30 January 2025
EMA/57953/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Dyrupeg

International non-proprietary name: pegfilgrastim

Procedure No. EMEA/H/C/006407/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

µg	microgram
ATC	Anatomical Therapeutic Chemical
ATM	Atmosphere
AU	Absorbance Unit
bp	Base Pairs
CA	Capto Adhere
CD	Circular Dichroism
CE	Capillary Electrophoresis
CEX	Cation Exchange
CEX-HPLC	Cation Exchange-High Performance Liquid Chromatography
CI	Critical Intermediate
	Confidence Interval
icIEF	Imaged Capillary Isoelectric Focusing
CPP	Critical process parameters
CQA	Critical Quality Attributes
CT	Clinical Trials
DF	Diafiltration
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ELS	Evaporative Light Scattering
ESI	Electrospray Ionization
EU	Endotoxin Units
	European Union
FF	Fast Flow
G-CSF	Granulocyte Colony Stimulating Factor
GMP	Good Manufacturing Practice
HMW	High molecular weight
ICH	International Conference on Harmonisation
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IFN	Interferon
IMP	Investigational medicinal product
ISO	International Organization for Standardization
LC	Liquid Chromatography
LC / MS	Liquid Chromatography / mass Spectrometry
LMW	low molecular weight
LOD	Limit of Detection
LOQ	Limit of Quantitation
MAA	Marketing Authorization Application
CSF	Colony Stimulating Factor
mM	millimolar

mol	mole
mPEG-PAL	Monomethoxy polyethylene glycol propionaldehyde
MS	Mass Spectrophotometry
NA	Not Applicable
nm	Nanometers
NMT	No More Than
OP	Operational Parameter
Pa	Pascals
PAR	Proven Acceptance Ranges
PEG	Polyethylene glycol
Pegfilgrastim	Pegylated Filgrastim
PFS	Pre-Filled Syringe
pg	picogram
Ph. Eur.	European Pharmacopoeia
pI	Isoelectric Point
PP	Performance Parameter
ppb	parts per billion
ppm	Parts Per Million
PPQ	Process Performance Qualification
rHu G-CSF	Recombinant Human Granulocyte Colony Stimulating Factor
RMP	Reference Medicinal Product
	Risk management plan
RP	Reference Product
RP-HPLC	Reverse Phase High Performance Liquid Chromatography
SC	Subcutaneous
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEC-HPLC	Size Exclusion High Performance Liquid Chromatography
SE-HPLC	Size Exclusion High Performance Liquid Chromatography
SmPC	Summary of Product Characteristics
Sv	Sedimentation Velocity
Tm	Transition Midpoint
TSE	Transmissible Spongiform Encephalopathy
UF	Ultrafiltration
UPLC	Ultra Performance Liquid Chromatography
WCB	Working Cell Bank
WHO	World Health Organization

Not all abbreviations may be used.

1. Background information on the procedure

1.1. Submission of the dossier

The applicant CuraTeQ Biologics s.r.o. submitted on 31 January 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Dyrupeg, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication:

Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukemia and myelodysplastic syndromes).

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 10(4) of Directive 2001/83/EC – relating to applications for a biosimilar medicinal product.

The application submitted is composed of administrative information, complete quality data, appropriate non-clinical and clinical data for a similar biological medicinal product.

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force for not less than 10 years in the EEA:

- Product name, strength, pharmaceutical form: Neulasta, 6 mg, Solution for injection in pre-filled syringe
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22-08-2002
- Marketing authorisation granted by:
  - European Union
- Marketing authorisation number: EU/1/02/227/004

Medicinal product authorised in the Union/Members State where the application is made or European reference medicinal product:

- Product name, strength, pharmaceutical form: Neulasta, 6 mg, Solution for injection in pre-filled syringe
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22-08-2002
- Marketing authorisation granted by:
  - European Union
- Marketing authorisation number: EU/1/02/227/004

