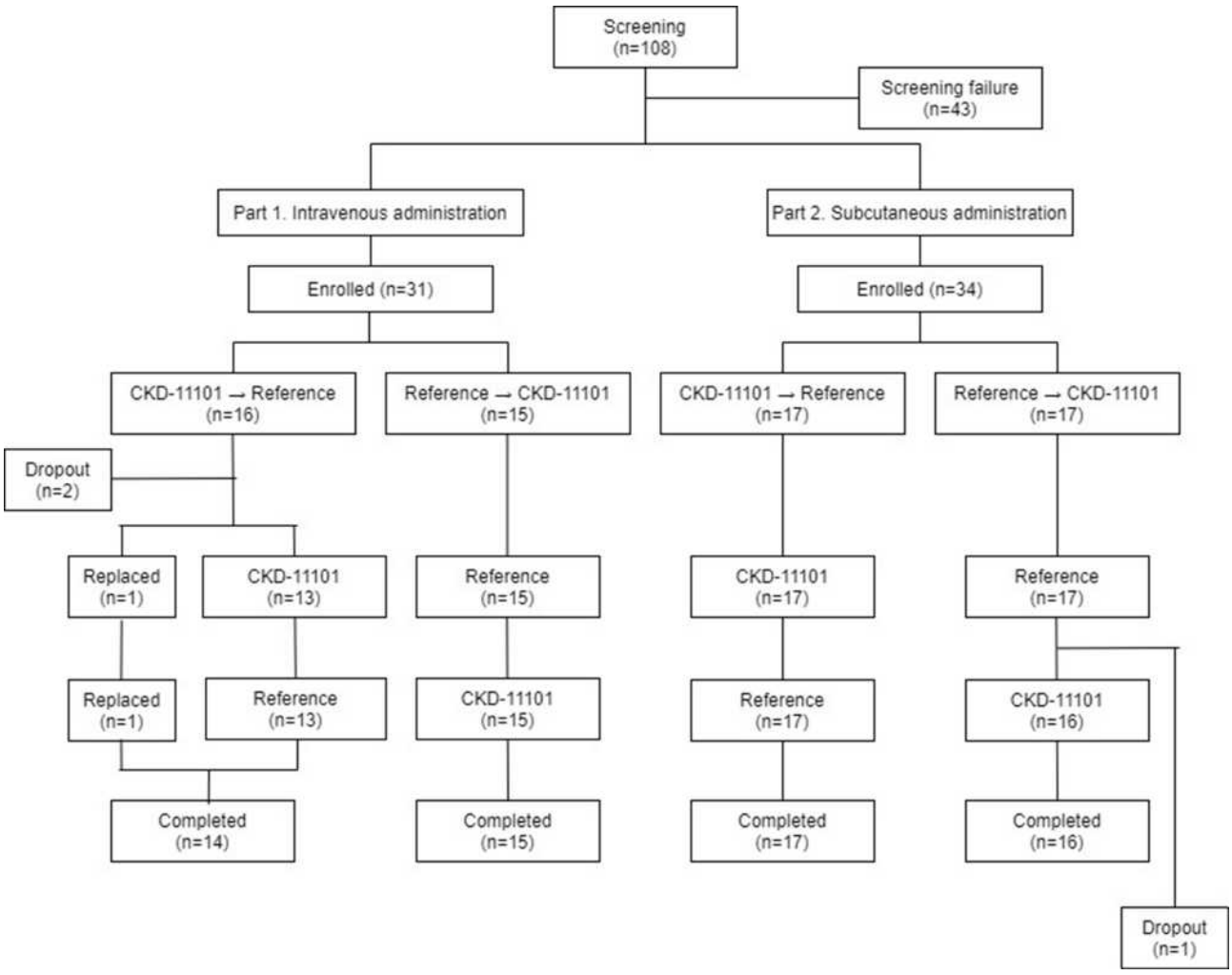


**Figure 1** Subject disposition.

control and standards samples. Biotinylated anti-EPO Ab was added to the plate and incubated for one hour at 25° C. After washing the microplate, a streptavidin conjugated to horseradish peroxidase (HRP) was added to the plate and incubated for one hour at 25°C. For the color development, tetramethylbenzidine (TMB) substrate solution was added to the plate. The reaction was stopped by adding stop solution and a microplate reader was used to detect the absorbance at 450 nm with a reference wavelength at 650 nm. The lower limit of quantification was 0.18 μg/L. The precision and accuracy of the standard curve was <8.3% and 97%–108%, respectively. The precision and accuracy of intra- and inter-assay were all within the acceptance criteria.

Immunogenicity (anti-drug antibodies) of study drugs was assayed using a validated enzyme-linked immunosorbent assay (ELISA) with a sensitivity of 230.726 μg/L for CKD-11101 and 309.560 μg/L for reference drug. Serum samples and quality control samples were incubated for two hours in the CKD-11101 or reference drug coated microplate. After washing the microplate, an anti-human IgG conjugated to HRP was added to the microplate as a detection antibody and incubated for one hour at 25°C. For the color development, TMB substrate solution was added to the plate and the reaction was stopped by adding 2N sulfuric acid. The microplate reader was used to detect absorbance at 450 nm.

