

Comparability of Biosimilar Filgrastim with Originator Filgrastim: Protein Characterization, Pharmacodynamics, and Pharmacokinetics

Fritz Sörgel^{1,2} · Arnd Schwebig³ · Johann Holzmann⁴ · Stefan Prasch⁴ · Pritibha Singh³ · Martina Kinzig¹

Published online: 3 April 2015

© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract

Background Biosimilars provide safety, purity, and potency similar to those of a reference biologic product.

Methods An array of protein analytical techniques was used to compare the physicochemical properties of proposed biosimilar filgrastim (EP2006), US-approved originator filgrastim, and EU-approved originator filgrastim. Biological characterization involved surface plasmon resonance spectroscopy analyses and in vitro proliferation assays. A randomized, double-blind, two-way crossover, phase I study in healthy volunteers assessed the pharmacodynamics, pharmacokinetics, and safety profiles of EP2006 and US-approved originator filgrastim (administered as a single subcutaneous 10 µg/kg injection).

Results EP2006 and originator filgrastim (US and EU approved) were highly similar with respect to primary, secondary, and tertiary protein structures; mass, size, purity, charge, and hydrophobicity. No differences in receptor binding affinity were observed, and all samples demon-

strated similar in vitro bioactivity. In the phase I study, no statistically significant differences between EP2006 and US-approved originator filgrastim were noted in pharmacodynamic or pharmacokinetic parameters, and all confidence intervals were within the equivalence boundaries. The two products had similar safety profiles.

Conclusion These studies provide robust evidence of the structural and functional similarity between the proposed biosimilar filgrastim (EP2006) and the US-approved originator filgrastim.

Key Points

Biosimilar filgrastim (EP2006) and originator filgrastim (US and EU approved) were highly similar with respect to primary, secondary, and tertiary protein structures; mass, size, purity, charge, and hydrophobicity. No differences in receptor binding affinity were observed, and all samples demonstrated similar in vitro bioactivity.

In a randomized, double-blind, two-way crossover, phase I study in healthy volunteers, no statistically significant differences between EP2006 and US-approved originator filgrastim were noted in pharmacodynamic or pharmacokinetic parameters, and all confidence intervals were within the equivalence boundaries. The two products had similar safety profiles.

These studies provide robust evidence of the structural and functional similarity between the proposed biosimilar filgrastim (EP2006) and US-approved originator filgrastim.

✉ Fritz Sörgel
fritz.soergel@ibmp.net

¹ IBMP-Institute for Biomedical and Pharmaceutical Research, Paul-Ehrlich-Strasse 19, 90562 Nürnberg-Heroldsberg, Germany

² Institute of Pharmacology, Faculty of Medicine, University of Duisburg-Essen, Essen, Germany

³ Hexal AG/Sandoz Biopharmaceuticals, Holzkirchen, Germany

⁴ Sandoz GmbH, Kundl, Austria

1 Introduction

Granulocyte colony-stimulating factor (G-CSF) is primarily used in clinical practice for the prevention of chemotherapy-induced neutropenia and for the mobilization of hematopoietic stem cells. The first G-CSF to be approved in the USA was filgrastim (Neupogen[®]; Amgen Inc., Thousand Oaks, CA, USA), a non-glycosylated protein. Biosimilars are approved biologics with safety, potency, and purity similar to those of a reference product. Although biosimilars of filgrastim are available in Europe and are in widespread use, no biosimilar G-CSF has been approved in the USA. One filgrastim biosimilar, EP2006 (Zarzio[®]; Sandoz GmbH, Kundl, Austria), was approved in Europe in 2009 for the same indications as those of its reference product, on the basis of extensive protein characterization and clinical studies in healthy volunteers and patients with breast cancer, which confirmed its similarity to the EU-approved originator product [1, 2].

Biosimilars provide a more affordable treatment option and, as global healthcare costs continue to rise, they are likely to become an increasingly important component of the therapeutic landscape [3]. Cost savings are already apparent in Europe, with an estimated €85 million saving across 17 EU countries in 2011 being associated with the introduction of biosimilar G-CSF [4]. This appears to have been accompanied by improved patient access, with anecdotal evidence of increased willingness by clinicians to use G-CSF as primary rather than secondary prophylaxis [2, 5]. Whether a similar experience will occur in the USA remains to be seen.

Development of a biosimilar product requires physicochemical and biological protein characterization, pharmacodynamic and pharmacokinetic studies in healthy volunteers, and clinical efficacy and safety data. In recent years, detailed physicochemical and biological comparisons of a biosimilar and its originator have been made possible by advances in analytical methods [1]. In fact, analytical similarity is now considered the most robust scientific basis for comparing different biologics, as it is more sensitive than clinical study endpoints in identifying even minor differences between products [6]. In addition, pharmacokinetic/pharmacodynamic studies provide critical data as part of a stepwise similarity exercise. Clinical experience in patients, although not described in this paper, is needed to provide confirmatory evidence of biosimilarity.

Here, we report the physicochemical and biological characterization of a proposed biosimilar filgrastim (EP2006) and US- and EU-approved originator filgrastim (Neupogen[®]). We also report the findings from a randomized, double-blind, phase I study that assessed the bioequivalence of EP2006 to US-approved originator filgrastim with respect to pharmacodynamics, pharmacokinetics, and safety.