Medicinal product which is or has been authorised in accordance with Union provisions in force and to which bioequivalence has been demonstrated by appropriate bioavailability studies:

- Product name, strength, pharmaceutical form: Neulasta, 6 mg, Solution for injection in pre-filled syringe

- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22-08-2002
- Marketing authorisation granted by:
  - European Union
  - (Union) Marketing authorisation number(s): EU/1/02/227/004

1.3. Information on paediatric requirements

Not applicable.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Scientific advice

The applicant received the following scientific advice on the development relevant for the indication subject to the present application:

Date	Reference
17 October 2019	EMA/CHMP/SAWP/546617/2019

The scientific advice pertained to the following quality, non-clinical, and clinical aspects:

- The classification and the quality control measures proposed for the intermediates to support a MAA; the control strategy for monitoring the in-process impurities and excipient to support the release of commercial batches; the proposed storage conditions of the drug substance; the approach for qualifying an in-house batch of the drug substance; the proposed approach to establish the analytical similarity of BP14 with Neulasta-EU to support a MAA.
- The proposed strategy to establish the non-clinical comparability of BP14 with Neulasta-EU to support a MAA.
- The proposed PK, PD, safety and immunogenicity pivotal clinical study to establish the clinical similarity of BP14 and Neulasta-EU and support a MAA; the proposed design aspects of the pivotal study in particular, the blinding strategy, the choice of dose, of PK sampling times, the proposed endpoints and statistical hypotheses, the approach for safety analysis and immunogenicity assessment, and the approach to adjusting PK results based on protein content.

1.6. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Tomas Radimersky Co-Rapporteur: Fátima Ventura

The application was received by the EMA on	31 January 2024
The procedure started on	29 February 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	21 May 2024
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	07 June 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	30 May 2024
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	27 June 2024
The applicant submitted the responses to the CHMP consolidated List of Questions on	13 September 2024
The following GMP inspection was requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GMP inspection at one manufacturing site in India was conducted between 8 April 2024 – 12 April 2024. The outcome of the inspection carried out was issued on	12 November 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	23 October 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	31 October 2024
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	7 November 2024
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	14 November 2024
The applicant submitted the responses to the CHMP List of Outstanding Issues on	20 December 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues	16 January 2025

to all CHMP and PRAC members on	
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	23 January 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Dyrupeg on	30 January 2025

2. Scientific discussion

2.1. About the product

Dyrupeg (also referred as BP14) is a proposed biosimilar to the reference medicinal product Neulasta, marketed by Amgen in Europe, Canada, and the USA. BP14 drug product (DP) is a covalent conjugate of the recombinant methionyl human granulocyte colony-stimulating factor (r-met-HuGCSF) and monomethoxy polyethylene glycol (m-PEG). Recombinant human GCSF is obtained via the microbial fermentation of *Escherichia coli* (*E. coli*) strain transfected with a genetically engineered plasmid containing the gene for the expression of human GCSF.

Pegfilgrastim is classified under the pharmacotherapeutic group immunostimulants, colony-stimulating factor; ATC Code: L03AA13.

The proposed indication for Dyrupeg is for reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (except chronic myeloid leukaemia and myelodysplastic syndromes).

2.2. Type of application and aspects on development

BP14 development program was designed to meet the recommendations in the following European Medicines Agency guidance on biosimilars:

- Guideline on similar biological medicinal products (CHMP/437/04 Rev 1; 2014)
- Biosimilar medicinal products containing recombinant granulocyte-colony stimulating factor (Annex to the guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues) (EMEA/CHMP/BMWP/31329/2005; 2006)
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev 1; 2014)
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (EMEA/CHMP/BWP/49348/2005; 2006)

2.3. Quality aspects

2.3.1. Introduction

The finished product is presented as solution for injection in a pre-filled syringe containing 6 mg of pegfilgrastim as active substance in 0.6 mL of solution for injection. The finished product is given in total (total use) in a single administration.

