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ORIGINAL ARTICLE

Pharmacokinetics, pharmacodynamics, safety, and immunogenicity of Pelmeg®, a pegfilgrastim biosimilar in healthy subjects

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Abstract

A pharmacokinetics (PK)/pharmacodynamics (PD) study (EudraCT number 2015-002966-21) was conducted to investigate the biosimilarity of Pelmeg® (pegfilgrastim), a biosimilar to EU-authorized Neulasta®, which is used in the clinic for prevention of chemotherapy-induced neutropenia. The single-dose, randomized, double-blind, two-way crossover study comprised 171 healthy male subjects, receiving Pelmeg and Neulasta (6 mg as subcutaneous injection) in a sequential manner. Primary PK endpoints were the area under the concentration curve from time zero to last measurable concentration ($AUC_{0\text{-last}}$) and the maximum concentration (C_{max}). The primary PD endpoint was the area under the effect curve ($AUEC_{0\text{-last}}$) for absolute neutrophil count (ANC). Safety and immunogenicity were also assessed. Comparability was demonstrated for both PK endpoints, with geometric mean ratios (test/reference) for $AUC_{0\text{-last}}$ and C_{max} of 95.2% and 92.8%, respectively. The corresponding confidence intervals (CIs; 94.3%) were [86.6%;104.7%] for $AUC_{0\text{-last}}$ and [84.4%;102.2%] for C_{max}, both being within the equivalence margin of 80.0% to 125.0%. Likewise, PD comparability was demonstrated, with the geometric mean ratio (test/reference) of $AUEC_{0\text{-last}}$ of 100.2%, with a corresponding CI (95%) of 98.7%-101.8%. No clinically meaningful differences were observed for safety and immunogenicity between Pelmeg and Neulasta. Pelmeg was found to be highly similar to the reference product.

KEYWORDS

biosimilar, filgrastim, myelosuppressive chemotherapy, Neulasta, neutropenia, oncology, pegfilgrastim, Pelmeg, supportive care

1 | INTRODUCTION

Chemotherapy impacts rapidly dividing cells by directly causing cell death and slowing or stopping proliferation. Due to these effects, many chemotherapy regimens are associated with myelosuppression, resulting in reduced production of neutrophils (and also other blood cells like erythrocytes and thrombocytes). Often such hematological toxicities can limit the delivery of the planned dose and

intensity of chemotherapy, which is crucial for tumor control and patient survival. In clinical practice, neutropenia is the main limiting factor for the applicability of chemotherapy.[1]

Thereby, both the duration of Grade 4 neutropenia (defined as absolute neutrophil count [ANC] of < 0.5 × 10^9/L) and the depth of the nadir after chemotherapy are correlated with the development of infectious complications.[2] Thus, an important goal in oncological practice is the prevention of neutropenia when administering chemotherapy.

Filgrastim is a recombinant human granulocyte colony-stimulating factor (G-CSF), which stimulates the production of neutrophil precursors, enhances the function of mature neutrophils, and ameliorates neutropenia and its complications.[3] Pegfilgrastim is a pegylated form of filgrastim, developed to increase the half-life. Pegfilgrastim retains the same biological activity as filgrastim and binds the same G-CSF receptor. A once-per-chemotherapy-cycle administration of pegfilgrastim was shown to be sufficient to reduce the duration of severe neutropenia as effectively as daily treatment with filgrastim.[4]

The efficacy and safety of pegfilgrastim for the prevention of chemotherapy-induced neutropenia were demonstrated in two pivotal Phase 3 studies with Neulasta,[2,5] leading to regulatory approval of Neulasta in the US and the EU.

Pelmeg (development code B12019) is a biosimilar pegfilgrastim. A comprehensive analytical, functional, and preclinical comparability program demonstrated a high degree of similarity of Pelmeg between and the reference product Neulasta. In the clinical development program, two comparative studies were conducted.

Study B12019-101 was the first-in-human trial for Pelmeg, a pharmacokinetics (PK)/ pharmacodynamics (PD) study. Objectives of the study were to demonstrate PK comparability of Pelmeg to Neulasta based on area under the concentration curve from time zero to last measurable concentration ($AUC_{0\text{-last}}$) and maximum concentration (C_{max}), to demonstrate PD comparability based on area under the effect curve ($AUEC_{0\text{-last}}$) for ANC-time curve, and to investigate immunogenicity and safety. The results from this study, as presented here, confirm the biosimilarity of Pelmeg to EU-authorized Neulasta.

2 | MATERIALS AND METHODS

This randomized, double-blind, single-dose, two-way crossover study in healthy subjects was conducted at two study sites in Germany, between October 2015 and April 2016.

The study was registered with EudraCT (number 2015-002966-21) and was conducted in accordance with the International Conference on Harmonisation Guideline for Good Clinical Practice E6, the European Clinical Trial Directives 2001/20/EC and 2005/28/EC, and applicable national and local regulatory requirements. The aspects of the study concerned with the investigational medicinal product met the requirements of EU Good Manufacturing Practice. The protocol and informed consent form were reviewed and approved by relevant ethics committees prior to implementation. Written informed consent was obtained from all subjects prior to screening.

