ORIGINAL ARTICLE

BJCP | BRITISH PHARMACOLOGICAL SOCIETY

A large multicentre, randomized, double-blind, cross-over study in healthy volunteers to compare pharmacokinetics, pharmacodynamics and safety of a pegfilgrastim biosimilar with its US- and EU-reference biologics

Anne Bellon[1] | Jessie Wang[2] | Andrej Skerjanec[3] | Maria Velinova[4] | Daniel Dickerson[5] | Ahad Sabet[6] | Ly Ngo[7] | Terry O'Reilly[8] | Charles Tomek[9] | Steven Schussler[2] | Stefanie Schier-Mumzhiu[1] | Sreekanth Gattu[1] | Sven D. Koch[1] | Celine Schelcher[1] | Miryana Dobreva[10] | Anca Boldea[11] | Roumen Nakov[1] | Gordon P. Otto[1]

[1]Hexal AG, Novartis, Holzkirchen, Germany

[2]Novartis, Princeton, NJ, USA

[3]Sandoz AG, Novartis, Basel, Switzerland

[4]PRAHS, Groningen, The Netherlands

[5]PRAHS, Lenexa, KS, USA

[6]PRAHS, Salt Lake City, UT, USA

[7]PRAHS, Marlton, NJ, USA

[8]Celerion, Tempe, AZ, USA

[9]Celerion, Lincoln, NE, USA

[10]Sandoz, Novartis, Sofia, Bulgaria

[11]Sandoz Pharma Services Romania SRL, Novartis, Bucharest, Romania

Correspondence
Anne Bellon, Hexal AG, Novartis, Holzkirchen, Building 18/3 reception, Industriestrasse 18, 83607, Holzkirchen, Germany.
Email: anne.bellon@sandoz.com

Aims: Recombinant PEGylated human granulocyte colony-stimulating factor (pegfilgrastim) is indicated for the reduction of chemotherapy-induced neutropenia and prevention of febrile neutropenia. Biosimilar pegfilgrastim is expected to reduce the financial burden of this complication of chemotherapy. The aim of this study was to demonstrate biosimilarity between Sandoz biosimilar pegfilgrastim and its US- and EU-approved reference biologics.

Methods: Phase I, randomized, double-blind, single-dose, 3-period, 6-sequence cross-over, multicentre study to evaluate the pharmacokinetics, pharmacodynamics, safety and immunogenicity of Sandoz biosimilar pegfilgrastim with US- and EU-references in healthy adults.

Results: Pharmacokinetic and pharmacodynamic similarity was demonstrated between the 3 biologics, as the 90% confidence interval for all primary pharmacokinetic and pharmacodynamic endpoint comparisons were contained within the predefined similarity margins of 0.80–1.25. Safety, immunogenicity and tolerability were also similar.

Conclusions: Sandoz biosimilar pegfilgrastim demonstrated pharmacokinetic and pharmacodynamic similarity to both US- and EU-reference biologics. No meaningful differences in safety, local tolerability and immunogenicity were identified.

KEYWORDS

bioequivalence, biologicals, medical oncology, pharmacodynamics, pharmacokinetics

1 | INTRODUCTION

Unmet medical needs in providing access to biologics can in part be addressed by the development and availability of biosimilars, which improve availability of well-established therapeutic and supportive care.[1] The affordability and sustainability of cancer care is expected to be enhanced by the successful uptake of biosimilar medicines.[2]

A biosimilar is a biological medicine shown to be highly similar to another already approved biological medicine (the *reference biologic*).[3] Biosimilar clinical development programmes differ from traditional reference biologic development in that Phase I studies demonstrating pharmacokinetic (PK) and pharmacodynamic (PD) similarity to the reference biologic in healthy volunteers or patients play a pivotal role.[4] To gain marketing authorization, a biosimilar must demonstrate similarity in terms of physicochemical characteristics and biological activity, as well as similar PK, PD, efficacy and safety in humans compared with the reference biologic via the *totality of evidence* approach.[3,5-7] If the biosimilar is shown to have similar efficacy and safety to its reference biologic in one indication, it may be granted authorization in all of the indications held by the reference biologic through the scientific principle of extrapolation, provided there is scientific justification.[5,8]

For a range of cancer types (such as pancreatic, gastric, and metastatic breast cancer) myelosuppressive chemotherapy remains a key therapeutic strategy.[9,10] Frequently, patients undergoing myelosuppressive chemotherapy experience neutropenic complications, including life-threatening febrile neutropenia. **Pegfilgrastim** (Neulasta, Amgen, Thousand Oaks, CA, USA) is a long-acting form of the recombinant methionyl human granulocyte colony-stimulating factor (**rG-CSF**) filgrastim covalently conjugated to polyethylene glycol (PEG). Pegfilgrastim, also called pegylated rG-CSF, is indicated in adult patients treated with cytotoxic chemotherapy for malignancies, in order to reduce the duration of neutropenia and incidence of febrile neutropenia.[11,12]

Biosimilars of pegfilgrastim have received marketing authorization from the European Medicine Agency, including Sandoz biosimilar pegfilgrastim (LA-EP2006; Ziextenzo), Accord Healthcare (INTP5; Pelgraz), Mylan (MYL-1401H; Fulphila), Coherus (CHS-1701; Udenyca) and Cinfa Biotech (B12019; Pelmeg). Fulphila and Udenyca have also been approved in 2018 by the US Food and Drug Administration (FDA). Recently, in November 2019, Ziextenzo received approval from FDA, based on, but not limited to, the clinical results presented in this article.

Using state-of-the-art analytical procedures, the structural, physicochemical and functional characteristics of Sandoz biosimilar pegfilgrastim have been demonstrated to be comparable to those of reference pegfilgrastim, referred to as the *reference biologic* in this article. An analytical bridge between European (EU-) and US-approved reference products and Sandoz biosimilar pegfilgrastim has also been performed, with analytical similarity established.

To demonstrate biosimilarity of Sandoz biosimilar pegfilgrastim to both US-reference biologic[12] as requested by FDA[13] and EU-reference biologics[11] as requested by the European Medicines Agency,[14] a 3-way clinical Phase I study is required to confirm PK and

PD profile similarity of the 3 biologics after a single dose subcutaneous administration in humans.

High interindividual PK variability has previously been reported for pegfilgrastim,[15-17] and was also recognized in the previous 2-arm clinical study conducted with Sandoz biosimilar pegfilgrastim.[18] The new 2018 European Medicines Agency *Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (rG-CSF)* clearly states that the intrasubject variability of pegfilgrastim PK properties is considerably lower than the intersubject variability.[19]

To circumvent this high interindividual PK variability, the study was designed as a 3-way cross-over study, so that variability in treatment differences could be reduced by having within-subject comparisons.