Other ingredients are sodium acetate, sorbitol (E420), polysorbate 20 (E432) and water for injection.

The product is available in pre-filled syringe (Type I glass), with a rubber plunger stopper, a plunger rod, a stainless-steel injection needle and a rubber needle cap with an automatic needle safety guard.

Although this dossier is not considered a Quality by Design application, certain elements of an enhanced approached were applied. Dyrupeg is referred to in the dossier as BP14.

2.3.2. Active substance

2.3.2.1. General information

The medicinal product is developed as a proposed biosimilar to the reference medicinal product Neulasta authorised by Amgen Europe B.V.

The international non-proprietary name (INN) of the active substance is pegfilgrastim. The active substance is a genetically engineered recombinant human granulocyte colony-stimulating factor (rHu-met-G-CSF) conjugated to monomethoxy polyethylene glycol (PEG). Pegfilgrastim is the long-acting form of filgrastim. The polyethylene glycol molecule covalently bound to filgrastim results in increased persistence *in vivo* compared to that of filgrastim due to decreased renal clearance. Filgrastim is an endogenous granulocyte-colony stimulating factor (GCSF) with selectivity for the neutrophil lineage. It is produced by monocytes, fibroblasts, and endothelial cells. Filgrastim exerts its therapeutic effects by regulating the production of neutrophils within the bone marrow, affecting the neutrophil progenitor cell proliferation and differentiation, carrying out selected end-cell functions (including enhanced phagocytic ability, priming of cellular metabolism associated with respiratory burst, antibody-dependent phagocytosis, and the increased expression of some of the cell surface antigens.

Filgrastim is an active substance (critical) intermediate produced in genetically modified bacteria *E. coli* as a single, non-glycosylated, polypeptide chain containing 175 amino acids and a molecular mass of 18,800 Da. Filgrastim has an α-helical structure with two intra-molecular disulfide bonds formed between cysteine residues and a single free cysteine. Filgrastim (rHu-met-G-CSF) is identical to natural human G-CSF, except for the presence of an additional methionine at the N-terminal end and the absence of glycosylation. Pegylated G-CSF is manufactured by conjugating an approximately 20,000 Da mPEG-ALD to the N-terminal methionine of G-CSF, resulting in a total molecular mass of 38,800 Da. The structure for the active substance is shown in Figure 1 below.