## PK Samples and Analysis

Serum samples for PK analysis of darbepoetin alfa were collected at 0 (Pre-dose), 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 168, and 264-hour after IV dose at each period (Part 1) and at 0 (Pre-dose), 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, 120, 168, 216, 264, and 360-hour after SC dose at each period (Part 2). The serum

sampling time points were different in part 1 and part 2. It is because that the half-life of darbepoetin alfa was 25 hours when administered intravenously, while the half-life was 48 hours when administered subcutaneously. The PK parameters were calculated by a non-compartmental method using WinNonlin 8.0 software (Pharsight, CA, USA). The maximum serum concentration ($C_{max}$) and the time to reach peak concentration ($T_{max}$) were directly obtained from the serum concentration-time profiles. The area under the concentration–ime curve from zero time to the last observed concentration ($AUC_{0–last}$) was determined by the trapezoidal method. The area under the curve from time zero to infinity ($AUC_{0–\infty}$) was estimated by the equation $AUC_{last} + C_{last}/\lambda_z$, where $\lambda_z$ was the terminal elimination constant. The terminal half-life ($t_{1/2}$) was calculated by the equation In(2) divided $\lambda_z$. The clearance and apparent clearance (CL and CL/F) were calculated by Dose/$AUC_{last}$, and the volume of distribution and apparent volume of distribution ($V_z$ and $V_z$/F) were calculated as the CL and CL/F divided by $\lambda_z$.

## PD Samples and Analysis

Whole blood samples for PD analysis were collected at 0 (Pre-dose), 8, 24, 48, 96, 168 and 264-hour after IV dose at each period (Part 1) and at 0 (Pre-dose), 8, 24, 48, 72, 120, 216 and 360-hour after SC dose at each period (Part 2). The time course of baseline-adjusted reticulocyte count (%) after administration of the test and reference drug was used for primary PD marker. The maximal baseline-adjusted reticulocyte count ($\Delta E_{max}$) was directly obtained from the observed data. The area under the baseline-adjusted reticulocyte count–time curve from time 0 to the last detectable time point ($\Delta AUEC_{0–last}$) was determined by the trapezoidal method. Other exploratory PD markers including hemoglobin (g/L), hematocrit (%) and red blood cell (RBC) count ($10^6/mm^3$) were baseline-adjusted and graphically compared between the treatments.

## Immunogenicity Evaluation

Anti-drug antibodies to CKD-11101 or darbepoetin alfa were determined at pre-dose, 12 days (Part 1) or 16 days (Part 2) post-dose at each study period.

## Tolerability Evaluation

The tolerability was assessed for the subjects who received at least one of the study drugs. Safety profiles were evaluated based on adverse events (AEs), physical examinations, assessment on injection site reactions, vital signs, 12-lead electrocardiograms (ECGs), clinical laboratory evaluations.

## Statistical Analysis

All statistical analyses were performed using the SAS® Version 9.4 (SAS Institute Inc., Cary, NC, USA). The baseline characteristics of demographic parameters and anemia parameters such as reticulocyte count, hemoglobin, ferritin and transferrin were analyzed using either *t*-test or Mann–Whitney *U*-test depending on whether normality is satisfied. Descriptive statistics were used to summarize study data and a general linear mixed effect model was developed to compare the primary PK parameters ($C_{max}$, $AUC_{0\text{-last}}$ and $AUC_{0–\infty}$) between test and reference drugs. Using the model, the geometric mean ratio (GMR) and its 90% CI of the test drug to reference drug were estimated for the primary PK parameters. If 90% confidence intervals (CIs) of the GMR for these parameters were within the range of 0.8-1.25, the PK characteristics of test and reference drugs were considered as equivalent. For the comparison of primary PD parameters ($\Delta E_{max}$ and $\Delta AUEC_{0\text{-last}}$ of reticulocyte count), the mean difference and its *p*-values of the test drug and reference drug were estimated for the primary PD parameters using a general linear mixed effect model. If the *p*-values of the mean difference for these parameters were above 0.05 the PD characteristics of test and reference drugs were considered as equivalent.[19] A *P*-value of less than 0.05 was considered as statistically significant.

# Results

## Subjects

In part 1, a total of 31 subjects were enrolled and 29 of whom completed the study as planned (Figure 1). In part 2, a total of 34 subjects were enrolled and 33 of whom completed the study as planned (Figure 1). The PK and PD analyses were done for 29 and 33 subjects who completed the study in part 1 and part 2, respectively. In part 1, one subject's baseline of reticulocyte result was missed during the CKD-11101 (test drug) period and that data was excluded from PD analysis. Tolerability analysis was performed for 29 and 34 subjects who administered the study drug more than once in part 1 and part 2, respectively.

The baseline characteristics of subjects were comparable between part 1 and part 2 (Table 1). The mean ± standard deviation (SD) values for baseline characteristics of subjects