2.1 | Study population

Healthy male subjects (as determined by medical history, physical examination including vital signs, electrocardiogram [ECG], and clinical laboratory testing), aged 18-55 years, with a body mass index (BMI) between 20.0 and 30.0 kg/m^2 (inclusive), and a weight between 60 and 100 kg (inclusive) were eligible to be included in the study. All subjects were to comply with the contraception requirements as specified in the protocol. Subjects were excluded if they had been previously treated with pegfilgrastim, or if they had known anti-drug antibodies (ADAs) to filgrastim, pegfilgrastim, or polyethylene glycol (PEG).

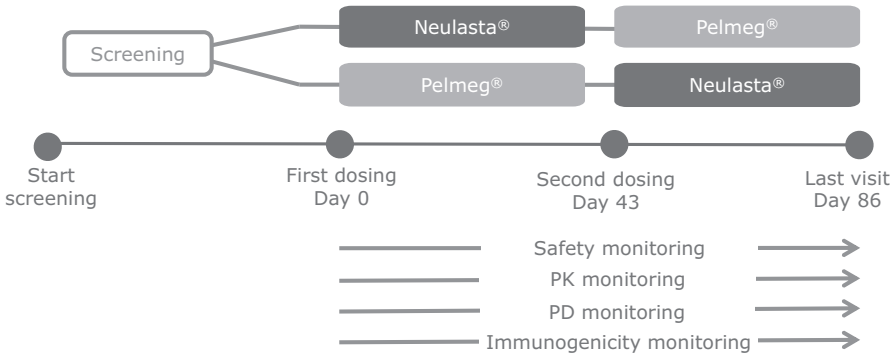
2.2 | Study design

The sample size was determined by the anticipated variability of the PK endpoints $AUC_{0\text{-last}}$ and C_{max}, for which the intra-individual coefficient of variation (CV) was expected to be high, but not exceeding 50% (based on [6]). A targeted power of 90%, an expected true test/reference ratio of 0.95-1/0.95, and biosimilarity limits of 80.0%-125.0% were assumed for the primary PK parameters. To account for the expected high variability of the PK parameters the study methodology was based on a two-stage design,[7,8] planning for a sample size recalculation after completion of Stage 1 and potential sample size adjustment for Stage 2. In Stage 1 of the study, 172 subjects were to be enrolled, whereas Stage 2 would allow the recruitment of additional subjects. With a total of 156 evaluable subjects in Stage 1 (assuming a drop-out rate of up to 10%), it could be assumed that the targeted power was already achieved based on Stage 1 only. However, under the given assumptions of a CV of 50% there was a probability of approximately 5.5% that the study needed to go into Stage 2. In case the variability of the primary PK parameters was higher than expected, the probability to enter into Stage 2 increased. According to the predefined decision rules, no Stage 2 was performed. The given sample size was also considered to be appropriate and sufficient in order to support the assessment of biosimilarity for the PD endpoint $AUEC_{0\text{-last}}$ for ANC. Assuming an expected true test/reference ratio of 0.95-1/0.95 and biosimilarity limits of 80.0%-125.0% for the 95% confidence intervals (CIs), 156 evaluable subjects provided at least 90% power to lie within the acceptance ranges, as long as the intraindividual CV does not exceed 49%.

The study design is shown in Figure 1.

Subjects were screened 2 to 28 days prior to administration of study drug. Eligible subjects were admitted to the study site and remained hospitalized until Day 5, while ambulatory visits were performed after Day 5 until Day 43. Each subject participated in two study periods, with sequential administration of Pelmeg followed by Neulasta or Neulasta followed by Pelmeg. Dosing was separated by

FIGURE 1 Study design B12019-101



a wash-out period of at least 6 weeks (maximum 8 weeks), corresponding to approximately 15 half-lives of pegfilgrastim. Subjects were randomized in a 1:1 ratio to sequentially receive Pelmeg and Neulasta or *vice versa*. Study drugs were administered as subcutaneous (s.c.) injections to the abdomen, at a dose of 6 mg (Pelmeg: 6 mg/0.6 mL, batch number 9201515003, Cinfa Biotech SL, Spain, and Neulasta: 6 mg/0.6 mL, batch number 1056658B, Amgen Europe BV, The Netherlands).

