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Meta-analysis of Pharmacokinetic/ Pharmacodynamic Results of 3 Phase 1 Studies with Biosimilar Pegfilgrastim

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Abstract

A meta-analysis using data from 3 phase 1 studies evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of Sandoz biosimilar versus US- and EU-reference pegfilgrastim. The studies included a single-dose, double-blind, 3-arm, parallel-group study (study 1); a single-dose, double-blind, 2-way crossover study (study 2); and a single-dose, double-blind, 3-way, 6-sequence crossover study (study 3). Healthy male and female subjects were randomized to receive the proposed biosimilar (all studies), US-reference biologic (studies 1 and 3), or EU-reference biologic (studies 1, 2, and 3). For PK parameters (area under the serum concentration–time curve from time of dosing and extrapolated to infinity, area under the serum concentration–time curve from the time of dosing to the last measurable concentration, and maximum observed serum concentration) and PD parameters (absolute neutrophil count area under the effect curve from the time of dosing to the last measurable concentration and maximum measured absolute neutrophil count) geometric mean ratios and 90% confidence intervals (CIs) for treatment comparisons were calculated using the meta-analysis approach with a fixed-effects model. PK/PD biosimilarity was concluded if the 90%CIs were within the equivalence margins of 0.80 to 1.25. The 90%CIs for the geometric mean ratios for the PK/PD parameters were all within the equivalence margins. Safety and tolerability were similar between the proposed biosimilar and the US- and EU-reference pegfilgrastim in healthy subjects. This meta-analysis of 3 phase 1 studies supports PK/PD similarity of Sandoz biosimilar pegfilgrastim to US- and EU-reference pegfilgrastim. No clinically meaningful differences in safety or tolerability were observed.

Keywords

biosimilar, granulocyte colony-stimulating factor, neutropenia, pegfilgrastim

Prophylactic use of granulocyte colony-stimulating factors (G-CSFs) is recommended for patients undergoing chemotherapy with a high risk (>20%) of developing febrile neutropenia, and in select other patients at lower risk.[1–3] Biosimilar G-CSFs provide opportunities to reduce health care costs, improve sustainability of cancer care, and expand patient access.[4] A recent meta-analysis of randomized clinical trials in patients with breast cancer undergoing myelosuppressive chemotherapy indicated that there were no significant differences in efficacy or safety between reference biologics of filgrastim and long-acting G-CSF pegfilgrastim and their respective biosimilars.[5] Long-acting G-CSF biosimilars are now starting to emerge for clinical use, with several pegfilgrastim biosimilars, including Sandoz biosimilar pegfilgrastim (LA-EP2006), receiving positive opinions in 2018 and 2019 from the European Medicines Agency and the US Food and Drug Administration.

Development of Sandoz biosimilar pegfilgrastim included both analytical and clinical comparability studies. Physicochemical and functional analyses using advanced, state-of-the-art analytical techniques demonstrated that Sandoz biosimilar matches the US- and EU-reference biologics (Neulasta; Amgen) with

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regard to structure and biological activities/functions. In the clinical setting, 2 phase 3, randomized, double-blind, confirmatory studies have been conducted in the sensitive patient population of patients with breast cancer receiving myelotoxic (neo) adjuvant chemotherapy (PROTECT-1 and PROTECT-2).[6,7] Both studies demonstrated the therapeutic equivalence of Sandoz biosimilar and reference pegfilgrastim, as assessed by the duration of severe neutropenia during cycle 1 as the primary end point.[5–7] Subsequent pooled analyses of results from the 2 studies showed no clinically meaningful efficacy and safety differences between the 2 biologics and also among patients of Asian origin.[8,9]

In addition to these analytical and phase 3 clinical confirmatory studies, 3 phase 1 studies have been conducted to evaluate the pharmacokinetic (PK) and pharmacodynamic (PD) biosimilarity of Sandoz biosimilar and reference pegfilgrastim in healthy subjects (Table S1). Study 1 was conducted with a parallel design, based on the information on reference pegfilgrastim available at the time. Although the study failed to demonstrate statistically significant biosimilarity between treatments, it revealed an unexpected high intersubject variability for the area under the serum concentration–time curve (AUC) and an AUC ratio >1.0 for the comparison between the biosimilar and reference pegfilgrastim. The observed PK variability of >70% was substantially greater than the coefficient of variation (35%) used for the sample size calculation for this study, indicating that the study was underpowered to demonstrate biosimilarity between the 2 pegfilgrastim treatments. The high degree of intersubject variability of the PK results has since been confirmed by others with the reference biologic.[10] The PD parameters (the absolute neutrophil count [ANC] area under the effect curve [$AUEC_{0\text{-last}}$] and ANC maximum effect attributable to therapy [E_{max}]) were entirely within the predefined equivalence margins when evaluated using the 95% confidence interval (CI) approach for comparison between treatments, indicating the PD similarity of Sandoz biosimilar and reference pegfilgrastim. No meaningful differences in safety, local tolerability, and immunogenicity were observed between Sandoz biosimilar and the reference biologics.

A key finding of study 1 was that a parallel design is not the most suitable design, and a crossover design is more appropriate for studies involving pegfilgrastim and other products with high PK intersubject variability whenever possible.