The aim of the article is to present and discuss results of the study which demonstrated PK/PD similarity, similar safety, and similar immunogenicity between Sandoz biosimilar pegfilgrastim and the approved US- and EU-reference biologics in healthy subjects, as well as similarity between US and EU references.

What is already known about this subject

- Sandoz biosimilar pegfilgrastim has been shown to be highly similar to EU-reference; however, similarity of pharmacokinetics and pharmacodynamics between Sandoz biosimilar and US-, and between US- and EU-reference pegfilgrastim has not previously been established.

What this study adds

- This large study demonstrates similarity for pharmacokinetics and pharmacodynamics and shows no meaningful differences in safety and immunogenicity between Sandoz biosimilar and US- and EU-reference pegfilgrastim, which provides bridging data for previous efficacy and safety studies where only EU-reference biologic has been used.

2 | METHODS

2.1 | Design

This was a Phase I, randomized, double-blind, single-dose, 3-treatment-period, 6-sequence cross-over, multicentre study designed to evaluate the PK, PD, safety and immunogenicity of Sandoz biosimilar pegfilgrastim and compare them to the US- and EU-reference biologics in 576 healthy male and female subjects, aged 18–55 years (Figure S1). The marketed dose of the pegfilgrastim reference product, 6 mg/0.6 mL in a prefilled syringe, was used in this

study. The study was performed in 1 site in the Netherlands and 5 sites across the USA. Subjects eligible for inclusion in this trial had to fulfil all of the following key criteria by Day −1 of Treatment Period I: healthy subjects aged between 18–55 years; physically and mentally healthy, as determined by medical history, physical examination and safety laboratory assessments; weight: ≥60 kg; body mass index 19.0–30.0 kg/m^2; and absolute neutrophil count (ANC) of 2–7 $\times$ 10^9 cells/L. Subjects fulfilling any of the following criteria at screening and baseline (Day −1) of Treatment Period I were not eligible for inclusion in this study: known previous exposure to filgrastim, pegfilgrastim, G-CSF or any analogue of these; known hypersensitivity to the study drug or any of its constituents, hypersensitivity to *Escherichia coli* derived proteins; history of an acute severe allergic reaction (e.g. anaphylaxis, delayed hypersensitivity reaction); history and/or current evidence of moderate-to-severe allergy requiring medical treatment; history and/or current evidence of clinically significant latex allergy; positive anti-drug antibody (ADA) for pegfilgrastim and/or filgrastim and/or PEG at screening; history or current evidence of any clinically significant finding that, in the opinion of the investigator, would preclude inclusion in the study; history or current evidence of any clinically significant condition that might interfere with the distribution, metabolism, or excretion of any of the investigational drugs. During the study, concomitant medication used to treat adverse events, hormonal contraceptives, hormone replacement therapy were allowed. Vitamins, minerals and nutritional supplements were permitted as per investigator's discretion. Use of any medications/nondrug therapies subsequent to screening was monitored and documented at every patient visit. The study duration per subject was at least 25 weeks. After screening, volunteers were randomized 1:1:1:1:1:1 to 1 of 6 treatment sequences (Figure S1).

In each treatment period, each volunteer received a single subcutaneous injection of 6 mg/0.6 mL from a prefilled syringe of either Sandoz biosimilar pegfilgrastim, EU-reference or US-reference biologics. Administration of the study drug was always performed in the morning between 8 am and 11 am on Day 1 of each treatment period and PK/PD samples were collected at fixed, predefined time points (with a limited window allowance) following the study drug administration.

This clinical study was designed, implemented, executed, and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations, and with the ethical principles laid down in the Declaration of Helsinki. Protocol was approved by local ethics committees. Eligible subjects were only included in the study after providing written informed consent. A description of the study design is summarized in the online supporting information.

2.2 | Bioanalytical methods for PK, PD and immunogenicity

Pegfilgrastim serum concentrations and ADAs from healthy subjects were quantified/detected in the Sandoz laboratory (Oberhaching, Germany) using fully validated immunoassays.

Pegfilgrastim drug concentrations were determined using a sandwich enzyme-linked immunosorbent assay consisting of a capture anti-filgrastim antibody and a horseradish peroxidase-labelled anti-filgrastim detection antibody. Absorption was read photometrically, which is directly proportional to the amount of conjugate bound to the antibody complex.

The detection of ADAs against pegfilgrastim was performed using a sandwich enzyme-linked immunosorbent assay. In brief, anti-pegfilgrastim antibodies bind to a biotinylated pegfilgrastim immobilized on a streptavidin-coated plate and to a digoxigenin-labelled pegfilgrastim. Following an initial screening of all serum samples, positive samples were further analysed in a confirmatory assay. Confirmed positive samples were then further characterized by determination of the antibody titre.

Neutralizing antibodies were determined with a cell-based assay using NFS60 cells. NFS60 cell proliferation is stimulated by the addition of pegfilgrastim. In the presence of neutralizing antibodies (NAbs) against pegfilgrastim, the growth factor is bound and cell proliferation is inhibited. The number of viable cells was quantified with CellTiter-Glo. To differentiate between neutralizing anti-pegfilgrastim antibodies and unspecific cytotoxicity, serum samples that induced a reduced cell proliferation were subsequently analysed.

For both PK and ADA assays, the coefficients of variation (%) of the mean OD of a duplicate determination had to be ≤20%. At the upper and lower limits of quantification (LOQs), a coefficient of variation of ≤25% was acceptable. For the pegfilgrastim drug concentrations, the calibration ranged from 1.5 (lower LOQ) to 48 ng/mL (upper LOQ). The limit of detection of the ADA assay was validated at 13 ng/mL.

ANCs were measured in local clinical laboratories using standardized flow cytometry-based routine haematology methods.

2.3 | Objective, primary and secondary endpoints

This Phase I study aimed to confirm that Sandoz biosimilar pegfilgrastim is similar to US- and EU-reference biologics in terms of PK, PD, safety and immunogenicity in healthy volunteers. The following treatment comparisons were undertaken: Sandoz biosimilar *vs* US-reference; Sandoz biosimilar *vs* EU-reference; and US-reference *vs* EU-reference. The last comparison represents the bridging requirements according to section 351(k)(2)(A) of the Public Health Service documents.

The PK co-primary endpoints were: AUC_{0-inf} (area under the plasma concentration–time curve from the time of dosing and extrapolated to infinity); AUC_{0-last} (area under the plasma concentration–time curve from the time of dosing to the last measurable concentration); and C_{max} (maximum plasma concentration). The PD co-primary endpoints were derived from ANC (corrected as well as non-corrected for baseline) over time: $AUEC_{0-last}$ (area under the effect curve measured from the time of dosing to the last measurable concentration); and E_{max} (maximum neutrophil count measured following administration of the study medication). To calculate the baseline corrected

ANC, individual baseline value measured before pegfilgrastim administration in each period was subtracted from individual ANC value measured at each time point after drug administration. All those were tested as co-primary endpoints for all pairwise comparisons among Sandoz biosimilar, US-reference, and EU-reference biologics. Similarity was to be demonstrated for PK/PD parameters if the 90% confidence intervals (CIs) for geometric mean (GM) ratios of all 5 co-primary endpoints and pairwise comparisons were contained within the predefined equivalence margins of 0.80–1.25.