2.3 | Endpoints and statistical analysis

The primary PK endpoints were $AUC_{0\text{-last}}$ and C_{max}. The primary PK analysis was performed on the model-based PK set (defined as all subjects with reliable PK data for both study periods ie without any important protocol deviation which would render the data between treatments incomparable). For $AUC_{0\text{-last}}$ and C_{max} the $(1–2\alpha)$% CI for the ratio of the test and reference products was to be contained within the equivalence margin of 80.0%-125.0%. The primary PK parameters were evaluated using an α1-level of 0.0284 (corresponding to a 94.32% CI for the test/reference ratio). For Stage 1, the 94.32% confidence limits were calculated based on the antilogs of the least square means and mean square error from a general linear model (GLM) analysis of variance with sequence, subjects within sequence, period and treatment as fixed effects on log-transformed data. In order to achieve a better approximation to a normal distribution, PK parameters related to concentrations (such as $AUC_{0\text{-last}}$ and C_{max}) were logarithmically transformed before analysis. Secondary PK endpoints included time to C_{max} (t_{max}), terminal elimination rate constant (λ_z), half-life ($t_{½}$); these were evaluated descriptively. The primary PD endpoint was $AUEC_{0\text{-last}}$ for ANC. The primary PD analysis was performed on the model-based PD set (defined as all subjects with reliable PD data for both study periods ie without any important protocol deviation which would render the data between treatments incomparable). Pelmeg and Neulasta were assumed to be comparable if the 95% CI of the test/reference ratio is within the equivalence margin of 80.0%-125.0%. The 95% confidence limits were calculated based on the antilogs of the least square means and mean square error from a GLM analysis of variance with sequence, subjects within sequence, period and treatment as fixed effects on log-transformed $AUEC_{0\text{-last}}$ of ANC data. To achieve a better approximation to a normal distribution, PD parameters related to concentrations (such as $AUEC_{0\text{-last}}$) were logarithmically transformed before analysis. The secondary PD endpoints maximum effect (E_{max}) and $t_{max,E}$ of ANC, and CD34 + counts were evaluated descriptively. Safety variables included adverse events (AEs), local tolerability, physical examinations, vital signs, 12-lead ECG, and laboratory safety assessments. Immunogenicity was investigated by assessment of ADAs. Safety results were summarized descriptively.

2.4 | Bioanalysis

2.4.1 | Analysis of pegfilgrastim concentrations

Blood samples for PK analysis were collected during the in-patient phase, predose and up to 96 hours postdose, and during the ambulatory visits in each period.

Pegfilgrastim concentrations in serum were determined using a standard quantitative enzyme-linked immunosorbent assay (ELISA) technique. The assay employed components from the R&D Systems' (Biotechne AG, Switzerland) Human G-CSF DuoSet ELISA kit. Microplates are coated with mouse anti-human G-CSF capture antibody which binds the G-CSF in the sample. After the analyte is bound it is detected using a biotinylated goat anti-human G-CSF detection antibody. The capture antibody is then bound by streptavidin-horseradish-peroxidase (HRP), which in turn enzymatically catalyses tetramethylbenzidine (TMB) conversion.

The determination was carried out over an expected calibration range of 0.20-8.00 ng/mL (samples above the calibration range could be diluted up to 400-fold). The lower limit of quantification of the assay was 0.2 ng/mL For low, medium, and high quality control, the intraday variability was between 3.9% and 6.5% while interday variability was between 3.1% and 4.3%. The method was validated in accordance with the European Medicines Agency (EMA) Guideline on Bioanalytical Method Validation[9] and the FDA Draft Guidance for Industry on Bioanalytical Method Validation [10].

2.4.2 | Analysis of ANC and CD34

Blood samples for determination of ANC were collected during the in-patient phase, predose, and up to 96 hours postdose, and during the ambulatory visits in each period. Determination of ANC from whole

blood was performed by fluorescent flow cytometry, using the automated hematology analyzer XT-2000i (SYSMEX) and reagents. Before samples from the clinical study were analyzed, quality control (QC) samples (including three concentration levels) were measured on each day of the analytical performance. Only after acceptance of QC samples, study samples were analyzed. The method was validated by the provider.

Blood samples for determination of CD34 + were collected on Day 1 (predose), and between Day 3 and Day 12 postdose. The frequency of CD34 + cells from whole blood was determined with a flow cytometry-based assay, using the BD Bioscience Stem Cell Enumeration Kit in combination with the FACS Canto Clinical Software. The kit is an FDA cleared in vitro diagnostic test which meets the ISHAGE Guidelines.[11] The sensitivity of the assay was determined as 2.7 CD34+ cells/μL. The assay was found to be precise, with ≤ 30% CV.

2.4.3 | Analysis of ADAs

Blood samples for ADA analysis were obtained on Day 1 predose, Days 8, 15, 22, 29 of each period, and Day 43 of the last period.