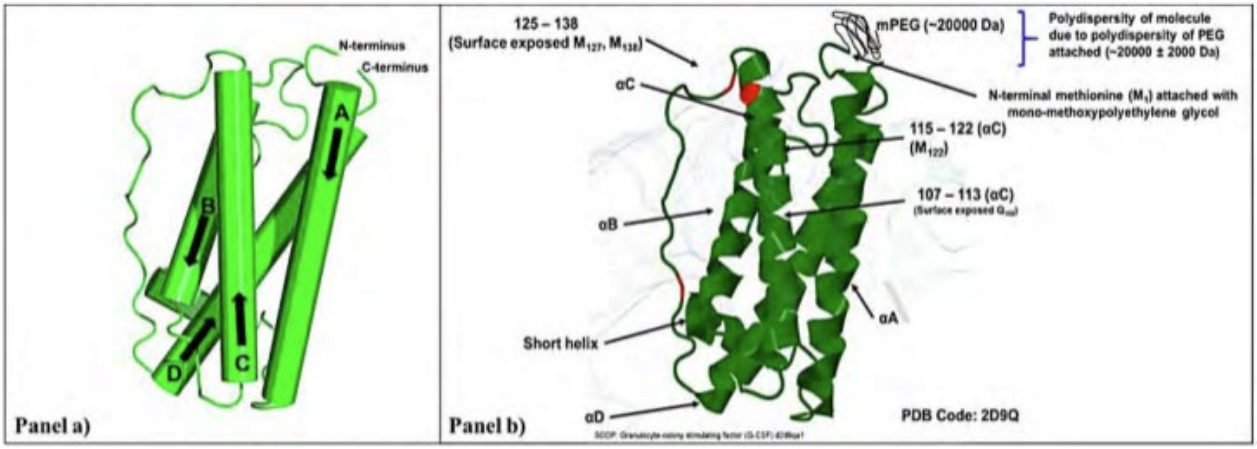


Figure 1: Active substance structure

2.3.2.2. Manufacture, characterisation and process controls

Manufacturers

The active substance is manufactured at CuraTeQ Biologics Private Ltd, Survey No 77 & 78, Indrakaran Village (Telangana), India. A major objection was initially raised as adequate proof of GMP was not provided.

During the procedure, a valid proof of GMP compliance has been provided for all the manufacturing sites, resolving the major objection.

Description of manufacturing process and process controls

The active substance (AS) manufacturing process has been adequately described. The filgrastim active substance intermediate is expressed in genetically modified *E. coli* cells The ranges of critical process parameters and the routine in-process controls along with acceptance criteria, including controls for microbial purity and endotoxin, are described for each step. The operational steps include seed generation, inoculum expansion, fermentation, harvest, recovery, purification and freezing of the active substance intermediate, filgrastim.

The upstream process starts with fed-batch fermentation of the working cell bank (WCB), followed by the harvest of the produced inclusion bodies (IBs). The IBs contain the active substance, which are manipulated (centrifugation, cell disruption and washing) until freezing at -20°C for storage.

The downstream part includes solubilization and refolding of IBs, chromatographic purification of filgrastim, PEGylation of filgrastim, bulk active substance preparation, filtration and storage.

Sanitisation of chromatography columns are described for each chromatographic step. Prior to product loading, the columns are tested for microbiological controls (bioburden, BET). Lifetime of the chromatography columns has been described in resin reusability studies and found acceptable.

During routine manufacturing, filgrastim critical intermediates and the active substance are not reprocessed or re-worked.

The active substance is filled and stored in PETG (polyethylene terephthalate co-polyester – glycol modified) bottles with HDPE (high-density polyethylene) closures. Bottles and closures are certified radiation-sterilised,

non-pyrogenic, non-cytotoxic, and comply with USP Class VI guidelines. A technical diagram of the PETG bottle and product certificate is included. Bottles are released based on in-house specifications.

Extractable/leachable studies have been performed for final active substance filter and suitability of storage container. Leachable samples were evaluated. For stability studies, the active substance is stored in PETG bottles with HDPE closure, representing the primary packaging container. The suitability of container closure system and active substance is confirmed by stability studies.

Control of materials

Source, history and generation of the plasmid clone is adequately described.

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. No human or animal derived materials are used in the active substance manufacturing process and acceptable documents have been provided for raw materials of biological origin used in the establishment of cell substrate. Media composition is provided, and their control is considered adequate.

A two tiered cell banking system is used and sufficient information is provided regarding testing of master cell bank (MCB) and working cell banks (WCB) and release of future WCBs. MCB and WCB are stored below -80 °C in an ultra-low temperature freezer, with acceptable frequency for viability testing and plasmid retention testing.

End of production cell banks (EPCB) have been tested for identity by phenotypic and genotypic characteristics, purity, viability, and plasmid retention of recombinant construct. Data for the gene copy number of EPCB, WCB and MCB were provided together with defined acceptance criteria for gene copy number. Genetic stability has been demonstrated for cells at and beyond the limit of cell age.

Control of critical steps and intermediates

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout the active substance manufacturing process is given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests. Actions taken if limits are exceeded are specified.

The control strategy is developed according to ICH guideline, applying the risk assessment approach: failure modes and effects analysis (FMEA) to identify potential operational parameters (critical process parameters (CPPs), key process parameters (KPPs), non-CPPs) and performance parameters (in-process control (IPCs), in-process test (IPTs)). Normal operating ranges (NOR) and proven acceptable ranges (PAR) are provided. Acceptance limits and in-process specification are appropriate, with defined actions if results are outside IPCSs and NORs.