Secondary endpoints included the descriptive statistics for PK parameters t_{max} (time to maximum observed concentration) and elimination half-life ($t_{1/2}$), and for PD parameter $t_{max,E}$ (time to the maximum effect attributable to the therapy under investigation), as well as safety, immunogenicity, and local tolerance at the injection site.

Additional analyses included evaluation of the impact of immunogenicity on PK, PD, and safety; and comparing the primary PK and PD parameters of the ADA-positive analysis set, as defined below, with the parameters of the ADA-negative analysis set, as well as comparing safety in the 2 populations.

2.4 | Statistics

Data from a previous Phase I cross-over study made an assumption of 14, 14 and 10% difference in GM of AUC_{0-inf}, AUC_{0-last} and C_{max} between Sandoz biosimilar pegfilgrastim and reference biologics.[20] The estimated difference for PD was 5% for both AUEC and E_{max}. Since the study duration of ≥25 weeks is relatively long for a PK/PD study, and it was assumed that most subjects would experience pain as an adverse event (AE; bone pain, back pain, headache, and myalgia), there was a high risk of volunteers dropping out with time. The drop-out rate was estimated to be 25%, and included subjects lost to follow-up and subjects potentially excluded from the full analysis set for other reasons (e.g. major protocol deviations). Based on the above criteria, the estimated variability and correlation among the co-primary endpoints, a total of 576 randomized healthy male and female subjects were planned to be enrolled to achieve at least 432 evaluable subjects (with 72 per sequence). This resulted in an overall power of 90% to conclude that similarity would be given for all co-primary endpoints in all pairwise comparisons (biosimilar vs US-reference, biosimilar vs EU-reference, US-reference vs EU-reference). PK parameters of pegfilgrastim and PD parameters deriving from ANC were calculated using noncompartmental analysis with Phoenix WinNonLin (Version 8.0).

For the analyses of the primary endpoints, analyses of variance were performed on the natural logarithm transformed primary PK parameters AUC_{0-inf}, AUC_{0-last} and C_{max}, and primary PD parameters ANC $AUEC_{0-last}$ and E_{max}, separately. The mixed effect ANOVA model included treatment, sequence and period as fixed effects and subject nested within sequence as a random effect. The analyses were performed using PROC MIXED in SAS version 9.4. Descriptive analyses were provided for each PK and PD parameter, safety and immunogenicity endpoints.

2.5 | Analysis sets

The safety analysis set (SAF) includes all randomized subjects who received the investigational medicinal product (IMP) at least once. Subjects were analysed according to the treatment they received. The full analysis sets for PK (FAS-PK) and PD (FAS-PD), included all subjects who received study drugs and who had at least 1 of the evaluable PK or PD parameters defined as primary endpoints in at least 2 periods without a major protocol violation. Primary PK and PD analyses in the study were based on FAS-PK and FAS-PD, respectively. The ADA-positive analysis set includes all subjects who received the IMP and had a confirmed positive result for ADA against pegfilgrastim and/or filgrastim and/or PEG at any time point (Day 1 predose, Day 15, Day 28) in any period. Safety analysis for AEs on the ADA-positive analysis set included treatment-emergent AEs (TEAEs) from the moment the assessment of ADA positivity was made (same day assessment was included). In contrast, the PK and PD summary statistics were performed on the PK and PD parameters of the subjects included in the ADA-positive analysis set from the period(s) following the assessment of ADA positivity on Day 15 and/or Day 28, and also included the data from the current period if the assessment of ADA positivity was made on Day 1. The ADA negative analysis set included all subjects who received the IMP and had negative results for ADA at all times in all periods.

2.6 | Safety

TEAEs were defined as AEs with a date of onset occurring on or after the first study drug administration until 4 weeks after last study drug administration, or conditions present prior to the first study drug administration that increased in severity, changed from being not suspected to being suspected of study drug relationship, or developed into serious AEs (SAEs) after study drug administration.
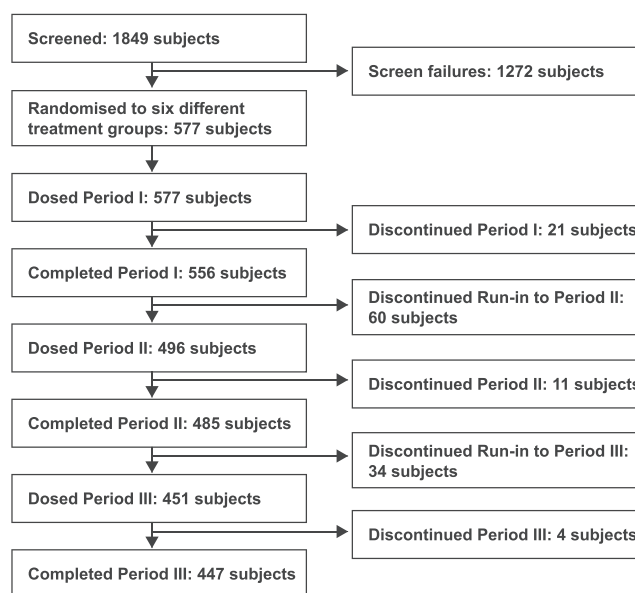
FIGURE 1 Patient disposition of study participants

TABLE 1 Baseline characteristics of the 6 different treatment sequences in the SAF