Anti-pegfilgrastim antibodies in serum were detected with an immunoassay using electroluminescence. The testing concept involved a multi-tiered approach. Initially, samples were subjected to a run-specific screening assay. If a sample result exceeded the cut point of the screening assay, then the sample was considered as ADA-reactive and was advanced to the next tier. Otherwise, the sample was considered negative, and no further tests were required on the sample. All samples that were ADA positive in the screening assay were subsequently tested in a confirmatory assay. In the confirmatory assay, samples were tested in parallel with four different competitive inhibitors (Pelmeg, Neulasta, Filgrastim, PEG6000). Samples that gave a percentage inhibition value equal to or greater than the confirmatory cut point were classified as positive for the respective competitive inhibitor. Relative sensitivity was demonstrated and controlled using an anti-Pelmeg whole molecule affinity-purified antibody reagent, in combination with an anti-PEG positive control antibody reagent. A conservative test strategy was applied to classify samples as ADA positive if any reactivity with Pelmeg, Neulasta, filgrastim, or PEG6000 was detected in a confirmatory assay. All confirmed positive samples were further characterized for ADA titer in a ligand-binding assay format and for neutralizing capacity in a cell-based assay (NSF-60 assay). The methods were developed in accordance with the EMA Guideline on immunogenicity assessment of therapeutic proteins (EMEA/CHMP/BMWP/14327/2006 Rev 1, May 2017).[12]

2.5 | Compliance with design and statistical analysis requirements

The study was designed to enroll equal subject numbers for each treatment sequence, and subjects were randomized in a 1:1 ratio. Inclusion and exclusion criteria were predefined in the protocol. As there was a visible difference between the syringes for the test and reference products, drug administrations were performed by an unblinded team of medics and medically trained staff members, who were not involved in any further study activities, and in a way that the subjects remained blinded. Subjects, investigator staff, persons performing the assessments or being responsible for determining dosing regimen and staff of the sponsor or data analysts, remained blinded from the time of randomization until database lock.

3 | RESULTS

3.1 | Demographics and baseline characteristics

A total of 172 subjects were randomized and enrolled in the study (86 subjects for each treatment sequence). One subject was randomized but

TABLE 1 Analysis sets

	Treatment sequence		
	Pelmeg-Neulasta	Neulasta-Pelmeg	Total
Safety set	85	86	171
PK set	84	85	169
PD set	84	83	167
Model-based PK set	79	82	161
Model-based PD set	79	82	161

Abbreviation: PD, pharmacodynamics; PK, pharmacokinetic.

TABLE 2 Demographics and baseline characteristics

	N = 161
Age (years)	
Median	42
Min; max	19, 55
Weight (kg)	
Median	81.5
Min, max	61.3, 99.3
Height (cm)	
Median	179
Min; max	165, 197
BMI (kg/m^2)	
Median	25.6
Min; max	20.0, 30.0
Smoking status n (%)	
Yes	29 (18.0)
No	132 (82.0)

All subjects in this study were male and white. Thus, subject distribution by sex and race is not shown. Numbers are based on the primary analysis set (ie the model-based PK set; numbers are identical for the model-based PD set).
Abbreviations: BMI = body mass index, Max = maximum, Min = minimum, N = number of subjects.

not treated, due to tachycardia in the predose ECG. Of the 171 subjects who received study medication, 8 subjects discontinued the study prematurely (5 for "personal reasons," 2 were lost to follow-up, 1 due to a protocol violation). A total of 163 subjects completed both study periods.

All subjects who received a dose of study medication were included in the safety set, whereas subjects who received a dose of study medication and who had adequate and reliable PK data from at least one study period were included in the PK set. Subjects who had evaluable PD data from at least one study period were included in the PD set. The model-based PK/ PD sets, used for the primary PK and PD analyses, respectively, included only subjects with data from both study periods, and without any protocol deviations which would render the data incomparable between treatments. Analysis sets are shown in Table 1. Demographics and baseline characteristics are shown in Table 2 for the primary analysis set (model-based PK and model-based PD set).

3.2 | Pharmacokinetics

Results are presented for the primary analysis set, the model-based PK set. This set includes all subjects with reliable data for both study periods, and without any important protocol deviation. Of the 171 subjects who received study medication, 10 were excluded from the model-based PK set, because they either discontinued prematurely and had reliable data for one study period only, or could not provide full PK profiles, for example, due to missing visits. Mean serum concentrations of pegfilgrastim after administration of Pelmeg and Neulasta were very similar, with maximum serum concentrations at around 24 hours postdose (Figure 2).

The results for the statistical analysis of the primary PK parameters are shown in Table 3. There was no relevant difference in the exposure of pegfilgrastim after administration of Pelmeg and Neulasta, as the 94.32% CIs for the ratio of the test and reference products were fully contained within the equivalence margin of 80.0%-125.0%. The primary PK endpoint of this study was met and PK comparability between test and reference was shown.

In addition to the prespecified analysis of $AUC_{0\text{-last}}$, also $AUC_{0\text{-inf}}$ was analyzed, using the same model as described for the primary analysis. For this analysis, data from 143 subjects were available after Neulasta treatment and from 143 subjects after Pelmeg treatment (model-based PK set); there were 127 subjects with $AUC_{0\text{-inf}}$ data from both periods. The geometric mean ratio was 92.1%, and the 94.32% CIs for the ratio of the test and reference products were 82.9 and 102.2, hence fully within the equivalence margin of 80.0%-125.0%. Secondary PK parameters were evaluated descriptively and were found to be very similar for Pelmeg and Neulasta. Time to C_{max} was 16.0 hours with Pelmeg and Neulasta, λ_z was 0.018 l/h with Pelmeg and 0.017 l/h with Neulasta, and $t_{½}$ was 39.1 h with Pelmeg and 40.2 hours with Neulasta.