A second PK/PD study (study 2 hereafter) was subsequently conducted to address the limitations of study 1 and was based on previous findings and new data that had emerged on the reference biologic (EudraCT No. 2015-003752-51).[11] A crossover design was used in study 2, enabling each subject to serve as its own control, thereby reducing variability. In addition, the sample size estimation was revised for study 2 based on intrasubject variability derived from a recently published crossover bioequivalence study with another proposed biosimilar of pegfilgrastim.[12] Heterogeneity of the subject population was further reduced by implementing a tighter inclusion criteria for ANC and body mass index (BMI). PK/PD similarity of Sandoz biosimilar pegfilgrastim vs reference pegfilgrastim was subsequently confirmed in this crossover study. No meaningful differences in safety, local tolerability, and immunogenicity were observed between the 2 biologics.[11]

Study 3 was a clinical study conducted to confirm that the PK/PD of Sandoz biosimilar pegfilgrastim is similar to that of both US- and EU-reference biologics. Owing to the high interindividual PK variability previously reported for pegfilgrastim,[10] this study was designed as a 3-way, 6-sequence crossover study to circumvent the interindividual variability and take into account intraindividual variability instead.[13] PK/PD similarity was demonstrated among the 3 biologics, as the 90%CIs for all PK and PD comparisons were within the predefined similarity margins[14] of 0.80 to 1.25. No meaningful differences in safety, local tolerability, and immunogenicity were observed among the 3 biologics.

Since the designs of the 3 studies were different and the sensitivity of the bioanalytical assay varied slightly among the studies, using subject-level data for combined/integrated analysis is not meaningful. Therefore, the meta-analysis method[15,16] was employed to perform a combined analysis, which can provide a more precise estimate of biologics comparisons than the individual studies.

The clinical pharmacology profile of pegfilgrastim is known to be unique in terms of its nonlinear PK profile, differential behavior in men and women, and demonstrating variability driven by several factors. Owing to the variable and unique features of pegfilgrastim, there has been a continuous discussion within the scientific community about the PK/PD similarity in smaller patient studies, robustness of the results, and the impact of such variable PK profiles on clinical parameters such as safety. This meta-analysis was conducted with 3 phase 1 studies to support similarity of PK and PD properties of Sandoz biosimilar with US- and EU-reference pegfilgrastim, to confirm robustness of the biosimilarity data in a larger cohort, and thereby address all aspects of residual uncertainty regarding the biosimilarity of Sandoz pegfilgrastim. Safety data were also evaluated as a pooled analysis. With data from 1040 healthy subjects, this is the largest-known evaluation of PK/PD parameters and safety of a biosimilar of pegfilgrastim.

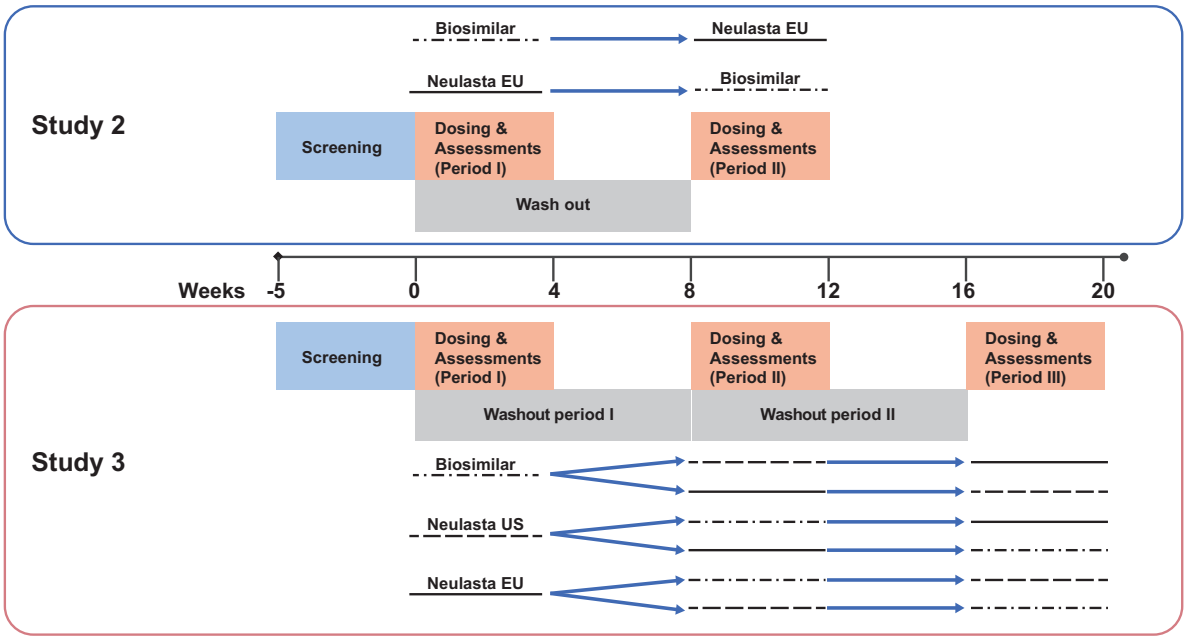


Figure 1. Study designs of study 2 and study 3 (crossover study designs).