**Table 1** Demographic Characteristics

| **Intravenous Administration (Part 1)** | **CKD-11101 → Reference (n=16)** | **Reference → CKD-11101 (n=15)** | **Total (n=31)** | **P-value** |
|---|---|---|---|---|
| Age (years) | 28.1 ± 5.4 [21 - 41] | 27.1 ± 4.5 [22 – 38] | 27.6 ± 4.9 [21 – 41] | 0.583[a] |
| BMI (kg/m$^2$) | 22.2 ± 2.3 [18.1-25.5] | 22.4 ± 1.7 [19.7–25.1] | 22.3 ± 2.0 [18.1-25.5] | 0.225[a] |
| Reticulocyte count (%) | 1.14 ± 0.33 [0.58 – 1.68] | 1.04 ± 0.26 [0.65 – 1.60] | 1.09 ± 0.29 [0.58 – 1.68] | 0.374[a] |
| Hemoglobin (g/dL) | 16.0 ± 0.6 [15.2 – 17.2] | 15.8 ± 1.0 [13.7 – 17.4] | 15.9 ± 0.8 [13.7 – 17.4] | 0.762[a] |
| Ferritin (ng/mL) | 85.8 ± 56.6 [16.1 – 185.6] | 83.9 ± 65.3 [27.1 – 290.2] | 84.9 ± 59.9 [16.1 – 290.2] | 0.891[b] |
| Transferrin (mg/dL) | 274.16 ± 33.81 [217.74 – 353.00] | 277.24 ± 40.60 [218.61 – 367.50] | 275.65 ± 36.65 [217.74 – 367.50] | 0.820[a] |
| **Subcutaneous Administration (Part 2)** | **CKD-11101 → Reference (n=17)** | **Reference → CKD-11101 (n=16)** | **Total (n=33)** | **P-value** |
| Age (years) | 27.2 ± 8.2 [21-50] | 26.9 ± 6.6 [22–46] | 27.1 ± 7.3 [21–50] | 0.706[b] |
| BMI (kg/m$^2$) | 22.9 ± 1.9 [20.2–26.6] | 22.1 ± 1.4 [19.1–24.4] | 22.5 ± 1.7 [19.1-26.6] | 0.208[a] |
| Reticulocyte count (%) | 1.06 ± 0.36 [0.53 – 2.11] | 0.93 ± 0.22 [0.67 – 1.66] | 1.00 ± 0.30 [0.53 – 2.11] | 0.180[b] |
| Hemoglobin (g/dL) | 15.7 ± 0.9 [13.5 – 17.2] | 15.8 ± 0.7 [14.4 – 17.5] | 15.7 ± 0.8 [13.5 – 17.5] | 0.561[a] |
| Ferritin (ng/mL) | 102.4 ± 65.5 [25.6 – 264.1] | 74.6 ± 52.4 [22.9 – 177.4] | 88.9 ± 60.3 [22.9 – 264.1] | 0.119[b] |
| Transferrin (mg/dL) | 251.00 ± 35.47 [195.26 – 317.18] | 283.21 ± 35.53 [212.68 – 334.88] | 266.62 ± 38.58 [195.26 – 334.88] | 0.990[b] |

**Notes**: All values are expressed as mean ± standard deviation and [minimum– maximum]. [a]P-value was calculated by *T*-test. [b]P-value was calculated by Mann–Whitney *U*-test
**Abbreviations**: CKD-11101, test drug; Reference, reference drug.

who participated in part 1 were 27.6 ± 4.9 years for age, 22.3 ± 2.0 kg/m$^2$ for BMI, 1.09 ± 0.29% for reticulocyte count, 15.9 ± 0.8 g/dL for hemoglobin, 84.9 ± 59.9 ng/mL for ferritin and 275.65 ± 36.65 mg/dL for transferrin. Those values of subjects who participated in part 2 were 27.1 ± 7.3 years for age, 22.5 ± 1.7 kg/m$^2$ for BMI, 1.00 ± 0.30% for reticulocyte count, 15.7 ± 0.8 g/dL for hemoglobin, 88.9 ± 60.3 ng/mL for ferritin and 266.62 ± 38.58 mg/dL for transferrin. All the subjects were male in both part 1 and part 2 and both the baseline characteristics of subjects were not significantly different between the sequence group both in part 1 and part 2 (Table 1).

## PK Analysis

The systemic exposure to darbepoetin alfa was similar after single IV or SC administration of the test (CKD-11101) or reference drug given as 60 μg prefilled syringe. The mean serum concentration–time profiles of darbepoetin alfa were superimposable both in part 1 and part 2 (Figure 2A and B, respectively).

In part 1, the mean serum concentration-time profiles were similar after a single IV administration of test or reference drug (Figure 2A). The serum concentration of darbepoetin alfa reached a maximal level at a median of 0.25-hours post-dose and eliminated following a multi-exponential decrease pattern in both test and reference drug (Figure 2A). The primary PK parameters were comparable between the test and reference drug (Figure 3A–C and Table 2). The GMRs of test drug to reference drug and its 90% CI for $C_{max}$, $AUC_{0\text{-last}}$ and $AUC_{0-\infty}$ were 1.05