Characteristic	Biosimilar → US-reference → EU-reference n = 96	Biosimilar → EU-reference → US-reference n = 97	US-reference → biosimilar → EU-reference n = 96	US-reference → EU-reference → biosimilar n = 95	EU-reference → biosimilar → US-reference n = 96	EU-reference → US-reference → biosimilar n = 97
Age (y)						
Mean (SD)	32.5 (10.50)	35.5 (11.39)	34.2 (10.72)	33.9 (10.11)	33.9 (9.78)	33.6 (10.55)
Median	30.0	33.0	31.0	30.0	32.0	31.0
Min, max	18, 54	18, 55	18, 55	20, 55	18, 54	18, 55
Sex, n (%)						
Male	59 (61.5)	60 (61.9)	62 (64.6)	68 (71.6)	68 (70.8)	61 (62.9)
Female	37 (38.5)	37 (38.1)	34 (35.4)	27 (28.4)	28 (29.2)	36 (37.1)
Race, n (%)						
American Indian or Alaska native	0	1 (1.0)	2 (2.1)	0	0	1 (1.0)
Asian	2 (2.1)	1 (1.0)	0	3 (3.2)	4 (4.2)	4 (4.1)
Black or African American	26 (27.1)	22 (22.7)	26 (27.1)	20 (21.1)	23 (24.0)	25 (25.8)
Native Hawaiian or other Pacific islander	0	0	0	0	0	1 (1.0)
White	59 (61.5)	72 (74.2)	63 (65.6)	70 (73.7)	66 (68.8)	58 (59.8)
Multiple	9 (9.4)	1 (1.0)	3 (3.1)	2 (2.1)	3 (3.1)	7 (7.2)
Other	0	0	2 (2.1)	0	0	1 (1.0)
Ethnicity, n (%)						
Hispanic or Latino	10 (10.4)	15 (15.5)	21 (21.9)	13 (13.7)	14 (14.6)	16 (16.5)
Not Hispanic or Latino	86 (89.6)	82 (84.5)	74 (77.1)	81 (85.3)	81 (84.4)	81 (83.5)
Not reported	0	0	1 (1.0)	0	0	0
Unknown	0	0	0	1 (1.1)	1 (1.0)	0
Weight (kg)						
Mean (SD)	76.97 (10.096)	75.12 (9.838)	78.90 (10.621)	77.92 (10.919)	78.83 (11.046)	76.51 (9.719)
Median	75.35	73.80	78.85	79.00	78.00	75.90
Min, max	60.6, 101.9	60.1, 106.5	60.0, 113.0	60.0, 102.1	60.4, 111.7	61.3, 103.6
Height (cm)						
Mean (SD)	173.7 (9.40)	172.2 (9.40)	174.2 (10.43)	175.4 (8.84)	175.2 (9.63)	171.6 (10.10)
Median	174.0	173.0	175.0	177.0	175.0	172.0
Min, max	153, 199	150, 192	153, 201	156, 194	147, 196	146, 195
BMI (kg/m^2)						
Mean (SD)	25.48 (2.357)	25.34 (2.665)	25.98 (2.509)	25.28 (2.703)	25.66 (2.572)	26.02 (2.871)
Median	25.20	25.20	25.85	25.50	25.70	26.10
Min, max	20.0, 30.9	19.4, 29.9	20.6, 30.0	19.4, 30.0	20.1, 30.0	19.5, 30.0
Baseline absolute neutrophil count (10^9/L)						

TABLE 1 (Continued)

Characteristic	Biosimilar → US-reference → EU-reference n = 96	Biosimilar → EU-reference → US-reference n = 97	US-reference → biosimilar → EU-reference n = 96	US-reference → EU-reference → biosimilar n = 95	EU-reference → biosimilar → US-reference n = 96	EU-reference → US-reference → biosimilar n = 97
Mean (SD)	3.22 (1.07)	3.27 (1.21)	3.18 (1.12)	3.14 (1.02)	3.18 (1.03)	3.31 (1.20)
Median	3.00	3.10	3.05	3.00	3.00	3.10
Min, max	1.50, 7.40	1.60, 9.80	1.26, 7.47	1.52, 7.50	1.35, 7.70	1.80, 8.30

Height was determined at screening. Weight and BMI were determined on Day −1 of Period I; if missing, screening measurements were used

Baseline ANC were measured on Day 1 Pre-dose of Period 1.

BMI, body mass index; SAF, safety analysis set; SD, standard deviation.

3 | RESULTS

3.1 | Demographics and baseline characteristics

A total of 577 subjects were randomized to the 6 treatment sequences (95–97 subjects per sequence) and administered study drug in Period I. Overall, 556 subjects (96.4%) completed Period I, 485 subjects (84.1%) completed Period II and 447 subjects (77.5%) completed Period III (Figure 1).

In the SAF (n = 577), subject demographic characteristics were well balanced across the 6 treatment sequence groups at baseline (Table 1). In the FAS-PK (n = 496), FAS-PD (n = 496) and ADA-negative (n = 479) analysis sets, subject demographics were also balanced across the treatment sequence groups at baseline. Overall, 130/577 subjects (22.5%) discontinued the study prematurely, which was below the assumed drop-out rate of 25% used to estimate the required study sample size. Subjects who received study drug in only Period I (n = 81) were excluded from the FAS-PK and the FAS-PD due to absence of results for the primary PK/PD parameters in subsequent treatment periods, leading to lack of intraindividual comparison.

3.2 | PK

Following subcutaneous administration of a single dose of 6 mg, pegfilgrastim concentrations were increasing up to about 12 hours where a plateau was reached, followed by a slight decrease from 36 to 48 hours and a more rapid decline until the last measurable concentrations (Figure 2A). In the 3 treatment periods combined, primary PK parameter (AUC_{0-inf}, AUC_{0-last} and C_{max}) GM values were comparable among the 3 biologics groups (Table 2), with ratios of point estimates for the comparisons, i.e. between 0.98 and 1.07 for AUC_{0-inf}, between 0.98 and 1.06 for AUC_{0-last}, and between 0.98 and 1.05 for C_{max}. For each pairwise comparison, the 90% CIs of the GM ratios for AUC_{0-inf}, AUC_{0-last} and C_{max} were all contained within the predefined PK similarity margins of 0.80–1.25 (Figure 3A). The values of the secondary PK parameters t_{max} and $t_{1/2}$ were comparable for Sandoz biosimilar, US-reference, and EU-reference biologics (Table 2). Median t_{max} was 12 hours in the 3 treatment groups, and GM $t_{1/2}$ was similar among the 3 biologics at ~17 hours.

3.3 | PD

Following subcutaneous administration of a single dose of 6 mg, neutrophil counts were increasing up to 60 hours followed by a decrease with a return to initial baseline values at around 336 hours (Figure 2B). In the 3 treatment periods combined, primary PD parameter ($AUEC_{0-last}$ and E_{max}) GM values were comparable among the 3 biologics groups (Table 2), with ratios of point estimates for the comparisons at 1.00 for $AUEC_{0-last}$ and 1.00–1.01 for E_{max}. For each pairwise comparison, the 90% CIs for the ratios of GM for the primary PD parameters $AUEC_{0-last}$ and E_{max} were all contained within the predefined PD similarity margins of 0.80–1.25 (Figure 3B). The values of the secondary PD parameter $t_{max,E}$ were
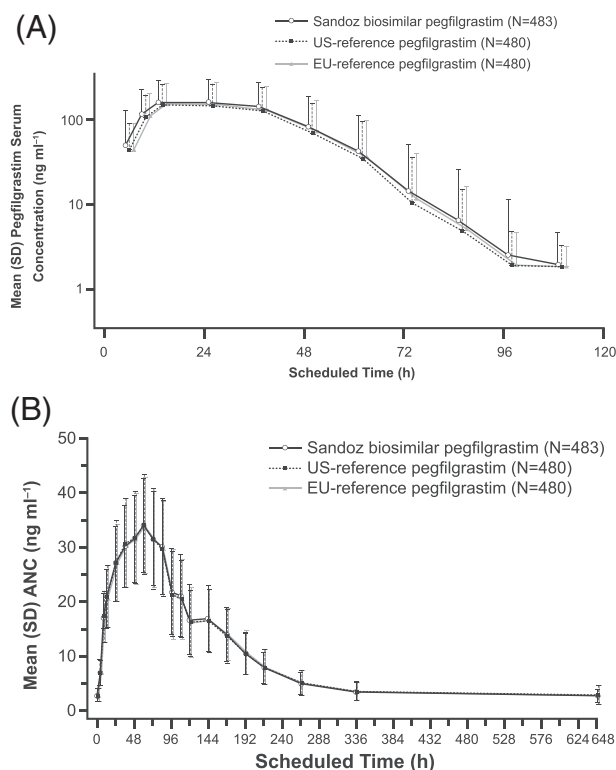
FIGURE 2 Pharmacokinetic and pharmacodynamic absolute neutrophil count (ANC) profiles following a fixed single subcutaneous injection of 6 mg of the proposed pegfilgrastim biosimilar or the reference products pegfilgrastim in healthy volunteers