Methods

Study Design and Conduct

The 3 PK/PD studies included in this meta-analysis were independently conducted, single-dose, randomized, double-blind studies. Study 1 was a 3-arm, parallel-group study, conducted at a single center in Germany (LA-EP06-101; EudraCT No. 2009-018051-16). Subjects were screened for eligibility 2 to 21 days before being randomized 1:1:1 to receive Sandoz biosimilar pegfilgrastim, US-reference pegfilgrastim, or EU-reference pegfilgrastim. Study 2 was a 2-way crossover study (LA-EP06-103; EudraCT No. 2015-003752-51).[11] The study design has previously been described and comprised 2 treatment/assessment periods of 4 weeks each separated by a washout period of 8 weeks (Figure 1).[11] In brief, subjects were screened for eligibility over a period of up to 5 weeks before being randomized 1:1 to receive 1 of the 2 treatment sequences: Sandoz biosimilar pegfilgrastim followed by reference pegfilgrastim (EU-sourced) or vice versa. Study 3 was a 6-sequence, 3-way, crossover multicenter (6 centers) study (EudraCT number: 2016-003549-27).[14] After screening, healthy subjects were randomized 1:1:1:1:1:1 to 1 of the 6 treatment sequences, starting with Sandoz biosimilar, US-reference, or EU-reference pegfilgrastim. The study duration per subject was ≥ 25 weeks, including screening (≤ 5 weeks), followed by 3 assessment periods (including treatment) of 4 weeks each separated by 2 washout periods (Figure 1). In all studies, pegfilgrastim was administered as a single 6-mg subcutaneous injection on day 1 of the treatment period after ≥10 hours of fasting.

The studies were conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice, applicable local regulations, and the Declaration of Helsinki. Study protocols were approved by independent ethics committees, and all subjects provided written informed consent. For study 1, ethical approval was by Lageso Ethikkommission, Berlin (Germany) (ID: ZS EK 12 087/10); for study 2, ethical approval was by Central Committee on Research Involving Human Subjects code, The Netherlands (ID: NL55704.056.15); and for study 3, ethical approval was by the Midlands Independent Review Board (United States) and Medisch Ethische Toetsings Commissie van de Stichting Beoordeling Ethiek Biomedisch Onderzoek (The Netherlands).

Study Populations

Studies 1, 2, and 3 were conducted in healthy male and female subjects. In study 1, subjects were aged between 18 and 55 years, weighed between 50 and 99.9 kg, had a BMI of 19.0 to 29.9 kg/m^2, and had an ANC and hematologic variables within the normal reference ranges. Subjects in study 2 were aged 18 to 45 years, weighed ≥60 kg, had a BMI of 19.0 to 28.0 kg/m^2, had an ANC of 2.0 to 7.0 cells/μL, and had hematologic variables within the normal reference ranges. Subjects in study 3 were aged between 18 and 55 years, weighed ≥60 kg, had a BMI of 19.0 to 30.0 kg/m^2, had an ANC of 2.0 to 7.0 cells/μL, and had hematologic variables within the normal reference ranges.

Key exclusion criteria included prior exposure to pegfilgrastim, filgrastim, or other G-CSFs; history of clinically significant diseases; history or presence of

conditions with the potential to interfere with drug distribution, metabolism, or excretion; and abnormal vital signs or laboratory results. In addition, subjects were excluded from study 2 if they tested positive for antidrug antibodies at the end of the first treatment period (day 28), or if their body weight fluctuated by more than 5% between the 2 treatment periods. These exclusion criteria also applied for study 3 on day −1 of the second and third treatment periods compared with day −1 of the previous treatment period.

Study End Points and Assessments

Study End Points. Studies 1, 2, and 3 sought to demonstrate the biosimilarity between Sandoz biosimilar pegfilgrastim and reference pegfilgrastim, in terms of PK and PD. The primary end points for the meta-analysis were the PK parameters AUC measured from time of dosing and extrapolated to infinity ($AUC_{0\text{-inf}}$), AUC measured from the time of dosing to the last measurable concentration ($AUC_{0\text{-last}}$), and maximum observed serum concentration (C_{max}) and PD parameters derived from the ANC over time—$AUEC_{0\text{-last}}$ and E_{max} (maximum neutrophil count measured following administration of the study medication). Secondary end points of studies 1, 2, and 3 also included safety and immunogenicity.

Pharmacokinetic and PD Assessments. In study 1, blood sampling for PK and PD analysis was performed over a 2-week period, with samples taken at 15 minutes before dosing, and at 30 minutes, and then every 4 hours after dosing on day 1 and day 2. From 36 hours after dosing, samples were taken every 12 hours thereafter until day 6, every 24 hours from day 6 to day 10, every 48 hours from day 10 to day 12, and every 72 hours from day 12 to day 15. In studies 2 and 3, blood sampling for PK and PD analysis was performed over a 2-week period, with samples taken at 15 minutes before dosing and at 4, 8, and 12 hours after dosing on day 1, every 12 hours thereafter until day 6, and every 24 hours from day 6 to day 15. Samples for PK analysis were collected into separator tubes and stored frozen at ≤−70°C until the time of analysis. In studies 1 and 3, serum sample analysis was performed by Hexal AG, and in study 2 by Nuvisan GmbH. PK end points were determined using a commercial sandwich enzyme-linked immunosorbent assay specifically validated for pegfilgrastim consisting of a capture anti-filgrastim antibody and a horseradish peroxidase–labeled anti-filgrastim detection antibody. Absorption was read photometrically, which is directly proportional to the amount of conjugate bound to the antibody complex (Table S2A).[13,14]

Samples for PD analysis were collected into potassium ethylenediaminetetraacetic acid tubes, kept at room temperature, and analyzed locally. PD assessment included analysis of changes in ANC using a validated commercial flow cytometer. PD ANC was measured by a validated method using commercial flow cytometers. These analyzers use flow cytometry to differentiate the white blood cell population into different categories, one of which is neutrophils. As cells pass through the laser beam, forward scattered light is generated. Forward scattered light is proportional to the cell size and the side-scattered light provides information on internal structure such globularity and granularity (Table S2B).[13,14]

Safety and Tolerability Assessments. Safety assessments were performed during the 28-day postdose follow-up period in all 3 studies and included adverse event recording, laboratory safety tests (hematology, clinical chemistry, and urinalysis), and monitoring of vital signs. Serious adverse events were documented up to 30 days after the last dosing of study drug. Local tolerance at the injection site was evaluated by the subject using a visual analog scale and by the investigator using the injection site reaction score. Immunogenicity assessments were performed in all 3 studies to evaluate binding and neutralizing antibodies. However, because of differences in the assay and analytical methods, such as cutoff points between studies, it is not appropriate to pool immunogenicity results and are hence not presented here.