2 Methods

2.1 Protein Characterization

Filgrastim was expressed using a recombinant *Escherichia coli* strain. Inclusion bodies were solubilized, and refolding was performed using a glutathione redox system. Protein purification was achieved via the application of multiple orthogonal modes of chromatography.

An array of analytical methods was used to compare EP2006 (480 µg/0.8 mL and 300 µg/0.5 mL solutions for injection) and the originator drug product obtained from the US market (480 µg/0.8 mL and 300 µg/0.5 mL) and the EU market (480 µg/0.5 mL and 300 µg/0.5 mL). The procedures were designed to identify any differences in the protein structure, mass, size, charge, hydrophobicity, receptor binding, and bioactivity of the test substances. The analyses included N-terminal Edman sequencing, peptide mapping with ultraviolet/mass detection, circular dichroism spectroscopy, 1D-¹H-nuclear magnetic resonance (NMR) spectroscopy, matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF-MS), size exclusion chromatography (SEC), sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), cation exchange chromatography (CEX), reversed-phase high-performance liquid chromatography (RP-HPLC), surface plasmon resonance (SPR) spectroscopy, and an in vitro bioassay, all of which have been described in detail previously [1]. Mass spectrometric detection of intact filgrastim and GluC-digested filgrastim, respectively, was performed by coupling RP-HPLC to an Orbitrap instrument (Thermo Fisher Scientific, Waltham, MA, USA).

In addition to these previously described methods, sub-visible protein particle determination was performed using micro-flow imaging (MFI) performed on a ProteinSimple MFI system (ProteinSimple, Santa Clara, CA, USA). Samples were evaporated before use for 20 min, and 1 mL sample volumes were analyzed. ProteinSimple MFI View Analysis Suite software was used for data evaluation. Clarity analysis was investigated by nephelometry, using a Hach Lange system (Hach Company, Loveland, CO, USA). Samples were injected as they were, and the procedure described in European Pharmacopoeia item 2.2.1 was used. Isoelectric focusing was conducted to evaluate charge.

Process-related impurities were assayed using established analytical technology. Host cell proteins (HCPs) were determined using an enzyme immunological method with lower limits of quantification (LLOQs) of 25 ppm (drug substance) and 50 ppm (drug product), respectively. Residual DNA was assayed using the ThresholdTM System (Molecular Devices Corp., Menlo Park, CA, USA).

Bacterial endotoxins were quantified using a suitable preparation of limulus amoebocyte lysate (LAL). Product-related variants were characterized and monitored using state-of-the-art analytical technology.

2.2 Pharmacodynamics and Pharmacokinetics

2.2.1 Study Design and Population

A single-center, randomized, double-blind, two-way crossover, phase I study with two treatment periods was conducted to determine the pharmacodynamics, pharmacokinetics, and safety of EP2006 and US-approved originator filgrastim (Neupogen®) following a single subcutaneous injection in healthy subjects. The study was conducted at PharmaNet Canada Inc. (Montréal, QC, Canada) and was in compliance with Good Clinical Practice, Good Laboratory Practice, local regulatory requirements, and the Declaration of Helsinki. The study protocol was reviewed and approved by the Institutional Review Board. All participants gave written informed consent before the initial assessment.

The subjects were healthy adult non-smokers or ex-smokers (defined as not having smoked for at least 6 months before study drug administration), aged between 18 and 49 years, with body weight between 50 and 109.9 kg and a body mass index (BMI) between 19 and 29.9 kg/m². The subjects had no prior exposure to recombinant human G-CSF products.

Subjects attended a screening visit less than 28 days before the first dosing of the study drug in period 1. Upon arrival at the unit in period 1, subjects were randomized 1:1 (using a computer-generated list) to receive a single subcutaneous injection of 10 µg/kg body weight of EP2006 or originator filgrastim. The study drug was administered in the morning on day 1 during each period. There was a wash-out period of at least 28 days between the two study drug periods. In both periods, eligible subjects resided in the clinical unit for at least 12 h prior to dosing through 36 h following dosing. Thereafter, the subjects returned to the clinical unit on an out-patient basis for scheduled pharmacodynamic, pharmacokinetic, and safety assessments on days 3 through 8, 11, and 15 of each period. A follow-up examination was performed on day 28 after dosing in period 2.

The subjects, investigator staff, persons performing the assessments, laboratory personnel, and data analysts remained blinded from the time of randomization through database lock. Unblinding was permitted only in the possible event of subject emergencies and at the conclusion of the study.

2.2.2 Objectives

The primary objective was to compare the neutrophil response in terms of the pharmacodynamic parameters: the

area under the effect on the absolute neutrophil count (ANC)–time curve from time zero to 120 h (AUEC_{0–120h}) and the maximum observed effect (E_{max}) following single subcutaneous injections of EP2006 and originator filgrastim. A second primary objective was to evaluate the following pharmacokinetic parameters: the area under the curve from time zero to the time of the last measurable concentration (AUC_{0–last}) and the maximum observed serum concentration (C_{max}). Pharmacokinetic equivalence was assessed as a secondary test after pharmacodynamic equivalence was shown. Secondary objectives included CD34+ cell counts, safety, immunogenicity, and local tolerance of both products.