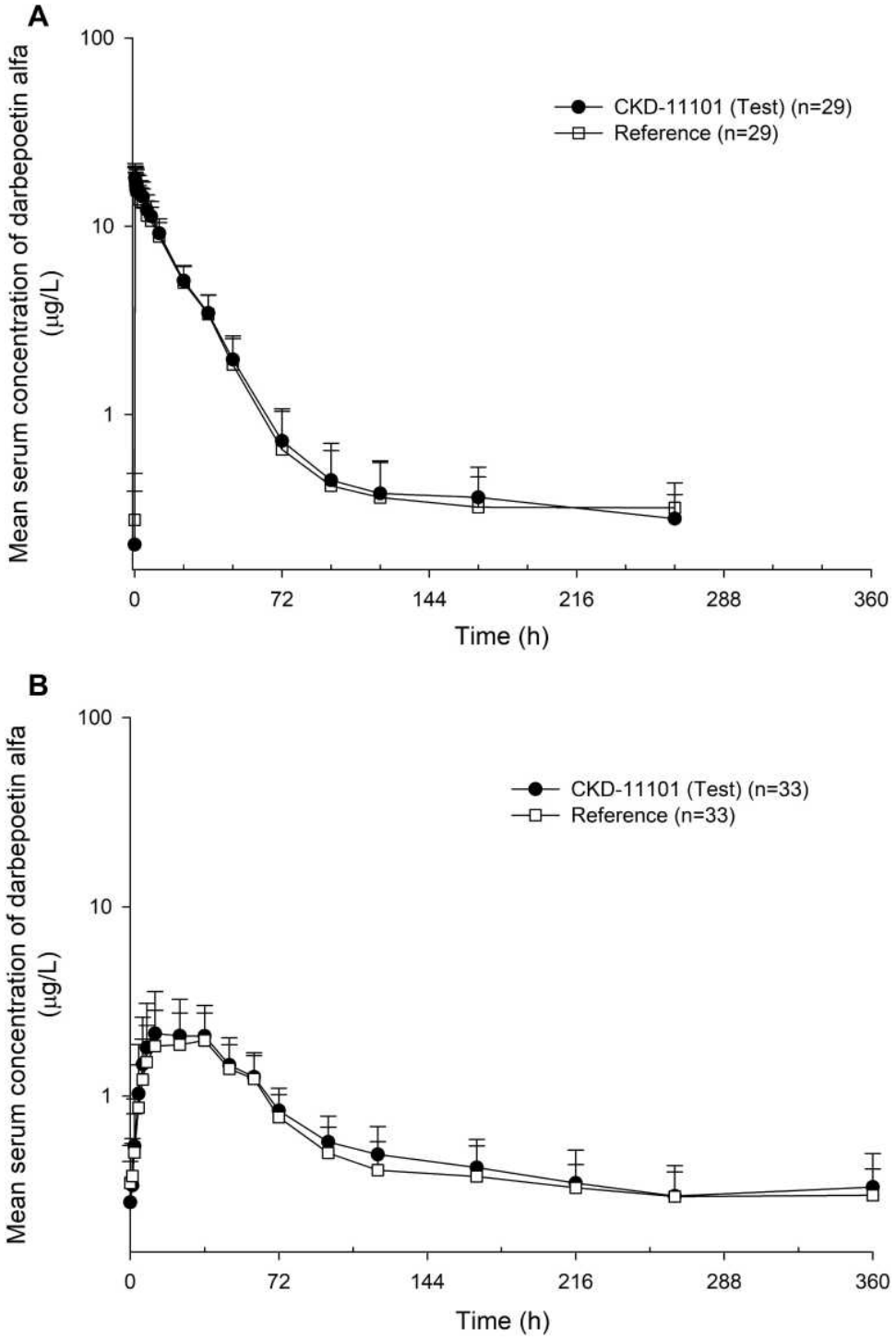


**Figure 2** Mean serum concentration-time profiles of darbepoetin alfa after a single intravenous (**A**) or subcutaneous (**B**) administration of CKD-11101 60 μg (test drug) or reference drug 60 μg.