Data for Meta-analysis

Data for the meta-analysis were sourced from the clinical trial reports of studies 1, 2, and 3. The meta-analysis focused on the primary PK and PD end point data. The data extracted included number of subjects, geometric means, and standard deviations under logarithms scale (geometric coefficient of variation in percentage) for the PK parameters $AUC_{0\text{-inf}}$, $AUC_{0\text{-last}}$, and C_{max}, and the PD parameters ANC $AUEC_{0\text{-last}}$ and E_{max} for each treatment, as well as geometric mean ratios and 90%CIs for each PK and PD parameter for the treatment comparison between Sandoz biosimilar pegfilgrastim and US-reference pegfilgrastim, between Sandoz biosimilar pegfilgrastim and EU-reference pegfilgrastim, and between US- and EU-reference pegfilgrastim. Details of how these data were calculated in study 2 have been published previously.[11] In line with the approach used for the individual studies, for studies 1 and 2, the meta-analysis included data from the PK and PD analysis populations, which included all subjects who completed the respective study (both periods for study 2), including completing PK/PD sampling, without a major protocol variation. For study 3, the meta-analysis included all subjects who received study drugs and who had at least 1 of the evaluable PK or PD parameters defined as the primary end point in at least 2 periods, without a major protocol violation.

Table 1. Demographic and Baseline Characteristics of Healthy Subjects in the Pooled Safety Analysis (Safety Analysis Set)

	Biosimilar Pegfilgrastim (N = 781)	US-Reference Pegfilgrastim (N = 604)	EU-Reference Pegfilgrastim (N = 772)
Sex, n (%)			
Female	285 (36.5)	223 (36.9)	277 (35.9)
Male	496 (63.5)	381 (63.1)	495 (64.1)
Ethnicity, n (%)			
Hispanic or Latino	86 (11.0)	82 (13.6)	88 (11.4)
Not Hispanic or Latino	692 (88.6)	519 (85.9)	681 (88.2)
Unknown	2 (0.3)	2 (0.3)	2 (0.3)
Not reported	1 (0.1)	1 (0.2)	1 (0.1)
Age, y			
Mean (SD)	32.8 (10.4)	34.8 (10.4)	33 (10.3)
Median (range)	30.0 (18-55)	33 (18-55)	30 (18-55)
Height, cm			
Mean (SD)	174.5 (9.5)	173.5 (9.7)	174.4 (9.7)
Median (range)	175.0 (146-203)	174.0 (146-201)	175.0 (146-203)
Weight, kg			
Mean (SD)	76.5 (10.5)	77.1 (10.6)	76.4 (10.3)
Median (range)	76.2 (51-113)	76.5 (52-113)	76.1 (54-113)
BMI, kg/m^2			
Mean (SD)	25.1 (2.6)	25.6 (2.7)	25.1 (2.6)
Median (range)	25.1 (19-31)	25.6 (19-30)	25.0 (19-30)

BMI, body mass index; SD, standard deviation.
For study 1, height, weight, and BMI were determined at screening.
For studies 2 and 3, height was determined at screening. Weight and BMI were determined on day-1 period 1; if missing, screening values were used. Subjects in the crossover design studies (studies 2 and 3) contributed to both treatments.

Statistical Methodology

A descriptive summary of demographic and baseline characteristics was provided for the pooled safety analysis set. For each primary PK parameter $AUC_{0\text{-inf}}$, $AUC_{0\text{-last}}$, and C_{max}, and primary PD parameter ANC $AUEC_{0\text{-last}}$ and E_{max}, the point estimates and 90%CIs of geometric mean ratios for treatment comparisons (biosimilar vs reference biologic) from the 3 studies were combined using a meta-analysis method with a fixed-effects model with weighted estimation.[15,16] The studies were weighted according to the inverse of the variance. Data were analyzed using the R system (version 3.4.0; The R Foundation for Statistical Computing, Vienna, Austria),[17] with the function "rma.uni" in R "metafor" package used in the calculation.[18] The combined point estimates and 90%CIs of geometric mean ratios for each PK and PD parameter were reported for all 3 pairwise comparisons. PK/PD biosimilarity was considered to be demonstrated if all CIs fell within equivalence margins of 0.80 to 1.25. Non–baseline-corrected PD parameters were used.

The incidence of treatment-emergent adverse events (TEAEs) was summarized (frequency of occurrence and number and percentage of subjects) by preferred term for each treatment for all causalities based on the pooled safety analysis set. The TEAE data were pooled directly by treatment in the pooled safety analysis set.

Results

Subject Demographics and Baseline Characteristics

Demographic and baseline characteristics of the safety analysis set encompassing subjects from the 3 studies are summarized in Table 1 and were similar between subjects who received biosimilar pegfilgrastim and those who received US- or EU-reference biologic.