2.2.3 Evaluation of Pharmacodynamics

For ANC assessment, blood samples (17 in total) were taken from each subject in both periods at 0.5 h before the study drug injection and at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 32, 48, 72, 96, and 120 h post-injection. For the evaluation of CD34+ cell counts, a total of ten blood samples were taken at 0.5 h before the injection and at 24, 48, 72, 96, 120, 144, 168, 240, and 336 h post-dose. The primary pharmacodynamic parameters measured for the ANC were AUEC_{0–120h} and E_{max} . The time to reach the maximum observed ANC effect ($t_{max,E}$) was also determined. The ANC was measured using a commercial flow cytometer (Advia 2120; Siemens AG, Munich, Germany). The CD34+ cell count was determined using a validated method with a commercial flow cytometer (BD LSRII; BD Biosciences, Franklin Lakes, NJ, USA) and an optimized CD34+ enumeration assay from the National Immune Monitoring Laboratory (University of Montreal, Montreal, QC, Canada). The CD34+ parameters measured were AUEC_{0–last}, E_{max} , and $t_{max,E}$.

2.2.4 Evaluation of Pharmacokinetics

Blood samples (16 in total) were taken for the pharmacokinetic assessment from each subject in both periods at 0.5 h before the injection and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 h post-injection. As well as the primary pharmacokinetic parameters of AUC_{0–last} and C_{max} , the area under the curve from time zero extrapolated to infinity (AUC_{0–∞}), time to reach C_{max} (t_{max}), elimination rate constant (K_{el}), and apparent terminal elimination half-life ($t_{1/2}$) were measured as secondary parameters. Pharmacokinetic analysis was performed using a validated enzyme-linked immunosorbent assay (Quantikine® Human G-CSF; R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany). The LLOQ was 0.039 ng/mL. The inter-day precision of the calibration standards of filgrastim ranged between 1.6 and 7.2 %, with accuracy between 99.0 and

101.0 %. The intra-day precision of the control samples ranged from 5.3 to 6.8 %. All control samples were within the predefined limits. The mean precision of the analyses of 20 incurred samples was 7.3 %. No sample value beyond ± 20 % was observed. Thus, the incurred sample reanalysis showed the reliability of the assay.

2.2.5 Evaluation of Safety

Adverse events (AEs) were recorded at each visit during the study. Laboratory tests, vital signs, an electrocardiogram, and local tolerance assessments were also performed during the study. To assess immunogenicity, blood samples taken before drug administration (0.5 h before the injection in periods 1 and 2) and afterward (at the follow-up visit) were analyzed for anti-filgrastim antibodies.

2.2.6 Statistical Analysis

This study was powered at 90 % for a sample size of 28 subjects for both pharmacokinetic and pharmacodynamic objectives. The safety population was defined as the group of subjects who received at least one dose of the study medication. The per-protocol (PP) analysis population included all subjects who received the study drug, provided evaluable pharmacodynamic profiles (for the ANC) and pharmacokinetic profiles, and completed the study without a major protocol violation. The primary pharmacodynamic/pharmacokinetic analyses were based on this PP population.

Descriptive statistics of the concentrations versus time, as well as all pharmacodynamic and pharmacokinetic parameters, were provided for each filgrastim product. An analysis of covariance (ANCOVA) was performed on the log-transformed ANC $AUEC_{0-120h}$, E_{max} , AUC_{0-last} , and C_{max} . The ANCOVA model included sequence, treatment, and period as fixed effects, and subject nested within sequence as a random effect. The log-transformed baseline value in each period (the ANC pre-dose value [or the check-in value if the pre-dose value was missing] for the ANC parameters and the pharmacokinetic parameters, and the CD34+ pre-dose value [or the check-in value if the pre-dose value was missing] for the CD34+ parameters) served as covariates in the model. The analysis was performed using SAS statistical analysis software (SAS Institute Inc., Cary, NC, USA).

Each ANCOVA included calculation of least-squares means (LSMs) for the treatments. The ratios of the LSMs were determined using exponentiation of the differences in the LSMs from the analyses of the corresponding log-transformed parameters. Consistent with Schuirmann's two one-sided tests for bioequivalence, 95 % (pharmacodynamic) and 90 % (pharmacokinetic) confidence intervals

(CIs) for these ratios were calculated for the ANC $AUEC_{0-120h}$, E_{max} , AUC_{0-last} , and C_{max} . Equivalence of biosimilar and originator filgrastim was to be concluded if the corresponding 95 % (pharmacodynamic) and 90 % (pharmacokinetic) CIs of the ratios of the LSMs of the parameters fell entirely within the predefined boundaries of 80–125 %.

Pharmacodynamics were further compared with respect to $t_{max,E}$ for the ANC, as well as $AUEC_{0-last}$, E_{max} , and $t_{max,E}$ for CD34+ cell counts. However, no formal hypothesis testing was applied to these parameters. All remaining pharmacokinetic parameters were analyzed descriptively. Therefore, all CIs reported for these secondary variables were interpreted only in an exploratory sense.