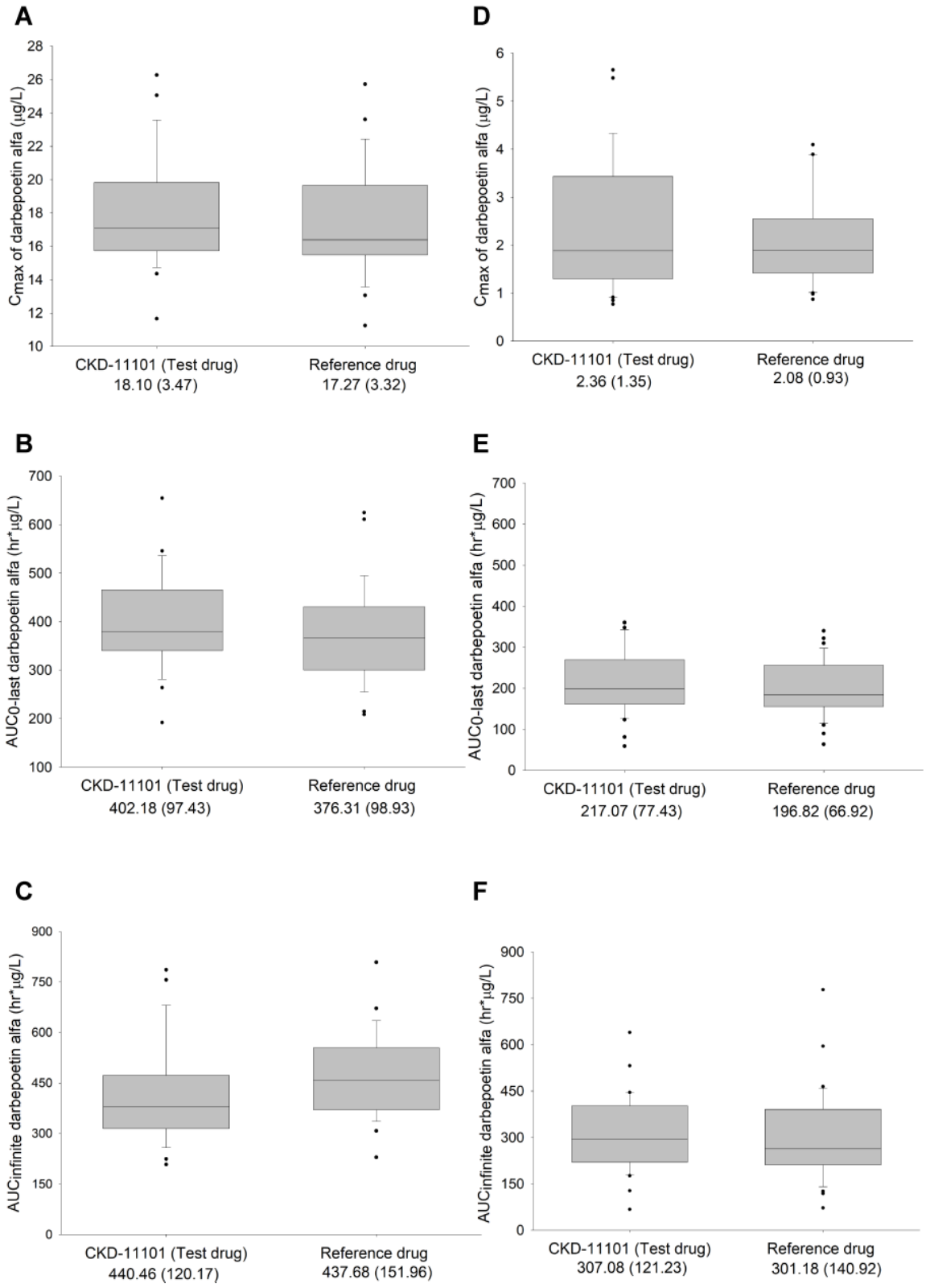


**Figure 3** Comparison of primary pharmacokinetic parameters between CKD-11101 (test drug) or reference drug after a single intravenous (**A**–**C**) or subcutaneous (**D**–**F**) administration. The values are expressed as mean (SD).

**Table 2** Pharmacokinetic Parameters of Darbepoetin Alfa After a Single Intravenous or Subcutaneous Administration of CKD-11101 60 μg (Test Drug) or Reference Drug 60 μg

| | PK Parameters | CKD-11101 60 μg (Test Drug) | Reference Drug 60 μg | Geometric Mean Ratio (90% CI) [b] |
|---|---|---|---|---|
| Intravenous Administration (part 1) (n = 29) | $T_{max}$ [a] (h) | 0.25 [0.23 – 1.05] | 0.25 [0.25 – 0.75] | - |
| | $C_{max}$ (μg/L) | 18.10 ± 3.47 | 17.27 ± 3.32 | 1.05 (1.03 – 1.07) |
| | $AUC_{0\text{-last}}$ (hr•μg/L) | 402.18 ± 97.43 | 376.31 ± 98.93 | 1.07 (1.04 – 1.11) |
| | $AUC_{0\text{-}\infty}$ (hr•μg/L) | 440.46 ± 120.17 | 437.68 ± 151.96 | 1.03 (0.96 – 1.10) |
| | $t_{1/2}$ (h) | 90.21 ± 74.43 | 159.03 ± 308.69 | - |
| | CL (L/h) | 0.15 ± 0.04 | 0.15 ± 0.05 | - |
| | Vz (L) | 16.23 ± 12.09 | 24.75 ± 34.35 | - |
| Subcutaneous administration (part 2) (n = 33) | $T_{max}$ [a](h) | 36 [12 – 36.07] | 36 [12 – 36.03] | - |
| | $C_{max}$ (μg/L) | 2.36 ± 1.35 | 2.08 ± 0.93 | 1.06 (0.97 – 1.16) |
| | $AUC_{0\text{-last}}$ (hr•μg/L) | 217.07 ± 77.43 | 196.82 ± 66.92 | 1.09 (1.03 – 1.15) |
| | $AUC_{0\text{-}\infty}$ (hr•μg/L) | 307.08 ± 121.23 | 301.18 ± 140.92 | 1.04 (0.94 – 1.14) |
| | $t_{1/2}$ (h) | 214.69 ± 96.74 | 259.21 ± 201.65 | - |
| | CL/F (L/h) | 0.24 ± 0.14 | 0.25 ± 0.14 | |
| | Vz/F (L) | 58.15 ± 40.49 | 71 ± 41.16 | |

**Notes**: All values are expressed as mean ± standard deviation. [a]$T_{max}$ was presented as median [min–max]. [b]Test/Reference.

(1.03–1.07), 1.07 (1.04–1.11) and 1.03 (0.96–1.10), respectively; and all of the values were fell within the conventional bioequivalence range of 0.80 - 1.25 (Table 2).

In part 2, the mean serum concentration-time profiles were similar after a single SC administration of test or reference drug (Figure 2B). The serum concentration of darbepoetin alfa reached a maximal level at a median of 36-hour post-dose and eliminated following a multi-exponential decrease pattern in both test and reference drug (Figure 2B). The primary PK parameters were comparable between the test and reference drug (Figure 3D–F and Table 2). The GMRs of test drug to reference drug and its 90% CI for $C_{max}$, $AUC_{0\text{-last}}$ and $AUC_{0-\infty}$ were 1.06 (0.97–1.16), 1.09 (1.03–1.15) and 1.03 (0.94–1.14), respectively; and all of the values were fell within the conventional bioequivalence range of 0.80 - 1.25 (Table 2).

## PD Analysis

In both study part 1 and 2, the time trend of PD markers (change of reticulocyte, hemoglobin, hematocrit, and red blood cell count from baseline) was similar after a single administration of test or reference drug (Figure 4, Supplemental Figure 1). The primary PD parameters ($E_{max}$ and $AUEC_{0\text{-last}}$) were not significantly different between the test and reference drug (all $p$-value >0.05, Table 3). The exploratory PD markers such as hemoglobin (g/L), hematocrit (%) and red blood cell (RBC) count ($10^6$/mm$^3$) parameters PD characteristics of darbepoetin alfa were similar to the known PK and PD characteristics of darbepoetin alfa.

The exposure-response relationship was also similar between the test and reference drug (Figure 5). The relationship between the mean serum concentration of darbepoetin alfa and the mean changes of reticulocyte from baseline showed a counterclockwise hysteresis pattern both in the test and the reference drug (Figure 5).