TABLE 2 Primary and secondary PK and PD parameters (FAS-PK and FAS-PD)

Parameter/comparison	Statistics	Biosimilar $n = 483$	US-reference $n = 480$	EU-reference $n = 480$
Primary PK parameters				
AUC_{0-inf} (h × ng/mL)	n	482[a]	480	479[b]
	GM (geo CV%)	4830 (125.7)	4470 (118.3)	4630 (117.4)
AUC_{0-last} (h × ng/mL)	n	482[a]	480	480
	GM (geo CV%)	4710 (131.8)	4370 (124.0)	4500 (123.7)
C_{max} (ng/mL)	n	483	480	480
	GM (geo CV%)	132 (108.2)	124 (106.0)	128 (103.6)
Secondary PK parameters				
t_{max} (h)	n	483	480	480
	Median (min, max)	12.10 (4.00, 36.75)	12.05 (8.00, 59.47)	12.03 (4.00, 36.10)
$t_{1/2}$ (h)	n	482[a]	480	479[b]
	GM (geo CV%)	17.2 (69.8)	17.2 (62.2)	17.0 (68.2)
Primary PD parameters[c]				
$AUEC_{0-last}$ (10^9 × h/L)	n	482[a]	479[b]	479[b]
	GM (geo CV%)	4100 (28.2)	4100 (28.6)	4060 (28.3)
E_{max} (10^9/L)	n	482[a]	480	480
	GM (geo CV%)	31.6 (28.1)	31.7 (29.3)	31.4 (28.6)
Secondary PD parameters[c]				
$t_{max,E}$ (h)	n	482[a]	480	480
	Median (min, max)	60.00 (8.00, 144.87)	60.00 (8.02, 150.38)	60.00 (24.00, 176.07)

[a]For 1 subject in the biosimilar group, no AUC_{0-inf}, AUC_{0-last}, $t_{1/2}$, nor PD data could be obtained in Period III;
[b]For 1 subject in the EU-reference group, no AUC_{0-inf} nor $t_{1/2}$ could be obtained in Period II. For 1 subject in the US-reference group and 1 subject in the EU-reference group, no $AUEC_{0-last}$ could be obtained in Period II;
[c]Baseline corrected ANC values.
ANC, absolute neutrophil count; AUC_{0-inf}, area under the plasma concentration–time curve from time of dosing and extrapolated to infinity; AUC_{0-last}, area under the plasma concentration–time curve to the last measurable concentration; $AUEC_{0-last}$, area under the effect curve measured from the time of dosing to the last measurable concentration; CI, confidence interval; C_{max}, maximum plasma concentration; E_{max}, maximum neutrophil count measured following administration of the study medication; FAS, full analysis set; geo CV%, coefficient of variation of the geometric mean in percent; GM, geometric mean; max, maximum; min, minimum; n, total number of subjects in each treatment group; PD, pharmacodynamic; PK, pharmacokinetic; $t_{½}$, apparent elimination half-life; t_{max}, time to the maximum observed serum concentration; $t_{max,E}$, time to the maximum effect attributable to the therapy under investigation.

comparable for Sandoz biosimilar, US-reference and EU-reference biologics (Table 2).

## 3.4 | Safety

3.4.1 | Adverse events

The safety profile of Sandoz biosimilar pegfilgrastim was similar to the safety profiles of the US- and EU-reference biologics (Table S1). The incidences and patterns of TEAEs were similar between the 3 biologics. The 5 most common TEAEs reported by subjects were headache, back pain, bone pain, myalgia, and pain in extremity. The most frequently affected system organ class was musculoskeletal and connective tissue disorders followed by nervous system disorders. Local study drug tolerance was good and similar in all treatment groups. The incidence of injection site reactions was low and similar in all

groups. There was a tendency for the incidences of TEAEs to decrease over the course of the study. TEAEs with a suspected causal relationship to study drug are presented by system organ class, preferred term and treatment group in Table 3.

A total of 11 subjects discontinued the study prematurely due to TEAEs (biosimilar group: 7/512 [1.4%], including 2 gunshot wounds described below; EU-reference group: 4/501 [0.8%]). Across all treatment groups, most TEAEs were mild. One subject (<1.0%) in each treatment group experienced a severe drug-related TEAE.

Treatment-emergent SAEs were reported in 3 subjects (0.6%) in the biosimilar group (completed suicide by fatal gunshot to the head; nonfatal gunshot wound to right chest and foot; appendicitis). The appendicitis case did not result in a change of study drug administration, and the subject completed the study. None of those SAEs were suspected to be related to the study drug. No treatment-emergent SAEs occurred in the reference groups.

(A)