Proposed hold time and storage conditions for upstream and downstream intermediates, process buffers, sterilised media and feed components are presented. Analytical procedures and qualification used during BP14 manufacturing process are described and found adequate.

Documentation regarding m-PEG (mPEG-pALD-20K) was provided and found adequate. mPEG-pALD-20K is of synthetic origin. Manufacture, raw materials, control of critical steps and intermediates, reference standard, release and stability testing were described in sufficient detail.

Process validation

The active substance manufacturing process has been validated adequately. Consistency in production has been shown on three consecutive full scale commercial batches. All acceptance criteria for the critical operational parameters and likewise acceptance criteria for the in-process tests are fulfilled demonstrating that the purification process consistently produces the active substance of reproducible quality that complies with the predetermined specification and in-process acceptance criteria.

There was no batch failure during validation and no excursion from NORs. The process validation shows consistent quality of the active substance.

A major objection raised during the procedure was addressed upon the submission of a valid GMP certificate following the GMP re-inspection of CuraTeQ Biologics Private Limited site in April 2024.

As part of the continued process verification (CPV) protocol, the applicant plans to evaluate process variability, repeatability and reproducibility from at least 30 batches to establish statistically derived control limits. The applicant is reminded that future changes to registered process parameters should be handled according to the variation guideline (2013/C 223/01, Guidelines on the details of the various categories of variations).

Data to support the hold time studies for process intermediates and buffers used during the routine manufacturing is provided. Hold time studies were performed using in-process samples from at-scale manufacturing of the active substance. Batch release data used in the hold time studies and details on resin reusability studies are provided and found acceptable. The samples were collected in containers representative of the proposed commercial batch container closure.

Manufacturing process development

The fermentation process was initially developed on laboratory scales. After development, several changes were introduced which included some changes from Process I to Process II, and the process was scaled up to commercial scale.

Manufacture of development, clinical and PPQ BP14 active substance batches has been performed using MCB as a starting material. For commercial active substance manufacture, WCB will be used. Comparability of the active substance and the finished product manufactured using either MCB or WCB is demonstrated based on characterisation data (3+3 active substance/finished product batches) for binding kinetics and secondary and tertiary structure, and based on process parameters, performance parameters for 3+3 upstream batches, and final finished product results.

FMEA risk assessment was performed for all active substance manufacturing process operational parameters to recognize potential CPPs. Risk assessment reports for upstream and downstream processes including all parameters are provided. Operational parameters were categorised as potential CPPs (pCPP) and non-CPPs based on a decision rule considering individual severity, occurrence and detectability scores. The classifications are adequately justified. Based on the FMEA analysis, the pCPPs were further evaluated in the characterisation study and found acceptable.

Process characterisation (PC) studies were performed via a univariate and/or multivariate approach using appropriately qualified scale down models (SDM). Quality data and statistical analysis of the results for the performed upstream and downstream PC studies are provided. Attributes evaluated in the process characterisation studies include purity by RP-HPLC and SEC-HPLC, yield, recovery, host cell protein (HCP) and host cell DNA (HCD). Purity by RP-HPLC included monitoring of product variants. Quality data for HCP and

Log reduction for CEX-I and CEX-II OP scale down model were provided. After the CEX-I and MMC chromatography steps, the HCP and HCD content in the MMC purification step samples is below the quantification limit, indicating significant clearance of HCP and HCD.

Characterisation

The characterization was performed for three at-scale, two clinical process II and three clinical process I active substance batches in comparison with the six batches of Neulasta reference product.