3 Results

3.1 Protein Characterization

3.1.1 Physicochemical Characterization

All drug substance samples analyzed by N-terminal Edman sequencing yielded the expected N-terminal sequence corresponding to the theoretical sequence of filgrastim (Met-Thr-Pro-Leu-Gly-Pro-Ala). N-terminal sequencing confirmed the identity of the primary structure in both EP2006 and originator samples. Peak patterns in peptide maps of batches of EP2006 and the originator drug product (480 μ g/0.8 mL for the US-approved originator and 480 μ g/0.5 mL for the EU-approved originator) were comparable in all samples, with no additional or missing peptides detected, indicating identical primary structure and disulfide bridging.

These results were confirmed using data from mass spectrometric analyses of the peptide maps, which demonstrated that the masses for each of the peptides generated by the GluC-digest were identical in the EP2006 and originator samples. Additionally, the values were comparable between the experimentally determined mass and the theoretical mass calculated from the amino acid sequence of each peptide. The chromatographic and mass spectrometric data of all samples were in agreement (Fig. 1). No significant differences in mass spectra and observed masses between the different samples were observed by either MALDI-TOF or RP-HPLC electrospray ionization (ESI) mass spectrometry. The molecular masses of all tested samples were in the ranges of 18,796.8–18,800.4 Da for MALDI-TOF (average mass) and 18,786.7992–18,786.8634 Da for RP-HPLC-ESI (mono-isotopic mass). These results are within the usual experimental error of the utilized instruments and demonstrate the identical primary structure of all samples.

Fig. 1 Overlay of reversed-phase high-performance liquid chromatograms of a Glu-C digest peptide map from biosimilar and originator filgrastim

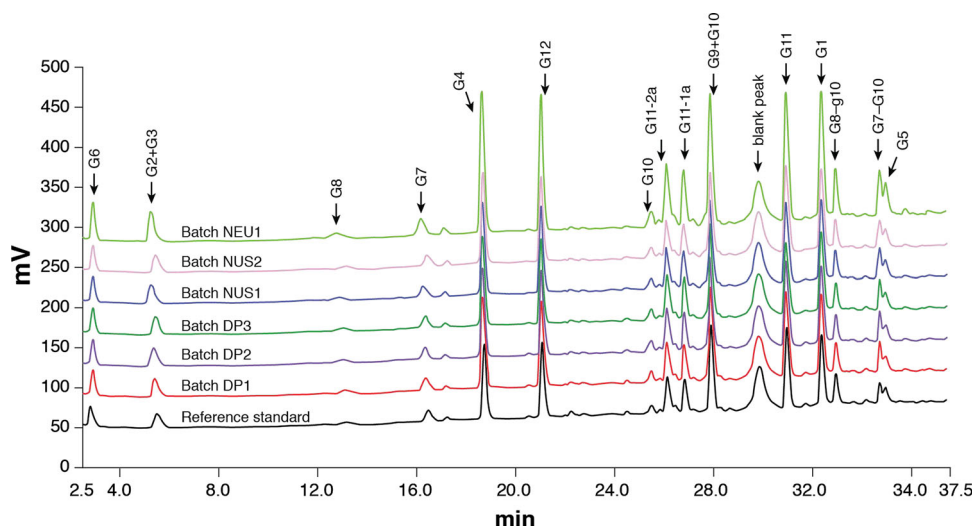
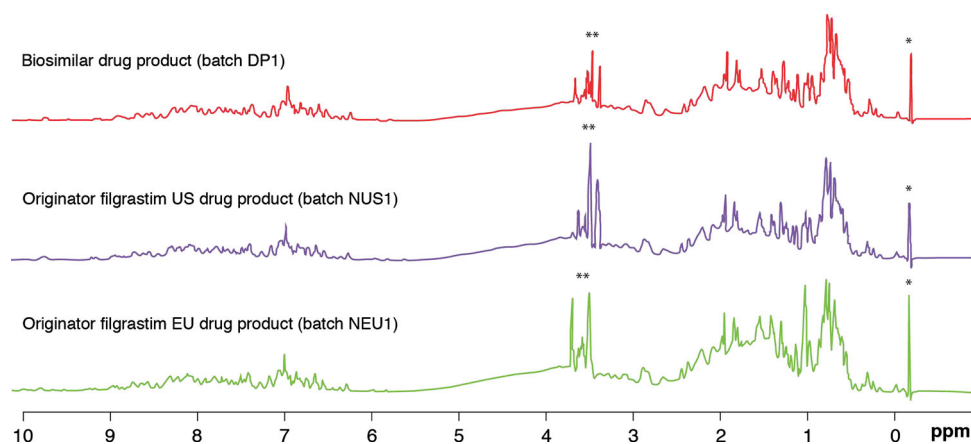


Fig. 2 1D- $\{^1\text{H}\}$ -nuclear magnetic resonance spectra of biosimilar filgrastim (batch DP1), US-approved originator filgrastim (batch NUS1), and EU-approved originator filgrastim (batch NEU1). ppm parts per million. *2,2,3,3-d $_4$ Sodium 3-(trimethylsilyl)propionate (d $_4$ -TSP) signal; **signals between 3.4 and 3.9 ppm correspond to formulation components, i.e., are not protein related



For all tested batches, the far ultraviolet circular dichroism spectra indicated a folded protein with a high proportion of α -helical secondary structure, as indicated by the pronounced minima at 208 and 222 nm. The high degree of congruence between the far ultraviolet circular dichroism spectra of biosimilar and originator filgrastim confirmed their similarity with respect to their secondary and tertiary structures. The NMR spectra of all tested samples also indicated a fully folded protein, with absence of signals >10 or <0 ppm, in agreement with the high content of the α -helical secondary structure. The biosimilar and originator NMR spectra corresponded to a high degree and showed no unexpected shift or broadening of signals. The amide regions, in particular, did not reveal any visible differences. These results demonstrated the structural similarity of biosimilar and originator filgrastim (Fig. 2).