(B)
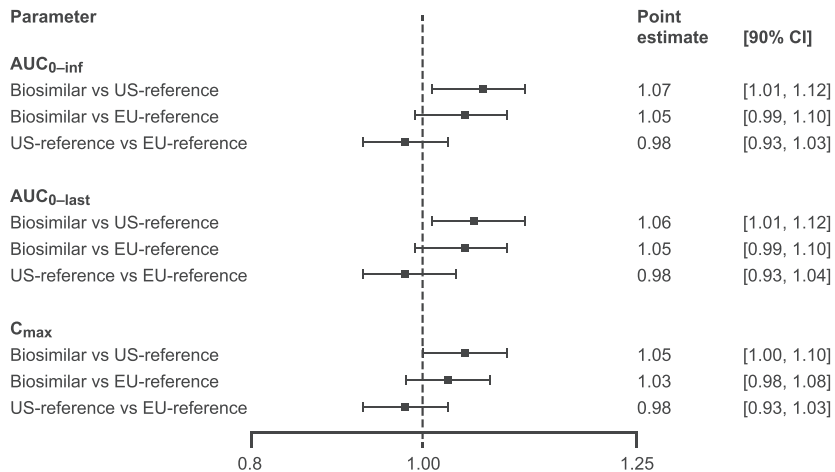
FIGURE 3 Comparisons of primary PK/PD parameters by treatment
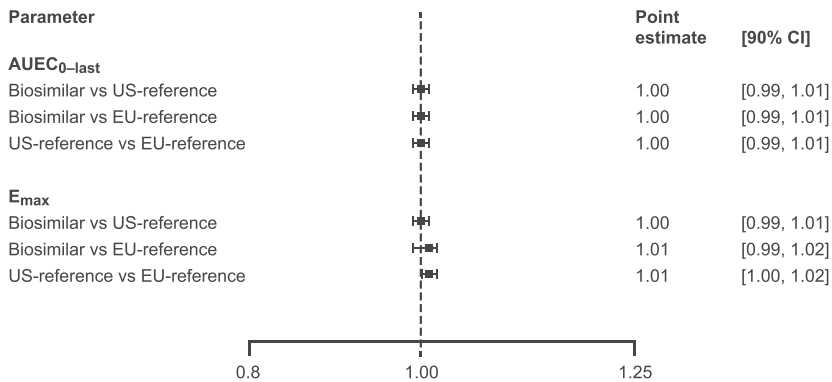
3.4.2 | Immunogenicity

The incidence of ADAs was low and similar across all treatment groups (biosimilar: 31/512 [6.1%]; US-reference: 36/511 [7.0%]; EU-reference: 39/501 [7.8%]). The overall numbers of subjects with confirmed positive ADA results diminished after subsequent administration in all groups (Table 4). Most confirmed positive ADAs were specifically directed against the PEGylated portion of the pegfilgrastim protein. In ADA-positive and ADA-negative subjects, values of primary PK and PD parameters were similar among treatment groups (data not shown). Three subjects developed pegfilgrastim NAbs in Period I only: 1 after biosimilar administration and 2 after administration of the EU-reference biologic. One subject dosed with EU-reference biologic discontinued the study in Period I due to NAb development (Table S2).

No treatment-emergent SAEs, severe TEAEs, or TEAEs leading to discontinuation were reported in patients included in the ADA-positive analysis set.

The incidence of TEAEs in ADA-positive subjects was similar across all treatment groups (biosimilar: 41/74 [55.4%]; US-reference: 45/78 [57.7%]; EU-reference: 40/76 [52.6%]).

4 | DISCUSSION

This large Phase I study achieved its primary objectives and demonstrated the PK/PD similarity of Sandoz biosimilar pegfilgrastim to both the US- and EU-reference biologics. No meaningful differences in safety, local tolerability or immunogenicity were identified between the 3 biologics. The study results also demonstrated PK/PD similarity between US- reference and EU-reference biologics, providing the PK and PD component of the scientific bridge between both biologics, allowing consideration of the results of the efficacy safety trials performed with the EU-reference biologic as comparator.[18,20,21]

PK similarity was demonstrated between Sandoz biosimilar and US-reference pegfilgrastim, Sandoz biosimilar and EU-reference pegfilgrastim, and US-reference and EU-reference pegfilgrastim for the primary PK parameters AUC_{0-inf}, AUC_{0-last}, and C_{max}, as the 90% CIs for the GM ratios of all 3 comparisons were contained within the predefined PK similarity margins (0.80–1.25). The values of the secondary PK parameters t_{max} and $t_{1/2}$ were consistent across the 3 treatment groups. GM values for $t_{1/2}$ of approximately 17 hours were calculated for all 3 treatment groups. These values are within the range of 15–80 hours reported in the Neulasta US prescribing information.[12]

TABLE 3 Incidence of most frequent (≥3% of subjects in any treatment group) TEAEs related to study drug by system organ class and preferred term (SAF)

Primary system organ class Preferred term	Biosimilar n = 512 n (%)	US-reference n = 511 n (%)	EU-reference n = 501 n (%)
Number of subjects with at least 1 related TEAE	424 (82.8)	428 (83.8)	413 (82.4)
Musculoskeletal and connective tissue disorders	345 (67.4)	363 (71.0)	350 (69.9)
Back pain	218 (42.6)	212 (41.5)	200 (39.9)
Bone pain	84 (16.4)	94 (18.4)	95 (19.0)
Myalgia	62 (12.1)	82 (16.0)	70 (14.0)
Pain in extremity	41 (8.0)	38 (7.4)	44 (8.8)
Arthralgia	30 (5.9)	40 (7.8)	33 (6.6)
Musculoskeletal pain	24 (4.7)	21 (4.1)	31 (6.2)
Musculoskeletal stiffness	21 (4.1)	14 (2.7)	13 (2.6)
Neck pain	19 (3.7)	20 (3.9)	16 (3.2)
Nervous system disorders	248 (48.4)	225 (44.0)	238 (47.5)
Headache	235 (45.9)	214 (41.9)	228 (45.5)
General disorders and administration site conditions	100 (19.5)	102 (20.0)	88 (17.6)
Injection site pain	29 (5.7)	27 (5.3)	30 (6.0)
Pain	22 (4.3)	21 (4.1)	19 (3.8)
Gastrointestinal disorders	44 (8.6)	28 (5.5)	38 (7.6)
Nausea	22 (4.3)	12 (2.3)	20 (4.0)
Metabolism and nutrition disorders	26 (5.1)	30 (5.9)	22 (4.4)
Hyperuricemia	23 (4.5)	25 (4.9)	21 (4.2)

Subjects experiencing multiple events within the same preferred term and/or system organ class are counted only once under those categories
SAF, safety analysis set; TEAE, treatment-emergent adverse event.

Within the predefined margins of PK similarity, the GM AUCs and the GM C_{max} for Sandoz biosimilar pegfilgrastim were approximately 5–7% higher than for the reference biologics; however, the slightly higher exposure did not translate into any apparent differences in the PD effect or the safety profile.

PD similarity was demonstrated between Sandoz biosimilar and US-reference pegfilgrastim, Sandoz biosimilar and EU-reference pegfilgrastim, and US-reference and EU-reference pegfilgrastim for the primary PD parameters ANC $AUEC_{0-last}$ and ANC E_{max}, as the 90% CIs of the GM ratios of all 3 pairwise comparisons were all contained within the predefined PD similarity limits (0.80–1.25).

TABLE 4 Summary of immunogenicity results (confirmed ADA positive) per treatment period (SAF)

	Biosimilar n (%)	US-reference n (%)	EU-reference n (%)
Treatment period I	n = 193	n = 191	n = 193
Total number of subjects with at least 1 positive ADA	29 (15.0)	30 (15.7)	35 (18.1)
Treatment period II	n = 166	n = 167	n = 163
Total number of subjects with at least 1 positive ADA	1 (0.6)	6 (3.6)	3 (1.8)
Treatment period III	n = 153	n = 153	n = 145
Total number of subjects with at least 1 positive ADA	1 (0.7)	0	1 (0.7)

ADA, anti-drug antibody; SAF, safety analysis set.