Pharmacokinetic and PD Treatment Comparison

The PK/PD analysis populations included in the meta-analysis encompassed 277 subjects from the parallel-group study (93 for Sandoz biosimilar, 93 for EU-reference pegfilgrastim, and 91 for US-reference), 169 from the 2-way crossover study, and 496 from the 3-way crossover study. Serum pegfilgrastim concentration and ANC profiles for representative studies are shown in Figures S1 and S2. The summary statistics for PK and PD parameters are provided in Table 2.

Point estimates and 90%CIs of geometric mean ratios from the meta-analysis of PK and PD

Table 2. Summary of PK/PD Parameters Using Meta-analysis Methods

Parameters	Statistics	Sandoz Biosimilar Pegfilgrastim			Combined Geometric Mean	US-Reference Pegfilgrastim			Combined Geometric Mean	EU-Reference Pegfilgrastim			Combined Geometric Mean
Study		1	2	3		1	2	3		1	2	3	
$AUC_{0\text{-last}}$	N	93	169	482		91		480		93	169	479	
(h.ng/mL)	Geometric mean	5767	7501	4710	5502	4998		4470	4555	5245	6556	4630	5120
	CV% geo-mean	106	107	132		112		124		106	110	124	
	Mean	8216	10371	7330		7213		6500		7277	8947	6740	
	SD	7277	8260	7140		6570		5900		5623	6441	6200	
$AUC_{0\text{-inf}}$	N	92	168	482		90		480		93	168	479	
(h.ng/mL)	Geometric mean	5834	7670	4830	5612	5020		4470	4558	5277	6739	4630	5183
	CV% geo-mean	105	104	126		112		118		105	104	117	
	Mean	8289	10476	7400		7251		6550		7297	9048	6820	
	SD	7294	8245	7130		6633		5890		5621	6423	6190	
C_{max} (ng/mL)	N	93	169	483		91		480		93	169	480	
	Geometric mean	158	209	132	153	145		124	126	155	189	128	145
	CV% geo-mean	121	88	108		117		106		113	89	104	
	Mean	229	271	184		207		168		215	239	173	
	SD	186	208	155		171		125		158	150	129	
Tmax (h)	N	93	169	483		91		480		93	169	480	
	Median	24.0	12.0	12.1		16.0		12.1		20.0	12.0	12.0	
	Min	9.06	4.08	4.00		8.00		8.00		8.00	8.00	4.00	
	Max	48.0	60.0	36.8		59.5		59.5		47.9	48.0	36.1	
t1/2 (h)	N	92	168	482		90		480		93	168	479	
	Geometric mean	40.5	13.9	17.2		38.4		17.2		41.1	14.1	17.0	
	CV% geo-mean	35	69	70		39.3		62		40	68	68	
	Mean	42.9	17.9	22.4		41.3		20.5		44.5	18.2	22.6	
	SD	16.1	18.9	29.9		16.9		14.1		19.7	20.4	33.9	
$AUEC_{0\text{-last}}$	N	93	169	482		91		479		93	169	479	
(h.10^9/L)	Geometric mean	5028	4953	6000	5617	5265		6010	5923	5091	4874	6000	5635
	CV% geo-mean	19.7	23.8[a]	22.2				22.3		22.7	22.9	21.7	
	Mean	5124	5090	6150				6160		5220	5002	6140	
	SD	1016	1210	1340				1380		1213	1144	1350	

(*Continued*)

Table 2. Continued

Parameters	Statistics	Sandoz Biosimilar Pegfilgrastim			Combined Geometric Mean	US-Reference Pegfilgrastim			Combined Geometric Mean	EU-Reference Pegfilgrastim			Combined Geometric Mean
Study		1	2	3		1	2	3		1	2	3	
E_{max} (10^9/L)	N	93	169	482		91		480		93	169	480	
	Geometric mean	38	36	35	35	39		35	35	38	35	35	35
	CV% geo-mean	24.6	25.2[a]	25.0		26.2		25.9		25	23.9[a]	25.5	
	Mean	39.0	36.6	35.8		40.1		35.9		38.9	36.4	35.7	
	SD	9.05	9.23	8.97		10.3		9.30		10.0	8.71	9.09	
$t_{max,E}$ (h)	N	93	169	482		91		480		93	169	480	
	Median	59.8	60.0	60.0		59.8		60.0		59.8	60.0	60.0	
	Min	36.0	36.0	8.00		23.9		8.02		36.0	24.0	24.0	
	Max	107	108.0	145		95.8		150		108	108	176	

$AUC_{0\text{-inf}}$, area under the serum concentration–time curve measured from time of dosing and extrapolated to infinity; $AUC_{0\text{-last}}$, area under the serum concentration–time curve measured from time of dosing to last measurable concentration; $AUEC_{0\text{-last}}$, area under effect curve measured from time of dosing to last measurable concentration; C_{max}, maximum observed serum concentration; CV%, coefficient of variation; E_{max}, maximum effect attributable to investigational medicinal product; PD, pharmacodynamic; PK, pharmacokinetic.
[a] Arithmetic CV%.

parameters for Sandoz biosimilar, US-reference, and EU-reference pegfilgrastim are shown in Figure 2. The 90%CIs of the geometric mean ratios for the primary PK parameters were all contained within the predefined equivalence margins (0.80-1.25) for all 3 comparisons: Sandoz biosimilar pegfilgrastim vs US-reference pegfilgrastim; Sandoz biosimilar pegfilgrastim versus EU-reference pegfilgrastim; and US-reference pegfilgrastim versus EU-reference pegfilgrastim, as illustrated in Figure 2. Similarly, the combined 90%CIs for the primary PD parameters were all contained within the predefined equivalence margins (0.8-1.25) for all 3 comparisons. The meta-analysis results therefore support PK and PD similarity between Sandoz biosimilar and US-reference pegfilgrastim, between Sandoz biosimilar and EU-reference pegfilgrastim, and between US- and EU-reference pegfilgrastim.