TABLE 3 Statistical analysis of primary PK parameters (model-based PK set, N = 161) and primary PD parameter (model-based PD set, N = 161)

	Pelmeg/Neulasta		
Parameter	Ratio (%)	94.32% CI	Intrasubject CV (%)[a]
PK parameters			
$AUC_{0\text{-last}}$	95.2	86.6;104.7	46.7
C_{max}	92.8	84.4;102.2	47.1
PD parameter			
$AUEC_{0\text{-last}}$	100.2	98.7;101.8	7.0

Abbreviations: ANC, absolute neutrophil count; $AUC_{0\text{-last}}$, area under the concentration time curve from time zero to last measurable concentration; $AUEC_{0\text{-last}}$, area under the effect time curve from time zero to last measurable concentration; CI, confidence interval; C_{max}, maximum concentration; CV, coefficient of variation; N = number of subjects; PD = pharmacodynamics; PK, pharmacokinetic.

[a]Intraindividual CV (%) estimated from the residual mean squares.

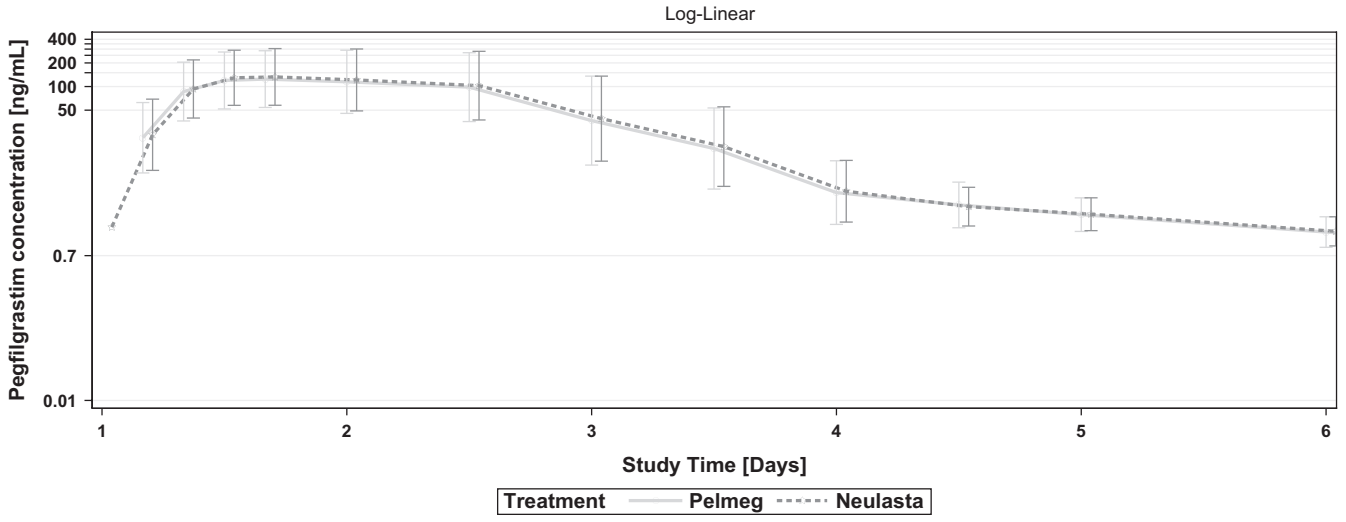


FIGURE 2 Geometric mean (geometric SD) serum concentrations of pegfilgrastim (model-based PK set, N = 161). Solid and dotted lines indicate the geometric mean serum concentrations with Pelmeg and Neulasta, respectively, up to 6 d postadministration. Error bars indicate geometric standard deviation (SD). N, number of subjects; PK, pharmacokinetic; SD, standard deviation