The PD marker ANC is a clinically relevant surrogate marker of efficacy in clinical practice.[22,23] To account for the circadian variability in ANC as described by Ackermann et al.,[24] the study medication was administered at a fixed, predefined time window for all participants in the study (see design section). Time points for PD blood sampling followed a schema with fixed, prespecified time points (with a limited window allowance) following the study drug administration. Therefore, the circadian variability in blood ANC is expected to be equally distributed between subjects and treatment sequences in the study.

Altogether, the equivalent PD profiles support the clinical equivalence shown in the confirmatory efficacy and safety studies of Sandoz biosimilar pegfilgrastim and the EU-reference biologic in patients with breast cancer.[25]

Although the study was not designed nor powered to detect sex-related differences, a posthoc subgroup analysis by sex was performed and showed no significant impact on the study outcome. However, higher variation in systemic drug exposure was observed in females, an observation confirmed in recently published European Medicines Agency draft guidance on rG-CSF products.[19]

The 3 biologics are also highly similar in terms of safety, as incidences and patterns of TEAEs were similar across all treatment groups. The most frequently reported TEAE was headache, followed by back pain, bone pain, myalgia and pain in extremity, all of which were reported with frequencies >10% across the treatment groups. All other TEAEs were reported with frequencies <10% by subjects in a treatment group. Particularly, the most frequently reported TEAEs (bone pain, myalgia and back pain) are consistent with the known safety profile of pegfilgrastim, commonly reported in the pegfilgrastim US prescribing information (bone pain and pain in extremity) and likely based on the mode of action of the drug.

The incidence of treatment-emergent SAEs was numerically slightly higher with the Sandoz biosimilar compared with US- and EU-

reference biologics, but importantly, none of the reported treatment-emergent SAEs were thought to be related to the study drugs. This was particularly true for the 2 cases of gunshot wounds that led to this numerical difference. Clinical safety laboratory findings, vital signs, electrocardiogram recordings, local study drug tolerance, injection site reactions and elicited immune responses to the study drugs were similar across all 3 biologics.

Numbers and proportions of subjects with confirmed positive ADAs were similar across the 3 biologics, and primary PK and PD parameters were comparable across the 3 biologics in ADA-positive and ADA-negative subjects. Most of the ADAs were directed against the PEGylated part of the molecule, as already noted by others.[19] This may be explained by exposure to widely used PEG as well as PEG derivatives in pharmaceutical and cosmetic products.

The similar PK and PD values across all 3 biologics groups suggest that ADA positivity does not have any measurable or clinically meaningful impact on PK or PD in any of the treatment groups. The overall numbers of subjects with newly confirmed positive ADA results decreased with the duration of subject study participation in all groups. The incidence of TEAEs and study drug related TEAEs in ADA-positive subjects was similar across all groups and was lower in ADA-positive subjects than in the overall SAF population, given the limitations of the comparison.

Three subjects developed NAbs in the first period (one after biosimilar administration and 2 after EU-reference biologic administration), but no NAbs were detected in subsequent testing. All subjects with ADAs, and in particular those with NAbs, were closely examined and it was concluded that the induced ADAs did not have any clinical relevance in terms of PK, PD or safety of the 3 study biologics. Limitations of this comparison include that ADAs for individual subjects varied across treatment periods and treatment groups, high interindividual variability, and small number of subjects with confirmed positive ADAs as compared to the overall SAF population. Observation periods between the SAF population and the ADA-positive set were also different, as only TEAEs occurring after the detection of ADAs were taken into consideration in the ADA-positive set for the TEAE analysis of the 2 analysis sets, which may have biased incidence rates.

The findings of the current study are consistent with those of the Phase I PK/PD study that compared Sandoz biosimilar with the EU-reference biologic exclusively[18] and with other Phase I studies comparing biosimilar pegfilgrastim medicines with the reference biologic (Neulasta). This includes published Phase I studies demonstrating similar PK/PD, safety, and immunogenicity between reference biologic and Cinfa Biotech biosimilar pegfilgrastim (B12019/Pelmeg),[26,27] Accord Healthcare biosimilar pegfilgrastim (INTP5/Pelgraz),[28,29] and Mylan biosimilar pegfilgrastim (MYL-1401H/Fulphila).[30] Crossover designs were used to compare the biosimilar of pegfilgrastim to both EU- and US-reference biologics.[29]

In summary, the primary objectives of this study were met, as PK and PD biosimilarity of Sandoz biosimilar pegfilgrastim with US- and EU-reference biologics was achieved. Similarity was also demonstrated between the US- and EU-reference biologics, providing the PK and PD components of the scientific bridge between both biologics, allowing consideration of the results of the efficacy safety trials[20,21] where Sandoz biosimilar pegfilgrastim was compared to only one of the 2 reference biologics. Secondary PK and PD parameter values were also close between Sandoz biosimilar pegfilgrastim, US- and EU-reference biologics, and no clinically meaningful differences were observed regarding safety and local tolerance among the treatment groups. The incidence of ADAs was similar across the treatment groups and ADAs had no impact on PK, PD or safety. In conclusion, Sandoz pegfilgrastim is biosimilar to US- and EU-reference biologics.

COMPETING INTERESTS

A.Be. is an employee of Hexal AG. J.W. is an employee of Novartis. M.V., D.D., A.Sa., L.N., T.O.R. and C.T. received compensation from Sandoz Biopharmaceuticals for their participation as Principal Investigators in the study. A.Sk. is an employee of Sandoz AG. S.S. is an employee of Sandoz Inc. S.S.-M., S.G., C.S. and R.N. are employees of Hexal AG. S.D.K. was an employee of Hexal AG at the time of study conduct and interpretation. M.D. is an employee of Sandoz Bulgaria. A.Bo. is an employee of Sandoz Pharma Services Romania SRL Romania. G.P.O. was an employee of Hexal AG.

CONTRIBUTORS

A.Be. was responsible for designing the study, data analysis, and writing the manuscript. J.W. was responsible for designing the study, statistical analysis, and writing the manuscript. M.V., D.D., A.Sa., L.N., D.K., T.O.R. and C.T. were Principle Investigators of the study and responsible for recruitment, safety of subjects and reviewing the manuscript. A.Sk., S.S., S.S-.M., S.G., S.D.K. and C.S. were responsible for designing the study, data analysis and writing the manuscript. M.D. and A.Bo. were responsible for data analysis and writing the manuscript. R.N. and G.P.O. were responsible for designing the study, data analysis and writing the manuscript. All authors approved the final manuscript for submission.

DATA AVAILABILITY STATEMENT

Research data are not shared.