Safety and Tolerability

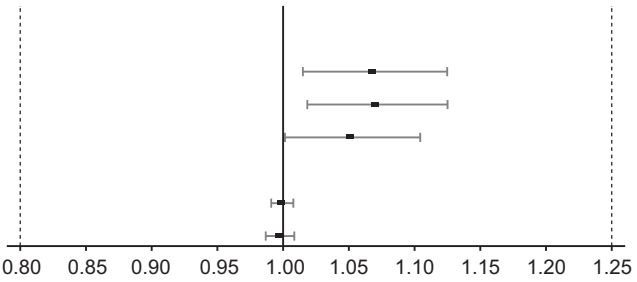
For safety analysis, no meta-analysis was performed, but only pooling of the data, which included all randomized subjects who received at least 1 dose of study drug in 1 of the 3 PK/PD studies. Safety and tolerability were found to be similar between the biosimilar, US-reference, and EU-reference biologic among this healthy subject population. TEAEs occurred in 89.4% of subjects who received Sandoz biosimilar pegfilgrastim, 88.2% who received US-reference pegfilgrastim, and 89.4% who received EU-reference pegfilgrastim (Table 3 and Table S3). TEAEs considered related to the study drug were reported in 85.8%, 84.3%, and 86.3% of subjects in the biosimilar, US-reference, and EU-reference pegfilgrastim groups, respectively. In the biosimilar group, 5 severe TEAEs (headache in study 1; neutropenia, appendicitis, gunshot wound, and suicide with gunshot wound in study 3) and 3 serious TEAEs (appendicitis, gunshot wound, and suicide with gunshot wound) were reported. The most common TEAEs occurring in subjects who received Sandoz biosimilar and EU-reference pegfilgrastim were headache (50.8% vs 44.9% vs 50.8%) in musculoskeletal and connective tissue disorders, and back pain (42.5% vs 46.7% vs 40.4%), bone pain (23.8% vs 15.6% vs 24.6%), and myalgia (19.3% vs 17.9% vs 22.8%) in nervous system disorders.

Discussion

This meta-analysis of 3 phase 1 studies conducted in healthy subjects provides further evidence of similar PK and PD characteristics between Sandoz biosimilar, US-reference, and EU-reference pegfilgrastim following a single 6-mg subcutaneous dose, as combined CIs for the geometric mean ratio are within the predefined equivalence margins (0.8-1.25) for all studied variables for each of the 3 comparisons. In addition, no clinically

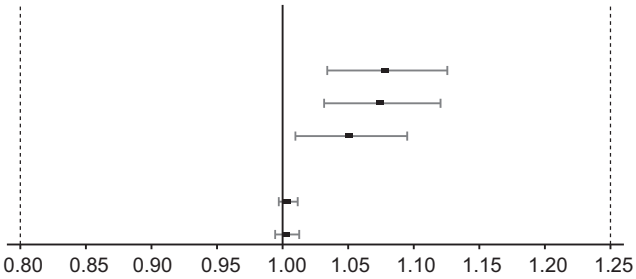
A) Sandoz biosimilar pegfilgrastim versus US-reference pegfilgrastim

Parameter	GMR	90% CI Lower	90% CI Upper	Forest Plot
PK: Pegfilgrastim				
$AUC_{0\text{-last}}$ (ng • h/mL)	1.0685	1.0150	1.1248	
$AUC_{0\text{-inf}}$ (ng • h/mL)	1.0704	1.0184	1.1251	
C_{max} (ng/mL)	1.0516	1.0014	1.1043	
PD: Absolute Neutrophil Count				
$AUEC_{0\text{-last}}$ (h*10^9/L)	1.9993	1.9909	1.0078	
E_{max} (10^9/L)	1.9976	1.9868	1.0085	



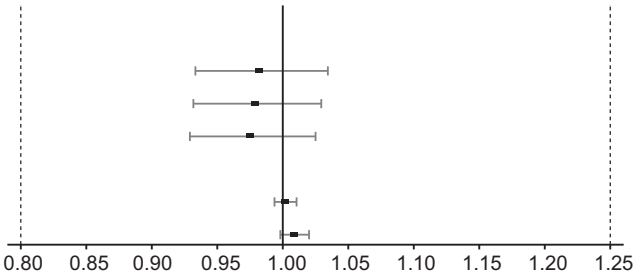
B) Sandoz biosimilar pegfilgrastim versus EU-reference pegfilgrastim

Parameter	GMR	90% CI Lower	90% CI Upper	Forest Plot
PK: Pegfilgrastim				
$AUC_{0\text{-last}}$ (ng • h/mL)	1.0789	1.0340	1.1256	
$AUC_{0\text{-inf}}$ (ng • h/mL)	1.0751	1.0317	1.1204	
C_{max} (ng/mL)	1.0515	1.0098	1.0950	
PD: Absolute Neutrophil Count				
$AUEC_{0\text{-last}}$ (h*10^9/L)	1.0043	1.9971	1.0115	
E_{max} (10^9/L)	1.0035	1.9943	1.0127	