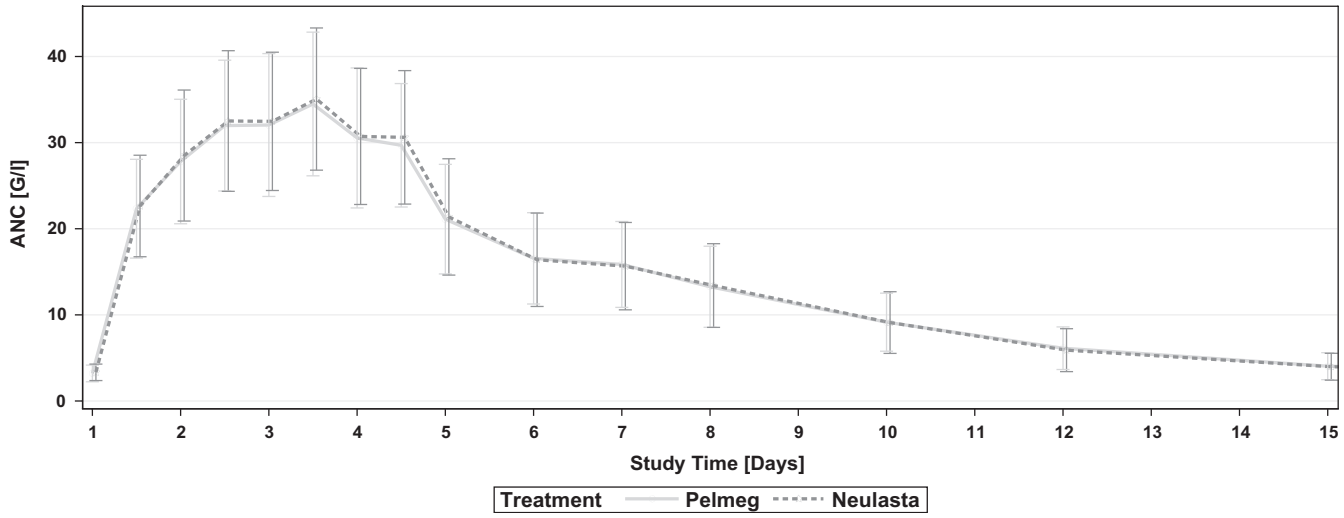


FIGURE 3 Mean (SD) ANC values until Day 12 (model-based PD set, N = 161). Solid and dotted lines indicate the absolute neutrophil counts (ANC) with Pelmeg and Neulasta, respectively, up to 12 days postadministration. Error bars indicate standard deviation (SD). ANC, absolute neutrophil count; N, number of subjects; PD, pharmacodynamics; SD, standard deviation

3.3 | Pharmacodynamics

Results are presented for the primary analysis set, which is the model-based PD set. This set includes all subjects with reliable data for both study periods, and without any relevant protocol deviation. Of the 171 subjects who received study medication, 10 were excluded from the model-based PD set, because they either discontinued prematurely and had reliable data for one study period only, or could not provide full PD profiles, for example, due to missing visits.

Mean ANC values after administration of Pelmeg and Neulasta are shown in Figure 3. ANC profiles were very similar after administration of Pelmeg and Neulasta. Starting from similar predose levels (around 3 G/L), comparable increases in mean ANC were observed. Peak levels were reached at around 3.5 days postdose and decreased thereafter. The predose level was reached again on Day 18. Results for the statistical analysis of the primary PD parameter are shown in Table 3. The geometric mean ratio of $AUEC_{0\text{-last}}$ was about 100% and the corresponding 95% CI was very close to 100%, indicating no difference with regard to ANC after administration of Pelmeg and Neulasta.

The primary PD endpoint of this study was met and PD comparability between test and reference was shown. Similar results after administration of Pelmeg and Neulasta were also observed for the secondary PD endpoints, geometric mean $AUEC_{0\text{-last}}$ and E_{max} of ANC, and CD34 + profiles.

3.4 | Safety

All 171 subjects dosed were included in the safety analysis. The percentage of subjects with any AE was comparable for Pelmeg and Neulasta (86.0% vs 81.3%, Table 4). In both groups, the majority of AEs were assessed as drug related by the investigator. In the majority of subjects, AEs were of mild or moderate severity. There were no deaths. One subject (treated with Pelmeg) reported the serious adverse event multiple injuries due to a car accident, assessed as unrelated to the study drug.

The pattern of AEs was similar for Pelmeg and Neulasta, with the majority of patients experiencing AEs in the System Organ Class of musculoskeletal and connective tissue disorders. Most commonly reported AEs (by preferred term) after both treatments were back pain, headache, nasopharyngitis, hypoglycemia, and pain in extremity. The safety results are summarized in Table 4.

Injection site reactions were reported for six subjects after administration of Pelmeg (injection site erythema, injection site hematoma, and injection site warmth), and for one subject after administration of Neulasta (injection site erythema). Injection site reactions were assessed as mild in all subjects. No clinically meaningful differences between treatments were observed for any safety assessments, including laboratory, ECG, or vital signs (data not shown).

3.5 | Immunogenicity

A special focus of the safety evaluation was immunogenicity, which was evaluated as a secondary endpoint. A summary of ADA results is shown in Table 5. Overall, 34 of 171 (19.9%) subjects in the safety set had confirmed ADA positive reactivity with PEG. Importantly, no anti filgrastim-reactive positive samples were detected in any subject. Thus, the detected signals appear to represent antibodies reactive with PEG, or with the PEG moiety of Pelmeg or Neulasta. No samples with neutralizing capacity in the NSF-60 cell-based assay were detected. Overall, all subjects with ADA positive signals were asymptomatic and these signals were considered not clinically relevant.