## Immunogenicity Evaluation

No subject was positive on the anti-CKD-11101 and anti-reference drug antibody assay before or after administration of the study drugs in part 1 and part 2.

## Tolerability Evaluation

Both test (CKD-11101) and reference drug were well tolerated in healthy subjects after a single IV or SC administration. In part 1, a total of 26 treatment emergent AEs (TEAEs) were reported and 15 (51.7%) subjects experienced ≥1 TEAE. The most commonly reported AEs were itching and headache after test drug administration, while throat irritation, rhinorrhea and bruise were reported after reference drug administration (Supplemental Table 1).

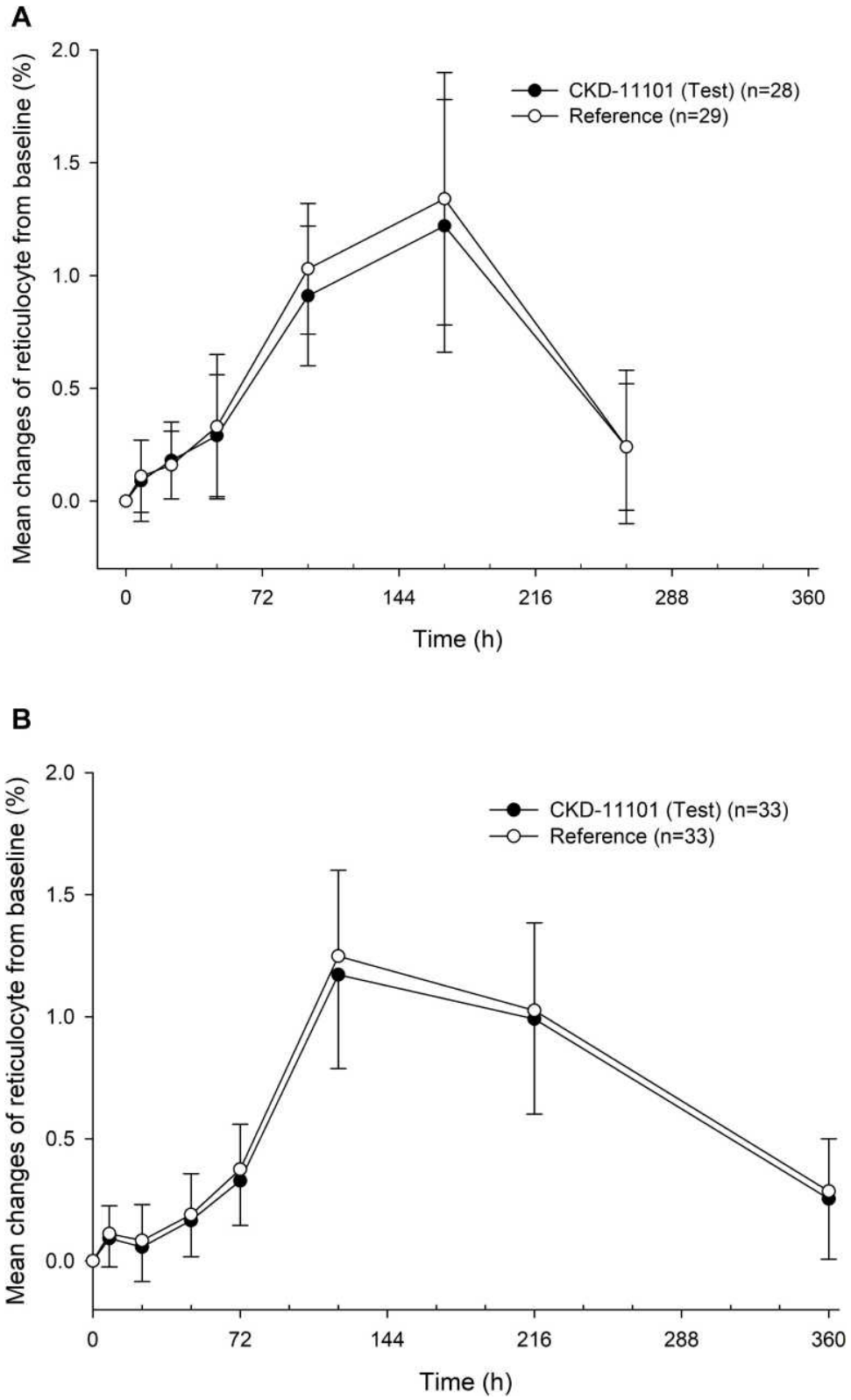


**Figure 4** Mean changes of reticulocyte from baseline after a single intravenous (**A**) or subcutaneous (**B**) administration of CKD-11101 60 μg (test drug) or reference drug 60 μg. Bars represent standard deviations.

**Table 3** Pharmacodynamic Parameters of Darbepoetin Alfa After a Single Intravenous or Subcutaneous Administration of CKD-11101 60 μg (Test Drug) or Reference Drug 60 μg

| | | CKD-11101 60 μg (Test Drug) | Reference Drug 60 μg | P-value[a] |
|---|---|---|---|---|
| Intravenous Administration (part 1) (n = 29) | $E_{max}$ (%) | 1.29 ± 0.50[b] | 1.43 ± 0.48 | 0.337 |
| | $AUEC_{0\text{-last}}$ (hr•%) | 174.30 ± 84.68[b] | 203.77 ± 75.49 | 0.247 |
| Subcutaneous Administration (part 2) (n = 33) | $E_{max}$ (%) | 1.28 ± 0.37 | 1.32 ± 0.32 | 0.435 |
| | $AUEC_{0\text{-last}}$ (hr•%) | 233.00 ± 83.40 | 252.02 ± 73.43 | 0.199 |

**Notes**: All values are expressed as mean ± standard deviation [a]P-value was calculated by ANOVA. [b]N = 28 for this statistics.