The active substance was characterized in terms of quantity, primary structure and identity. Molecular mass was analysed by ESI-MS. Results are given as ranges, which is attributed to the variability of molecular mass due to the Peg moiety. Peptide mass fingerprint was performed via trypsin peptide digestion. N-terminal sequence-RP-UPLC, N-terminal Truncated Fragments- RP-IPLC, Non-canonical Amino Acids-RP-IPLC, Site of PEGylation-RP-HPLC, N-terminal Truncated Fragments with LC-MS. Next, Di-Sulphide Bond-Non-Reducing RP-UPLC, Identity-SDS-PAGE, pI-icIEF, Immunoblotting was performed. Identity by SDS-PAGE and by icIEF is shown in sufficient detail. Higher order structure is performed by Far-UV CD, Near UV CD, FTIR, DSC, UV-Vis Spectra, Free cysteine by Ellman's Assay, Absolute Molar Mass by SEC-MALS. Level of higher order structure characterization is sufficient. Further, physicochemical properties, including size and charged variants, and variants generated by PEGylation process are investigated. Methods used are SE-HPLC and CE-SDS (size variants), CEX-HPLC (charge), RP-HPLC (oxidized impurities, reduced forms, deamidated impurities, and free filgrastim impurities). Provided results show minor differences between oxidation profile of Neulasta RMP and the applied medicinal product.

Functional activity was determined by Receptor Binding Kinetic Assay and by In-vitro Bioassay. The characterized batches are comparable. Brief description of the analytical methods used in characterization was provided.

2.3.2.3. Specification

Specifications include tests for appearance, protein concentration, pH, osmolality, identity by peptide mapping, potency by cell-based assay, purity by size (SEC and non-reduced CE), structural homogeneity (RP HPLC), charge heterogeneity (CEX-HPLC), safety - bioburden and endotoxin, impurity – HCP and host cell DNA (HCD). For HCP and HCD, the test will be performed in at the UF/DF (TFF II) stage and same shall be considered for active substance release. In case that results do not meet the acceptance criteria at UF/DF (TFF II) stage, the batch will be rejected. The HCP acceptance criterion has been tightened based on the performance of the developed process-specific HCP kit.

The limits of bacterial endotoxin and bioburden are acceptable, in line with compendial methods. Specifications for attributes such as physical appearance is based on the regulatory requirement and generated from historical batches. The specifications and acceptance criteria for release and stability (end of shelf-life) tests such as pH, osmolality, protein concentration, purity (SE-HPLC, RP-HPLC, CEX-HPLC and CE-SDS), and potency were demonstrated as clinically justified. For potency, process and analytical variation is considered for setting the specification.

Impurities were identified and characterized. Characterization of the product-related variant is presented and found acceptable. Charged variants are separated by CEX. Characterization by CEX was performed on PEGylation samples that were chosen for its higher level of impurities. Isolated impurity peaks were subjected to LC-MS analysis to identify the peak identities. Impurities based on hydrophobicity were separated by RP-HPLC. The results from spiked, controlled and stressed samples were evaluated using

potency and binding assays. It was concluded from the studies, that mono-, di- or tri-oxidized impurities are more than the specification limit, but have no significant impact on the functional activity of pegfilgrastim.

Deamidated impurities were characterized via Glu-C digest followed by peptide mapping LC-MS-MS. Individual deamidated fragments are characterized, including the potency testing. It was shown that deamidated samples have significantly reduced biological activity. Size variants characterization was performed using by SEC-MALS and SE-HPLC. HMW species were further analysed for the potency and by the CEX-HPLC. Disulphide bonds were sufficiently characterized using reduced spiked sample and analysed by RP-HPLC. Complete list acquired impurities is presented, confirming identity of the impurities. The characterization of product related impurities is considered sufficient.

Process related impurities are identified as follows: host cell protein (HCP), host cell DNA (HCD), antifoam, IPTD, free PEG, cyanide, bioburden and bacterial endotoxins. Relevant clearance data are provided and were found acceptable.

Polysorbate 20 is monitored as part of regular in-process testing (IPT for bulk active substance before active substance preparation) and is also included in finished product specification.

Analytical methods

The analytical methods used for release and stability testing of the active substance were described and found acceptable. Compendial methods were referenced (appearance, osmolality, and pH). The in-house analytical procedures used for active substance testing were validated as per ICH Q2 (R1) guidelines. Approved protocols and reports for each analytical method were provided. Reference was made to compendial methods, and description of the in-house methods is satisfactory.