4 | DISCUSSION

In line with the guidelines for biosimilar development, the focus of this clinical study was to confirm the biosimilarity of Pelmeg

TABLE 4 Summary of safety results (safety set, N = 171)

Subjects with AE, n (%)	Neulasta	Pelmeg	Total
Any AE	139 (81.3)	147 (86.0)	155 (90.6)
Drug-related AE	136 (79.5)	141 (82.5)	151 (88.3)
Serious AE	0 (0)	1 (0.6)	1 (0.6)
AE leading to discontinuation	0 (0)	0 (0)	0 (0.0)
Deaths	0 (0)	0 (0)	0 (0)
AEs by severity			
Mild	108 (63.2)	108 (63.2)	140 (81.9)
Moderate	111 (64.9)	119 (69.6)	136 (79.5)
Severe	2 (1.2)	2 (1.2)	4 (2.3)
Most common AEs by Preferred Term (≥2% of subjects in any of the treatment groups)			
Back pain	109 (63.7)	114 (66.7)	134 (78.4)
Headache	52 (30.4)	54 (31.6)	76 (44.4)
Nasopharyngitis	28 (16.4)	27 (15.8)	50 (29.2)
Hypoglycemia	37 (21.6)	33 (19.3)	49 (28.7)
Pain in extremity	29 (17.0)	18 (10.5)	41 (24.0)
Neck pain	14 (8.2)	8 (4.7)	21 (12.3)
Oropharyngeal pain	12 (7.0)	7 (4.1)	18 (10.5)
Myalgia	9 (5.3)	6 (3.5)	15 (8.8)
Musculoskeletal pain	8 (4.7)	5 (2.9)	13 (7.6)
Alanine aminotransferase increased	8 (4.7)	8 (4.7)	12 (7.0)
Blood pressure systolic increased	4 (2.3)	10 (5.8)	11 (6.4)
Fatigue	4 (2.3)	7 (4.1)	11 (6.4)
Nausea	6 (3.5)	6 (3.5)	10 (5.8)
Arthralgia	5 (2.9)	6 (3.5)	9 (5.3)
Feeling hot	4 (2.3)	5 (2.9)	9 (5.3)
Cough	4 (2.3)	6 (3.5)	9 (5.3)
Musculoskeletal chest pain	4 (2.3)	6 (3.5)	8 (4.7)
Diarrhea	3 (1.8)	5 (2.9)	8 (4.7)
Palpitations	5 (2.9)	2 (1.2)	7 (4.1)
Bone pain	5 (2.9)	1 (0.6)	6 (3.5)
Dizziness	4 (2.3)	2 (1.2)	6 (3.5)
Blood creatine phosphokinase increased	1 (0.6)	5 (2.9)	6 (3.5)
Gamma glutamyltransferase increased	4 (2.3)	5 (2.9)	6 (3.5)
Toothache	4 (2.3)	1 (0.6)	5 (2.9)
Hyperhidrosis	1 (0.6)	4 (2.3)	5 (2.9)

Note: Percentages are based on N. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 18.1.
Abbreviations: AE, adverse event; N, number of subjects.

TABLE 5 Summary of ADA results (safety set, N = 171)

Statistic		Subjects (%)
No. subjects (%) with ≥ 1 confirmed ADA positive sample		34 (19.9%)
No. subjects (%) positive with each competing antigen in confirmatory ADA assay	Neulasta + Pelmeg + PEG_{6000}	6 (3.5%)
	Pelmeg + PEG_{6000}	3 (1.8%)
	Neulasta + PEG_{6000}	1 (0.6%)
	PEG_{6000} only	24 (14.0%)
	Pelmeg only	0 (0%)
	Neulasta only	0 (0%)
	Filgrastim only	0 (0%)
No. subjects (%) positive in nAb assay		0 (0%)

Abbreviations: ADA = anti-drug antibody, nAb = neutralizing antibody, N = number of subjects.

as compared to the reference product Neulasta in a head-to-head comparison. Various factors have been taken into consideration when designing this first-in-human study of Pelmeg. The study was conducted in healthy subjects. Compared to cancer patients receiving chemotherapy, healthy subjects lack comorbidities and comedications, and are not immunosuppressed. Thus, they represent the most sensitive study population for conducting the PK and PD comparison. The use of a sensitive population is recommended by the Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: nonclinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev 1).[13] Also, with regard to assessing the potential immunogenicity of pegfilgrastim, healthy subjects are considered more sensitive than cancer patients, as the latter have a compromised immune system.

In both healthy and patient populations, the mechanism of action of pegfilgrastim is the same, whereby pegfilgrastim elicits its effects on hematopoietic cells by binding to specific cell surface receptors stimulating proliferation and differentiation of committed progenitor cells of the granulocyte-neutrophil lineage into functionally mature neutrophils. Because the bone marrow in a healthy subject population is functionally unimpaired (in comparison with patients undergoing myelosuppressive chemotherapy), the bone marrow of this subject population is expected to be more responsive to stimulation with G-CSF.[14]

The primary PD parameter ANC is an accepted surrogate marker and can be related to patient outcome to the extent that demonstration of a similar effect on the PD marker will ensure a similar effect on the clinical outcome (Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: nonclinical and clinical issues, EMEA/CHMP/BMWP/42832/2005 Rev 1).