C) US-reference pegfilgrastim versus EU-reference pegfilgrastim

Parameter	GMR	90% CI Lower	90% CI Upper	Forest Plot
PK: Pegfilgrastim				
$AUC_{0\text{-last}}$ (ng • h/mL)	0.9826	0.9333	1.0344	
$AUC_{0\text{-inf}}$ (ng • h/mL)	0.9794	0.9318	1.0294	
C_{max} (ng/mL)	0.9759	0.9291	1.0250	
PD: Absolute Neutrophil Count				
$AUEC_{0\text{-last}}$ (h*10^9/L)	1.0021	0.9937	1.0105	
E_{max} (10^9/L)	1.0090	0.9981	1.0201	



Geometric mean ratio (GMR) with error bars indicating 90% confidence interval (CI)

Figure 2. Forest plot of meta-analysis of PK and PD parameters for Sandoz biosimilar, US-reference, and EU-reference pegfilgrastim. Meta-analyses with fixed-effects model were used to derive the combined geometric means and 90% and 95%CIs. Non–baseline-corrected PD parameters were used. In study 2, the ANC PD values were involved in mixed model with baseline as a covariate when deriving ratios and CIs. *n = 92 for $AUC_{0\text{-inf}}$ in the Sandoz biosimilar pegfilgrastim treatment group of study 1. ANC, absolute neutrophil count; AUC, area under the serum concentration–time curve; $AUC_{0\text{-inf}}$, AUC measured from time of dosing and extrapolated to infinity; $AUC_{0\text{-last}}$, AUC measured from time of dosing to last measurable concentration; $AUEC_{0\text{-last}}$, area under the effect curve measured from time of dosing to last measurable concentration; CI, confidence interval; C_{max}, maximum observed serum concentration; E_{max}, maximum effect attributable to the investigational medicinal product; PD, pharmacodynamic; PK, pharmacokinetic.

meaningful differences in safety or tolerability were observed between biosimilar and reference biologics, or between the 2 reference biologics.

The meta-analysis results indicated that AUC and C_{max} were slightly higher (5%-7%) with Sandoz biosimilar pegfilgrastim compared with US- and EU-reference pegfilgrastim. This observation is similar to the results reported separately for studies 2 and 3.[11,13] Importantly, the 90%CIs for the ratio between the biosimilar and reference pegfilgrastim remained within the predefined biosimilarity margins for all primary PK parameters, and the slightly higher exposure did not translate into any differences in PD effects in the present study. Furthermore, no clinically meaningful

Table 3. Summary of TEAEs in Healthy Subjects in Pool (Studies 1, 2, and 3) (Safety Analysis Set)[a]

Number of Subjects With at Least 1	Biosimilar Pegfilgrastim N = 781, n (%)	US-Reference Pegfilgrastim N = 604, n (%)	EU-Reference Pegfilgrastim N = 772, n (%)
Pretreatment AE	4 (0.5)	3 (0.5)	5 (0.6)
TEAE	698 (89.4)	533 (88.2)	690 (89.4)
Severe TEAE	5 (0.6)	1 (0.2)	1 (0.1)
Serious TEAE	3 (0.4)	0	0
TEAE leading to study drug discontinuation	8 (1.0)	0	5 (0.6)
Serious TEAE leading to study drug discontinuation	2 (0.3)	0	0
Study drug–related TEAE	670 (85.8)	509 (84.3)	666 (86.3)
Study drug–related TEAE leading to study drug discontinuation	6 (0.8)	0	5 (0.6)
Study drug–related SAEs	0	0	0

AE, adverse event; PD, pharmacodynamic; PK, pharmacokinetic; SAE, serious adverse event; TEAE, treatment-emergent adverse event.
[a]For safety analysis, no meta-analysis was performed, but only pooling of the data. The pooled data include all randomized subjects who received at least 1 dose of study drug in 1 of the 3 PK/PD studies.

differences in efficacy and safety have been observed between Sandoz biosimilar pegfilgrastim, US-reference pegfilgrastim, and EU-reference pegfilgrastim in the 3 separate phase 3 clinical trials, providing further confirmation that this small difference in exposure does not translate into clinical relevance.[6–9] Phase 3 confirmatory studies comparing Sandoz biosimilar pegfilgrastim with reference pegfilgrastim showed that the mean duration of severe neutropenia in the first chemotherapy cycle (primary end point) was similar between the groups (1.05 ± 1.06 vs 1.01 ± 0.96 days), with a treatment difference of -0.04 days (95%CI, -0.19 to 0.11) that met the equivalence criteria (95%CIs within the defined margin of ± 1 day).[6–8] The most frequent TEAEs with a suspected relationship to pegfilgrastim were musculoskeletal and connective tissue disorders (biosimilar, 10.2%; reference, 9.7%), of which bone pain was the most frequent (biosimilar, 4.5%; reference, 6.1%).[6–8] Serious TEAEs with a suspected relationship to pegfilgrastim occurred at a low incidence in both groups.[6–8] These data show that the efficacy and safety data of Sandoz biosimilar pegfilgrastim are similar to those of the reference pegfilgrastim, thereby eliminating all residual uncertainty between the reference and biosimilar.