Among these people, 15 and 8 TEAEs were considered to be related to test and reference drug, respectively (Table 4). Itching, headache, and abdominal pain were observed after test drug administration and throat irritation and rhinorrhea were observed after reference drug administration of the drug-related AEs in the present study.

In part 2, a total of 37 TEAEs were reported and 15 (44.1%) subjects experienced ≥1 TEAE. Most common AEs were abdominal pain, diarrhea, headache, nasal congestion and upper respiratory tract infection for test drug treatment, while dyspepsia, dizziness, headache, skin nodule were commonly reported after reference drug administration (Supplemental Table 1). Among these people, 17 and 18 TEAEs were considered to be related to test and reference drug, respectively (Table 4). Headache was reported as a drug-related AE in both test and reference drug, and also abdominal pain and dizziness were reported. Most of the adverse events were mild in severity and there were no serious adverse events or unexpected adverse drug reaction. No clinically significant abnormalities were found in physical examinations, assessment on injection site reactions, vital signs, ECGs, or clinical laboratory evaluations.

## Discussion

This clinical study indicates that CKD-11101, a proposed biosimilar of reference drug, has comparable PK, PD and tolerability profile after a single IV or SC administration in healthy subjects. The similarity between CKD-11101 and reference drug was demonstrated by following evidence. First, the mean serum concentration-time profiles were superimposable after a single IV or SC administration of CKD-11101 or reference drug (Figure 2). Also, the GMRs of CKD-11101 to reference drug and their 90% CIs for the $C_{max}$, $AUC_{0\text{-last}}$ and $AUC_{0-\infty}$ were contained entirely within the conventional bioequivalence range of 0.80 - 1.25 (Table 2). Second, the time trend of PD markers was also similar between the CKD-11101 and reference drug treatments (Figure 4, Supplemental Figure 1), and no statistical difference was observed for primary PD parameters ($E_{max}$ and $AUEC_{0\text{-last}}$) between the CKD-11101 and reference drug treatments (all $p$-value >0.05, Table 3). Lastly, both of CKD-11101 and reference drug were well tolerated in these healthy subjects and there was no subject with the anti-drug antibody regardless of administration routes.

Although patients with CKD or patients receiving cytotoxic chemotherapy differ from the healthy subject with respect to their baseline hematologic status, this study was performed in healthy subjects to minimize possible confounding factors. However, if the PK and PD response in healthy subjects of two darbepoetin alfa are similar, efficacy in the CKD patients with anemia is expected similar. It is because the primary site of action for rhEPO is same regardless of disease status.[20,21] As expected, based on the results of this study, CKD-11101 showed equivalent efficacy and safety profile compared with reference drug for the treatment of renal anemia in the two randomized double-blind confirmatory clinical trials for CKD patients who are on or not on hemodialysis.[22,23]

In the present study, the PK and PD characteristics of darbepoetin alfa were similar to the known PK and PD characteristics of darbepoetin alfa.[16,18] In our study, the mean serum concentration of darbepoetin alfa was eliminated following a multi-exponential decrease pattern (Figure 2) and similar patterns were observed the previous clinical studies.[16,18] The PK parameters of darbepoetin alfa observed in our study were similar to known PK parameters of darbepoetin alfa.[16,18] The bioavailability of the SC dose calculated by the ratio of the $AUC_{0-last}$ of SC dose to that of IV dose was 52% to 54% and these values were also similar to a published clinical study result.[18] The