The retention time, determined by SEC of the major peak in all EP2006 and originator samples, corresponded to the retention time of the main peak in the reference standards. All tested biosimilar and originator samples were

practically devoid of high molecular weight variants. The electrophoretic mobility of the major band in all EP2006 and originator samples, as determined by SDS-PAGE, corresponded to that of the main band in the reference standard (in both the reduced mode and the non-reduced mode). No additional bands were detected. These retention time and electrophoretic mobility findings confirmed the identity of the compound and showed comparable purity of filgrastim in all EP2006 and originator samples.

Findings from the CEX and RP-HPLC analyses confirmed the identity of the tested substances with regard to charge and hydrophobicity. With both CEX and RP-HPLC, the retention times of the major peaks in all biosimilar and originator samples in the chromatograms of the tested batches corresponded to the retention times of the main peaks in the reference standards. All tested products were of similar high purity, with a low percentage of product-related variants. From RP-HPLC, the sum of the product-related substances and impurities was calculated as 1.7–2.0 % for EP2006 (480 $\mu\text{g}/0.8$ mL and 300 $\mu\text{g}/$

0.5 mL), 4.6–5.8 % for US originator filgrastim (480 µg/0.8 mL and 300 µg/0.5 mL), and 4.5–5.1 % for EU originator filgrastim (480 µg/0.5 mL and 300 µg/0.5 mL). Isoelectric focusing studies demonstrated that all analyzed drug substance samples had the same main band mobility confirming the identity.

No differences were found between EP2006 filgrastim and either US or EU originator filgrastim with respect to either the MFI or nephelometry analyses (data not shown).

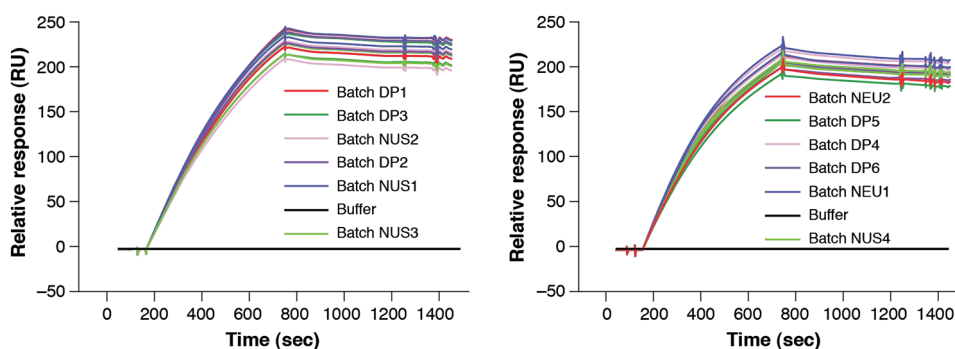
In addition, no differences were found with regard to low-level product-related variants and process-related impurities. Product-related variants identified in EP2006 were methionine oxidation variants, deamidation variants, succinimide variants, norleucine variants, N-terminal truncated variants, and high molecular weight variants (dimer and aggregation). These variants were also present in highly similar abundances in the reference product. However, two additional low-level variants (fMet and D → E misincorporation) that were identified in the reference product were absent in EP2006. The concentration of HCPs was below the LLOQ in all measured samples. The concentration of residual DNA was ≤ 200 pg/mg filgrastim in all samples, and the concentration of bacterial endotoxins was ≤ 2 IU/mg filgrastim in all measured samples.

3.1.2 Biological Characterization

The findings from the SPR spectroscopy, which was performed to investigate the kinetics of biosimilar and originator samples, are illustrated in Fig. 3. The absolute values of the kinetic rate constants varied only slightly between individual repeats of the experiment, and these variations were most likely due to aging of the sensor-chip surface during storage between experiments. Within each experiment, variability was low and no differences were detected between the biosimilar drug substance and the originator drug product with respect to the kinetic rate constants, k_{on} and k_{off} , or the equilibrium dissociation constant (k_d).

All tested samples of EP2006 and the originator showed similar in vitro bioactivity (data not shown).

Fig. 3 Sensorgram overlay of the receptor binding affinities of biosimilar and originator filgrastim, based on a surface plasmon resonance-based interaction assay using Biacore technology. RU resonance units



3.2 Pharmacodynamics and Pharmacokinetics

3.2.1 Subject Disposition and Baseline Characteristics

A total of 90 subjects were screened for this study. Of the 90 subjects, 28 (26 Caucasian; 20 males and eight females) were randomized, received at least one dose of study medication, and were included in the safety analysis. Two participants prematurely withdrew before completing both treatment periods (one from each group) without stating their reasons, and they were excluded from the pharmacodynamic and pharmacokinetic analyses. The 28 patients included in the safety analysis had a mean (\pm standard deviation) age of 37.1 ± 6.9 years (median 38 [range 19–49]), height of 169.8 ± 7.8 cm (median 169.3 [range 153–190]), weight of 72.6 ± 9.9 kg (median 70.3 [range 55–94]), and BMI of 25.2 ± 2.5 kg/m² (median 25.9 [range 19.6–29.7]).