The regulatory pathway for biosimilars in Europe and the United States is now well established. Evaluation of the "totality of evidence" available for a biosimilar is key to the assessment approach applied by regulatory agencies, including the US Food and Drug Administration and the European Medicines Agency. Within such frameworks, demonstration of biosimilarity requires the generation of a comprehensive array of comparability data, typically encompassing analytical studies demonstrating a high degree of similarity in protein structure; in vitro assays and in vivo preclinical studies demonstrating comparable biological activity; comparative PD and PK studies in humans; and confirmatory comparative clinical efficacy, safety, and immunogenicity studies in a sensitive patient population. The totality of evidence available to date demonstrated similar profiles for Sandoz biosimilar pegfilgrastim and both US- and EU-reference biologics. The present meta-analysis provides data supporting the PK and PD biosimilarity of Sandoz biosimilar to US- and EU-reference pegfilgrastim, consistent with the results previously reported in the phase 1 two-way crossover study[11] and the phase 1 three-way crossover study.[13] As described earlier, these data are complemented by state-of-the-art physicochemical and functional analyses demonstrating highly similar structure and biological function, and by 2 phase 3 clinical comparability studies that established no meaningful differences in efficacy, safety, or immunogenicity between Sandoz biosimilar and reference pegfilgrastim in patients with breast cancer receiving chemotherapy.[6–9]

In study 1, the 95%CIs of the ratios for the primary PK end point $AUC_{0\text{-last}}$ were outside the standard equivalence margins of 0.80 to 1.25 for the comparisons between Sandoz biosimilar pegfilgrastim and EU-reference, and between Sandoz biosimilar pegfilgrastim and US-reference. Similar differences were observed in secondary PK end points C_{max} and $AUC_{0\text{-inf}}$. Adjusting for weight and gender did not alter these evaluations. Retrospective evaluation of results revealed that the study was underpowered. Moreover, a major contributing factor to PK similarity not being demonstrated in study 1 was the unexpected high intersubject variability and the use of a parallel-group design. In study 2, a crossover design was implemented, enabling each subject to serve as their own control, thereby reducing the variability.[11] All primary PK and PD parameters fell

within the predefined equivalence margins, demonstrating the biosimilarity of Sandoz biosimilar and reference pegfilgrastim.[11] Similarly, study 3 was designed as a 3-way crossover study to circumvent interindividual variability and take into account intraindividual variability. PK and PD biosimilarity of Sandoz biosimilar pegfilgrastim with US- and EU-reference pegfilgrastim was achieved.[13] Similarity was also demonstrated between the US- and EU-reference pegfilgrastim, allowing the bridging of results from previous efficacy and safety studies where Sandoz biosimilar pegfilgrastim was compared with only 1 of the 2 reference medicines.

Although PK similarity was not demonstrated in study 1, when combined with the results of study 2 and study 3 for this meta-analysis, the 90% CIs for the ratio between the biosimilar and reference product remained within the predefined margins of biosimilarity for all primary PK and PD parameters, with no clinically meaningful differences in safety or tolerability identified.

PK and PD data from phase 1 crossover trials in healthy subjects have been reported for several other proposed pegfilgrastim biosimilars.[12,19–23] For all of these biosimilars studied, the PK and PD parameters met the predefined criteria for biosimilarity with reference pegfilgrastim. Consistent with other studies comparing PK and PD of proposed biosimilars and G-CSFs,[12,19–25] the 3 studies included in the present meta-analysis enrolled healthy subjects. Performing such studies in healthy subjects is the approach recommended by regulatory bodies when seeking to demonstrate PK/PD similarity. Use of a healthy subject population allows for greater control of variables that may influence the PK/PD of the drug than would be possible in patients, thereby providing greater sensitivity for detection of differences between a proposed biosimilar and the reference product. This population is further considered appropriate for such studies given that G-CSF therapies exert a measurable effect on a clinically relevant PD parameter—ANC—in healthy subjects. ANC changes are directly linked to the mechanism of action of pegfilgrastim,[26] and enhancing ANC levels is central to the management of chemotherapy-induced neutropenia with G-CSFs.[2]

G-CSF biosimilars, such as Sandoz biosimilar pegfilgrastim, have the potential to support the sustainability of therapeutic and supportive cancer care by providing lower-cost alternatives to their reference biologics, introducing price competition, increasing accessibility of biologic therapies to patients, and broadening the treatment and management options available to health care providers.[27–29] However, the degree to which biosimilars will impact cancer care sustainability is dependent on confidence and understanding among health care providers, patients, and health care systems.[30]

Limitations

Although the individual PK/PD data in the 3 studies were available, owing to differences in study design (parallel design for study 1 and crossover designs for studies 2 and 3), the 3 studies cannot be pooled directly. The meta-analysis provided an appropriate statistical method to evaluate the pooled analysis results. However, this meta-analysis has some limitations. First, the number of included trials is small (only 3). Second, there were some differences in the sensitivity of the bioanalytical assay used by these studies, which may lead to slight heterogeneity. Considering no significant heterogeneity, a fixed-effects model was used in the meta-analysis. Finally, as the number of subjects included in study 3 was more than that in studies 1 and 2, some potential bias from study 3 may exist. Therefore, the weighted method[15,16] was used in estimation in the fixed-effects model.

Conclusions

This meta-analysis further supports the already demonstrated PK/PD similarity of Sandoz biosimilar pegfilgrastim vs US- and EU-reference pegfilgrastim, and adds to the existing totality of evidence, including confirmatory data on similar clinical efficacy and safety between the biosimilar and reference biologics. Sandoz biosimilar pegfilgrastim presents a potential sustainable option for managing chemotherapy-induced neutropenia in patients with cancer.

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Conflicts of Interest

S.G. is an employee of Hexal AG (Sandoz, a division of Novartis) and hold Novartis shares. J.W. and R.A. are employees of Novartis. A.B., C.S., and R.N. are employees of Hexal AG (Sandoz, a division of Novartis).

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Data Sharing

Sandoz (A division of Novartis) is committed to sharing with qualified external researchers, access to patient-level data, and

supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided are anonymized to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations. This trial data availability is according to the criteria and process described at www.clinicalstudydatarequest.com

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Supplemental Information

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