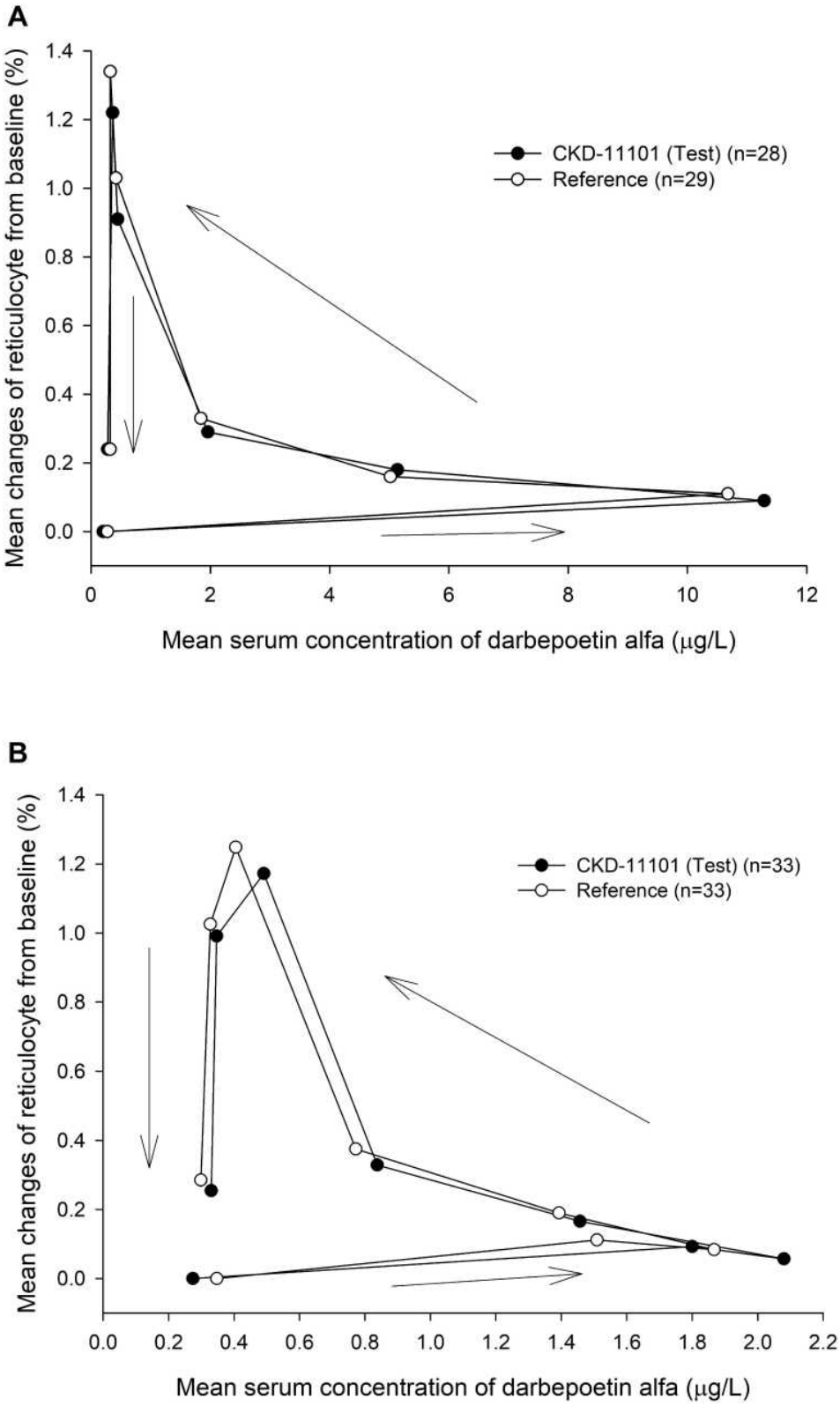


**Figure 5** Relationship between mean serum concentration of darbepoetin alfa and mean changes of reticulocyte from baseline after a single intravenous (**A**) or subcutaneous (**B**) administration of CKD-11101 60 μg (test drug) or reference drug 60 μg.

**Table 4** Summary of All Treatment Emergent Adverse Events

| | Part 1. Intravenous Administration | | Part 2. Subcutaneous Administration | |
|---|---|---|---|---|
| | CKD-11101 60 μg (Test Drug) (n=29) | Reference Drug 60 μg (n=29) | CKD-11101 60 μg (Test Drug) (n=33) | Reference Drug 60 μg (n=34) |
| Number of subjects with at least one TEAE (%)[a] | 8 (27.6) | 7 (24.1) | 11 (33.3) | 9 (26.5) |
| Number of TEAEs[b] | 16 | 10 | 19 | 18 |
| Number of subjects with at least one drug-related TEAE (%)[a] | 7 (24.1) | 5 (17.2) | 9 (27.3) | 9 (26.5) |
| Number of drug-related TEAEs[b] | 15 | 8 | 17 | 18 |

**Notes**: [a]Data are presented as the number of subject (percent of total subject within treatment groups). [b]Data are presented as the number of event.
**Abbreviation**: TEAE, treatment emergent adverse event.

PK and PD relationship showed a counterclockwise hysteresis pattern (Figure 5) which indicates a time delay between the serum concentration of darbepoetin alfa and the PD response related to the maturation of erythroid progenitors in bone marrow, and it was in agreement with the previous clinical study result.[18,24–27]

The reticulocyte count was evaluated as a primary endpoint for PD analysis in this study. Reticulocytes are immature red blood cell and develop and mature in the bone marrow, then circulate for about a day in the bloodstream before developing in the mature RBC.[25,28] Since the reticulocytes are produced primarily from EPO stimulation, it is physiologically appropriate that investigating the increase of reticulocytes to observe the activity of rhEPO as a PD marker.[25,28] The EMEA also recommend the reticulocyte as a recommended PD marker for assessment of the activity of EPO in a single-dose study although it is not a suitable endpoint for clinical efficacy studies.[29]

Both the CKD-11101 and reference drug were well tolerated in this study. There were no serious TEAEs or unexpected adverse drug reaction, and that no subject developed anti-drug antibody after treatment. Itching, headache, throat irritation, rhinorrhea and abdominal pain were the drug-related AEs observed in the present study. There were 6 and 2 cases of injection site reactions (eg, erythema, infiltration, edema and induration) after a single SC or IV dose of study drugs, respectively. All of the TEAEs and the injection site reactions were recovered spontaneously during the study. No clinically significant changes were observed in the physical examinations, vital signs, ECGs or clinical laboratory tests.

Collectively, these study results suggest that the CKD-11101 can be an alternative therapeutic option of reference drug for the treatment of anemia in the CKD patients and patients receiving cytotoxic chemotherapy.

## Conclusion

In conclusion, the CKD-11101 was well tolerated and showed comparable PK and PD characteristics with reference drug. The results of this study implies to support that the CKD-11101 and reference drug can be interchangeably used.

## Data Sharing Statements

The individual de-identified participant data supporting published results are available with approval from the corresponding author on reasonable request, at any time after publication.

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## Disclosure

Yu-Kyung Kwon is a full-time employee of Chong Kun Dang Pharmaceutical Corp., Seoul, Republic of Korea. The other authors report no conflicts of interest associated with this work.

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