3.2.2 Pharmacodynamics

The geometric mean ANC–time profiles (Fig. 4) and other pharmacodynamic parameters (Table 1) were comparable for both EP2006 and originator filgrastim. The respective mean values for the ANC AUEC_{0–120h} and E_{max} were 1493.06 and 25.97 with the originator compound, and 1541.28 and 26.06 with the proposed biosimilar. The 95 % CIs for ANC AUEC_{0–120h} (100.43 %; 105.78 %) and E_{max} (96.13 %; 104.70 %) were well within the predefined equivalence boundaries of 80–125 %; consequently, equivalence can be concluded with regard to pharmacodynamics. The ANCOVA of the log-transformed pharmacodynamic parameters of the ANC and CD34+ cell counts demonstrated that the outcome of the study was not influenced by any sequence effects.

With regard to CD34 cell counts, the 95 % CIs for AUEC_{0→last} (93.80 %; 111.55 %) and E_{max} (92.11 %; 119.64 %) were also within the predefined equivalence boundaries.

Fig. 4 Pharmacodynamic analysis: geometric mean absolute neutrophil count (ANC)–time profiles for biosimilar and US originator filgrastim

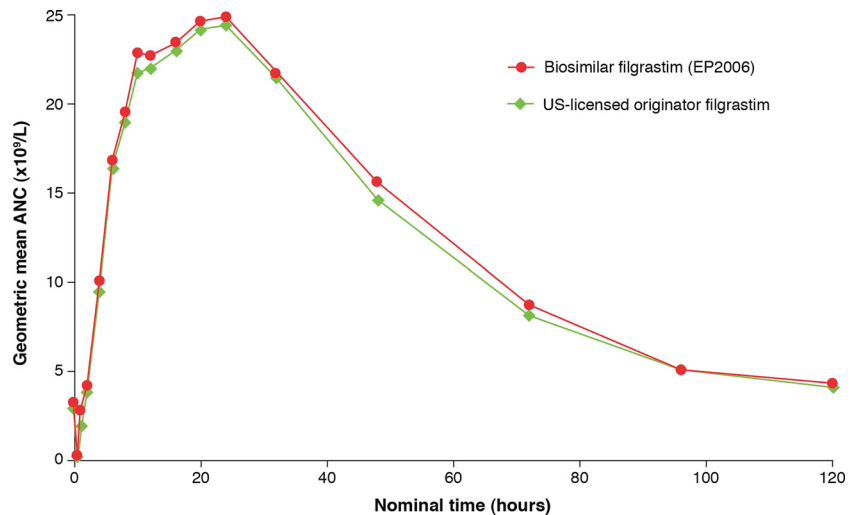


Table 1 Summary of pharmacodynamic and pharmacokinetic parameters of biosimilar and originator filgrastim

	Biosimilar, n = 26	Originator, n = 26
Pharmacodynamics		
Absolute neutrophil count		
AUEC _{0–120h} (×h 10 ⁹ /L) ^a	1523.64 [15.78 %]	1471.55 [17.89 %]
E _{max} (×10 ⁹ /L) ^a	25.67 [18.02 %]	25.49 [20.10 %]
t _{max,E} (h) ^b	20.0 [9.99–24.1]	20.0 [12.0–24.2]
CD34+		
AUEC _{0–120h} (h cells/μL) ^a	901.8 [38.60 %]	880.9 [38.20 %]
E _{max} (cells/μL) ^a	5.8 [50.36 %]	5.5 [46.82 %]
t _{max,E} (h) ^b	71.7 [24.1–169]	71.6 [24.1–168]
Pharmacokinetics		
AUC _{0–last} (ng h/mL) ^a	923.87 [20.28 %]	1043.52 [20.32 %]
AUC _{0–∞} (ng h/mL) ^a	927.67 [20.21 %]	1046.97 [20.27 %]
C _{max} (ng/mL) ^a	89.95 [22.74 %]	100.4 [24.31 %]
t _{max} (h) ^b	6.00 [3.99–8.04]	6.00 [3.99–8.02]
K _{el} (h ⁻¹) ^a	0.0806 [11.69 %]	0.0832 [13.73 %]
t _{1/2} (h) ^a	8.60 [11.69 %]	8.33 [13.73 %]

AUEC_{0–120h} area under the effect–time curve measured from time zero to 120 h, AUC_{0–last} area under the serum concentration–time curve from time zero to the time of the last measurable concentration, AUC_{0–∞} area under the serum concentration–time curve from time zero extrapolated to infinity, C_{max} maximum observed serum concentration, E_{max} maximum observed effect, K_{el} elimination rate constant, t_{1/2} apparent terminal elimination half-life, t_{max} time to reach C_{max}, t_{max,E} time to reach E_{max}

^a Geometric mean [geometric coefficient of variation]

^b Median [range]

3.2.3 Pharmacokinetics

The geometric mean filgrastim serum concentration–time profiles are displayed in Fig. 5, and other pharmacokinetic parameters are shown in Table 1. Bioavailability after administration of EP2006 filgrastim appeared to be slightly lower than that of the originator. Nevertheless, the 90 % CIs for AUC_{0–last} (84.39 %; 91.04 %) and C_{max} (84.00 %; 92.46 %) were within the standard equivalence boundaries of 80–125 %, confirming bioequivalence of both

treatments with respect to pharmacokinetics. No significant differences were detected between treatments for the secondary pharmacokinetic endpoints.