REFERENCES

1. McCamish M, Woollett G. Worldwide experience with biosimilar development. *MAbs*. 2011;3(2):209-217.

2. Lopes G, Vulto A, Wilking N, van Harten W, Meier K, Simoens S. Potential solutions for sustaining the costs of cancer drugs. *Eur Oncol Haematol*. 2017;13(02):102-107.

3. European Medicines Agency. Biosimilar medicines: Overview 2017. https://www.ema.europa.eu/en/human-regulatory/overview/biosimilar-medicines-overview. Accessed March 13, 2019.

4. Markus R, Liu J, Ramchandani M, Landa D, Born T, Kaur P. Developing the totality of evidence for biosimilars: regulatory considerations and building confidence for the healthcare community. *BioDrugs*. 2017;31(3):175-187.

5. Weise M, Bielsky MC, De Smet K, et al. Biosimilars: what clinicians should know. *Blood*. 2012;120(26):5111-5117.

6. Schneider CK. Biosimilars in rheumatology: the wind of change. *Ann Rheum Dis*. 2013;72(3):315-318.

7. U.S. Food and Drug Administration. Scientific considerations in demonstrating biosimilarity to a reference product: guidance for industry 2015. https://www.fda.gov/downloads/drugs/guidancecompliance regulatoryinformation/guidances/ucm291128.pdf. Accessed March 13, 2019.

8. Tesser JR, Furst DE, Jacobs I. Biosimilars and the extrapolation of indications for inflammatory conditions. *Biol Theory*. 2017;11:5-11.

9. Rumpold H, Winder T. Development of chemotherapeutics in oncology: is there anything new? *memo*. 2017;10(3):119-120.

10. Twelves C, Jove M, Gombos A, Awada A. Cytotoxic chemotherapy: still the mainstay of clinical practice for all subtypes metastatic breast cancer. *Crit Rev Oncol Hematol*. 2016;100:74-87.

11. European Medicines Agency. Neulasta summary of product characteristics. https://www.ema.europa.eu/en/documents/product-information/neulasta-epar-product-information_en.pdf. Accessed March 13, 2019.

12. U.S. Food and Drug Administration. Neulasta higlights of prescribing information. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125031s180lbl.pdf. Accessed March 13, 2019.

13. Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product – USFDA Guidance for Industry - December 2016.

14. CHMP Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues -EMEA/CHMP/BMWP/42832/2005 Rev1–18 December 2014.

15. Yang BB, Morrow PK, Wu X, Moxness M, Padhi D. Comparison of pharmacokinetics and safety of pegfilgrastim administered by two delivery methods: on-body injector and manual injection with a prefilled syringe. *Cancer Chemother Pharmacol*. 2015;75(6):1199-1206.

16. Melhem M, Delor I, Pérez-Ruixo JJ, et al. Pharmacokinetic-pharmacodynamic modelling of neutrophil response to G-CSF in healthy subjects and patients with chemotherapy-induced neutropenia. *Br J Clin Pharmacol*. 2018;84(5):911-925.

17. Brekkan A, Lopez-Lazaro L, Yngman G, et al. A population pharmacokinetic-pharmacodynamic model of pegfilgrastim. *AAPS J*. 2018;20(5):91.

18. Nakov R, Gattu S, Wang J, Velinova M, Schaffar G, Skerjanec A. Proposed biosimilar pegfilgrastim shows similarity in pharmacokinetics and pharmacodynamics to reference pegfilgrastim in healthy subjects. *Br J Clin Pharmacol*. 2018;84(12):2790-2801.

19. European Medicines Agency. Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (rG-CSF) Draft - dated 26 July 2018. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-similar-biological-medicinal-products-containing-recombinant-granulocyte-colony_en.pdf Accessed 8 August 2019.

20. Blackwell K, Donskih R, Jones CM, et al. A comparison of proposed biosimilar LA-EP2006 and reference pegfilgrastim for the prevention of neutropenia in patients with early-stage breast cancer receiving myelosuppressive adjuvant or neoadjuvant chemotherapy: pegfilgrastim randomized oncology (supportive care) trial to evaluate comparative treatment (PROTECT-2), a phase III, randomized, double-blind trial. *Oncologist*. 2016;21(7):789-794.

21. Harbeck N, Lipatov O, Frolova M, et al. Randomized, double-blind study comparing proposed biosimilar LA-EP2006 with reference pegfilgrastim in breast cancer. *Future Oncol*. 2016;12(11):1359-1367.

22. Gascon P, Fuhr U, Sörgel F, et al. Development of a new G-CSF product based on biosimilarity assessment. *Ann Oncol*. 2010;21(7):1419-1429.

23. da Silva AR. Role of pharmacokinetics: pharmacodynamics in biosimilar assessment. In: Bonate PL, Howard DR, eds. *Pharmacokinetics in drug development: problems and challenges in oncology*. Vol.4 Switzerland: Springer; 2016:175-188.

24. Ackermann K, Revell VL, Lao O, Rombouts EJ, Skene DJ, Kayser M. Diurnal rhythms in blood cell populations and the effect of acute sleep deprivation in healthy young men. *Sleep*. 2012;35(7):933-940.

25. Blackwell K, Gascon P, Jones CM, et al. Pooled analysis of two randomized, double-blind trials comparing proposed biosimilar LA-EP2006 with reference pegfilgrastim in breast cancer. *Ann Oncol*. 2017;28(9):2272-2277.

26. Roth K, Meyer N, Wessels H, Hoefler J, Jankowsky R. Comparability of pharmacodynamics and immunogenicity of B12019, a proposed pegfilgrastim biosimilar to Neulasta (abstract). *Blood*. 2017;130(supplement 1):1002.

27. Roth K, Gastl B, Lehnick D, Jacob K, Jankowsky R. Demonstration of pharmacokinetic and pharmacodynamic comparability in healthy volunteers for B12019, a new proposed pegfilgrastim biosimilar (abstract). *Blood*. 2016;128(22):5079.

28. Desai K, Catalano T, Rai G, Misra P, Shah N. Confirmation of biosimilarity in a pharmacokinetic/pharmacodynamic study in healthy volunteers for an analytically highly similar pegfilgrastim. *Clin Pharmacol Drug Dev*. 2016;5(5):354-363.

29. Desai K, Misra P, Kher S, Shah N. Clinical confirmation to demonstrate similarity for a biosimilar pegfilgrastim: a 3-way randomized equivalence study for a proposed biosimilar pegfilgrastim versus US-licensed and EU-approved reference products in breast cancer patients receiving myelosuppressive chemotherapy. *Exp Hematol Oncol*. 2018;7:22.

30. Waller CF, Tiessen RG, Lawrence TE, et al. A pharmacokinetics and pharmacodynamics equivalence trial of the proposed pegfilgrastim biosimilar, MYL-1401H, versus reference pegfilgrastim. *J Cancer Res Clin Oncol*. 2018;144(6):1087-1095.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.