The 6 mg dose of pegfilgrastim used in this study (ie the approved product dosage) is in the ascending part of the dose-response profile for AUC and C_{max},[15,6] and is therefore considered to be sufficiently

sensitive for assessment of PK. In order to account for the expected high variability of the relevant PK parameters,[6] the study methodology was based on a two-stage design, planning for a sample size recalculation after completion of Stage 1 and potential sample size adjustment for Stage 2.

For this study, the general principles for demonstration of bioequivalence were applied. Thus, the equivalence margins as used in standard clinical bioequivalence studies, that is, 80.0%-125.0%, were considered appropriate for the PK and the PD parameters (Guideline on the investigation of bioequivalence CPMP/EWP/QWP/1401/98 Rev.1/Corr).[16] For the PD parameter, 95% CIs were used. Finally, the crossover design helped to minimize variability of the PK and PD parameters.

For all primary PK endpoints ($AUC_{0\text{-last}}$ and C_{max}) and the PD endpoint ($AUEC_{0\text{-last}}$ of ANC), biosimilarity of Pelmeg and Neulasta was shown. Of note, PD comparability was also demonstrated when applying a tighter acceptance interval of 90.0%-111.0% (as suggested by the Draft EMA Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor, EMEA/CHMP/BMWP/31329/2005 Rev 1, July 2018).[17] The variability of PK parameters was high, as previously suggested by a study in the literature.[6] The safety profile of Pelmeg was characterized by AEs that are known adverse drug reactions of Neulasta, mainly musculoskeletal disorders and headache. Thereby, the frequencies and pattern of AEs was similar between Pelmeg and Neulasta, and in line with the product information for Neulasta. Drug-related hypoglycemia was reported in around 20% of subjects after administration of Pelmeg and Neulasta. Of note, all events of hypoglycemia were transient, asymptomatic and did not require medical intervention. A relatively high frequency of hypoglycemia was seen with both treatments due to the stringent reporting approach for laboratory AEs in this study and is not considered of clinical relevance. No clinically relevant differences between Pelmeg and Neulasta have been reported with respect to clinical laboratory parameters, vital signs, and cardiovascular safety.

Anti-drug antibodies directed against the PEG moiety were seen in 34 subjects (balanced between treatments). These signals were of relatively low magnitude and were not associated with clinical signs or symptoms. No filgrastim-reactive ADAs were detected in any subject receiving Pelmeg or Neulasta. This is in line with postmarketing experience for both pegfilgrastim and filgrastim, which has demonstrated an absence of clinically impactful immunogenicity associated with the use of either product, even in fully immune competent populations. The literature reports the results from a prospective 5-year study of 6768 peripheral blood stem cell donors who were treated with G-CSF and 2726 bone marrow donors who were not treated with G-CSF.[18] The results of that study showed that peripheral blood stem cell donors were not at increased risk for developing an autoimmune disease when compared to bone marrow donors. In addition, the US FDA has stated that they are unaware of reports of neutralizing antibodies to G-CSF products, concluding that the literature indicates that G-CSF products are low risk for causing ADA-related severe adverse effects (FDA, Transcript of FDA Adcom for Zarxio.[19]

The safety data set for Pelmeg was reviewed in detail for AEs that could potentially be immune mediated, with a particular emphasis on hypersensitivity reactions. There were no AEs classified as hypersensitivity or drug hypersensitivity in subjects treated with either Pelmeg or Neulasta, and local tolerability was good. The results from this pivotal PK/PD study supported the initiation of a second clinical study with Pelmeg (Study B12019-102, EudraCT No.: 2015-005022-19), which aimed to further investigate the immunogenicity and PD comparability after administration of Pelmeg and Neulasta to healthy subjects. The results from this study are reported separately.

5 | CONCLUSION

This comparative PK/PD study in healthy subjects has demonstrated biosimilarity between Pelmeg and Neulasta for PK and PD at the clinical dose of 6 mg. No clinically meaningful differences in the safety or immunogenicity profiles were observed.

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DISCLOSURE

This study was funded by Cinfa Biotech, now part of the Mundipharma network of independent associated companies. KR, HW, and RJ are employees of Cinfa Biotech, now part of the Mundipharma network of independent associated companies. At the time of the study, BG was an employee of Cinfa Biotech. JH is an employee of Staburo GmbH. DL is an employee of the University of Lucerne.

AUTHOR CONTRIBUTIONS

RJ, KR, HW, DL, JH, and BG contributed to the conception and design of the study. DL and JH contributed to the statistical analysis of the data. All authors contributed to the interpretation of the data. All authors contributed to drafting of the manuscript, revised the manuscript critically for intellectual content, and approved the final submitted version. ® Pelmeg is a registered trademark of Cinfa Biotech, SL (a member of the Mundipharma network of independent associated companies). ® Neulasta is a registered trade mark of Amgen.

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