3.2.4 Safety

A total of 68 treatment-emergent AEs were reported by 21 of the 28 subjects (75 %) during the study, with the incidence being similar with the two treatments, such that 37 AEs were reported by 13/27 subjects (48 %) with EP2006

Fig. 5 Pharmacokinetic analysis: geometric mean filgrastim concentration–time profiles for biosimilar and US originator filgrastim

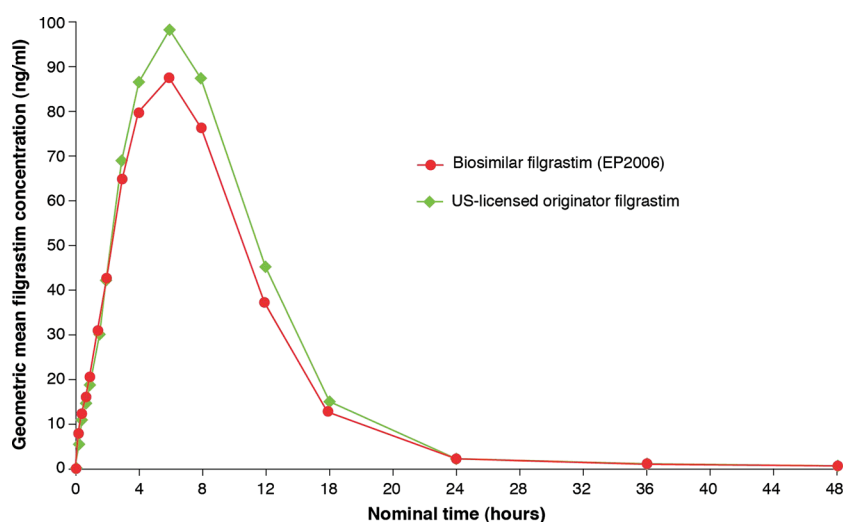


Table 2 Treatment-emergent adverse events (TEAEs)

	Biosimilar, <i>n</i> = 26	Originator, <i>n</i> = 26
Number of subjects with at least one TEAE (<i>n</i> [%])	13 [48.1 %]	14 [51.9 %]
Number of treatment-related TEAEs (<i>n</i>)	25	23
Most frequently reported TEAEs (<i>n</i> [%])		
Injection site hematoma	1 [3.7 %]	2 [7.4 %]
Injection site erythema	2 [7.4 %]	0
Vessel puncture site hematoma	2 [7.4 %]	0
Orthostatic heart rate response increased	5 [18.5 %]	4 [14.8 %]
Back pain	3 [11.1 %]	2 [7.4 %]
Myalgia	0	2 [7.4 %]
Headache	3 [11.1 %]	3 [11.1 %]
Upper respiratory tract infection	0	2 [7.4 %]

filgrastim and 31 AEs were reported by 14/27 subjects (52 %) with the originator. Most of the reported AEs were mild (*n* = 64), with only four being considered moderate.

No relevant differences in the severity, type, or pattern of AEs were observed between the two treatments. None of the reported AEs was considered severe, significant, or serious. Of the AEs observed, 48 were judged to be related to the drug treatment: 25 with EP2006 filgrastim and 23 with the originator. Overall, the most commonly observed AEs occurred with similar frequencies with both treatments (Table 2); Most of these AEs (e.g. back pain, myalgia) were expected with the use of filgrastim or were related to study procedures or to the study drug route of administration.

Overall, no safety issues were observed with respect to AEs, clinical laboratory measurements, vital signs, electrocardiographic features, and local tolerance assessment results, and no relevant differences were observed between the two treatments. No anti-filgrastim antibodies were detected in any of the subjects.

4 Discussion

Biosimilar development involves an iterative, stepwise, target-directed approach resulting in a product that is highly similar to the approved reference biologic. In addition, a lack of clinically meaningful differences between the biosimilar and the originator biologic needs to be demonstrated. The first step in showing similarity of a biosimilar to its originator product is extensive physico-chemical and biological characterization—now possible using an array of analytical techniques [7–10]. Such characterization has become feasible because of advances in the analytical methods that can be used to describe complex monoclonal antibodies, as well as smaller, simpler proteins, such as filgrastim.

In the analytical assessments described here, the primary protein structures of the proposed biosimilar and originator filgrastim were shown to be identical, according to the results of peptide mapping and other tests. Circular dichroism and NMR spectroscopy showed that both products have comparable, similar secondary and tertiary

structures. Additionally, CEX and RP-HPLC confirmed the identity with regard to charge and hydrophobicity, and showed that all tested products were of similar high purity, with a low percentage of product-related variants.

Bioactivity was shown to be similar with the two products, with both treatment groups demonstrating comparable binding to the G-CSF receptor in an SPR-based receptor affinity test and comparable biological activity in an *in vitro* cell proliferation assay. Previous protein characterization studies comparing the proposed biosimilar filgrastim (EP2006) with EU originator filgrastim had similar findings [1]. Since filgrastim is a non-glycosylated protein, state-of-the-art analytical methods can characterize its structure and function with a high degree of confidence regarding similarity.

Both the ANC and CD34+ cell counts have been validated as surrogate markers of filgrastim efficacy [2]. In our bioequivalence study, EP2006 filgrastim showed pharmacodynamic and pharmacokinetic equivalence to US-approved originator filgrastim following a single subcutaneous dose of 10 µg/kg in healthy adults. These results are in line with previous protein characterization and phase I studies, which established the bioequivalence of EP2006 and EU-approved filgrastim [1, 2]. Those studies with the EU-approved originator as a comparator demonstrated similarity across doses ranging from 1–10 µg/kg in both single-dose and multiple-dose settings for subcutaneous and intravenous administration.

The observed safety profile of EP2006 filgrastim in the described phase I study in healthy volunteers was similar to that of the originator with respect to AEs, clinical laboratory measurements, vital signs, electrocardiographic features, and local tolerance assessments. Furthermore, immunogenicity was not detected, with no anti-filgrastim antibodies observed in any subjects.

5 Conclusion

Extensive analytical and biological characterization demonstrates a high degree of similarity between the proposed biosimilar filgrastim (EP2006), US-approved originator filgrastim and EU-approved originator filgrastim. Equivalence of the proposed biosimilar filgrastim and US-approved filgrastim has also been shown with regard to pharmacokinetic and pharmacodynamic effects. These data, together with previous data comparing EP2006 and EU-approved filgrastim, provide robust evidence of similarity between EP2006 and US-approved originator filgrastim.

Conflicts of interest Fritz Sörgel and Martina Kinzig have conducted analytical and clinical research funded by Sandoz, and Fritz

Sörgel has received honoraria for presenting at Sandoz-sponsored symposia at international congresses. Arnd Schwebig and Pritibha Singh are employees of Hexal AG/Sandoz Biopharmaceuticals. Johann Holzmann and Stefan Prasch are employees of Sandoz GmbH.

Acknowledgments These studies were funded by Sandoz. Medical writing support was provided by Andy Bond of Spirit Medical Communications Ltd (Manchester, UK), supported by Sandoz.

Author contributions All authors made substantial contributions to the conception or design of the work and/or the acquisition, analysis, or interpretation of data (phase I data: Fritz Sörgel, Martina Kinzig, Arnd Schwebig, Pritibha Singh; protein characterization data: Johann Holzmann, Stefan Prasch). All authors were involved in developing and critically revising the content of the manuscript, and all provided final approval of the version submitted for publication. Arnd Schwebig is the guarantor for the overall content.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Sörgel F, Lerch H, Lauber T. Physicochemical and biologic comparability of a biosimilar granulocyte colony-stimulating factor with its reference product. *BioDrugs*. 2010;24:347–57.
- Gascon P, Fuhr U, Sörgel F, Kinzig-Schippers M, Makhson A, Balsler S, Einmahl S, Muenzberg M. Development of a new G-CSF product based on biosimilarity assessment. *Ann Oncol*. 2010;21:1419–29.
- Blackstone EA, Fuhr JP Jr. Innovation and competition: will biosimilars succeed? The creation of an FDA approval pathway for biosimilars is complex and fraught with hazard. Yes, innovation and market competition are at stake. But so are efficacy and patient safety. *Biotechnol Healthc*. 2012;9:24–7.
- Gascón P, Tesch H, Verpoort K, Rosati MS, Salesi N, Agrawal S, Wilking N, Barker H, Muenzberg M, Turner M. Clinical experience with Zarzio® in Europe: what have we learned? *Support Care Cancer*. 2013;21:2925–32.
- Verpoort K, Möhler TM. A non-interventional study of biosimilar granulocyte colony-stimulating factor as prophylaxis for chemotherapy-induced neutropenia in a community oncology centre. *Ther Adv Med Oncol*. 2012;4:289–93.
- McCamish M, Woollett G. The continuum of comparability extends to biosimilarity: how much is enough and what clinical data are necessary? *Clin Pharmacol Ther*. 2013;93:315–7.
- Chirino AJ, Mire-Sluis A. Characterizing biological products and assessing comparability following manufacturing changes. *Nat Biotechnol*. 2004;22:1383–91.
- Brockmeyer C, Saldi A. Binocrit: assessment of quality, safety and efficacy of biopharmaceuticals. *EJHP Pract*. 2009;15:34–40.
- Visser J, Feuerstein I, Stangler T, Schmiederer T, Fritsch C, Schiestl M. Physicochemical and functional comparability between the proposed biosimilar rituximab GP2013 and originator rituximab. *BioDrugs*. 2013;27:495–507.
- Holzmann J, Hausberger A, Ruppelchert A, Toll H. Top-down MS for rapid methionine oxidation site assignment in filgrastim. *Anal Bioanal Chem*. 2013;405:6667–74.