Extinction coefficient used for determination of protein was defined as mean of experimentally gained results for extinction coefficients for active substance, finished product and RMP. BET method was validated in line with Ph. Eur.

HCD and HCP testing methods are described. The applicant has performed a process-specific HCP validation based on the antibody coverage boundaries. For the active substance, the HCP coverage is 100.00%. The applicant is recommended to tighten the acceptance criterion of NMT 50 ng/mg HPC, given the maximal amount of HCP found in PPQ batches is 5 ng/ml and no results of HCP in clinical studies were provided (quality recommendation 2).

The summary of validation procedures does not include range parameter for the relative potency by in-vitro cell proliferation assay. But it has been acknowledged that the approved protocol and the relevant report describe the range calculation and provide the result.

The applicant provided detailed plans on their efforts to develop and implement an endotoxin assay based on recombinant Factor C. It is recommended to proceed with the provided plan to develop and implement an endotoxin assay based on recombinant Factor C (quality recommendation 1).

Batch analysis

Batch analysis data on 30 batches manufactured by Process I or Process II of the active substance were provided.

Six process I batches that are listed (CTDS 1-7) are used for process consistency evaluation, finished product analytical similarity and stability. Twelve process II batches were used for clinical campaign, stability, analytical similarity and photostability. Three commercial process batches are used for PPQ campaign,

analytical similarity and stability. Nine commercial post-PPQ batches are used in-house studies, MCB/WCB comparability studies and hold time studies. Batch results are presented and are consistent. Additionally, 15 commercial batches of mPEG (NOF Corporation) were analysed.

All batch analysis results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

Details on reference standards and materials used in development and routine manufacturing were provided and found acceptable. The primary reference standard was adequately characterized in terms of identity, purity, higher order structure, stability, protein related variants and impurities and biological potency. Relative potency of the primary reference standard was calibrated against the active substance, WHO international standard NIBSC (12/188) and Neulasta EU batch. Overall characterization strategy is sufficiently robust and acceptable.

2.3.2.4. Stability

The stability results indicate that the active substance is stable and justify the proposed shelf life and storage conditions in the proposed container. Overall, the proposed shelf-life of 24 months when stored at proposed long-term conditions (-20 ± 5°C) protected from light is considered acceptable.

Stability studies are carried out in line with ICH Q5C guidelines. For stability studies, the active substance is stored in representative containers of the primary packaging (PETG bottles with HDPE closure). Stability study protocols for long-term (3 clinical and 3 PPQ active substance batches, -20 ± 5°C), accelerated (1 clinical active substance batch 5 ± 3°C, at 40 ºC / 75% RH) and stress stability studies (1 clinical active substance batch, 25 ± 2ºC, 60 ± 5% RH) and photostability study were provided, control timepoints, analytical tests and acceptance criteria for individual quality attributes were appropriately defined.

Tested attributes and acceptance criteria for formal long-term stability study are aligned with the active substance specifications. Additional data is generated with three process I active substance batches up to 24 months of real time data with representative clinical and PPQ batches. Further PPQ batches stability data (up to 36 months) and prolonging the shelf-life should be submitted as a variation.

The photostability study (3 clinical batches) under light stress conditions at 25 °C in line with ICH guideline Q1B, showed minor increase in %HMW and %LMW, slight decrease in main peak purity tested by RP-HPLC and significant increase in charged variants tested by IEX-HPLC (specifically acidic variant species) compared to control samples.

The applicant committed to place at least one commercial batch on real-time stability annually and also to inform the competent authorities immediately in case of out-of-specification results.

2.3.3. Finished medicinal product

2.3.3.1. Description of the product and pharmaceutical development

Dyrupeg finished product (FP) is a clear and colourless, preservative free, visible particles free, sterile solution for subcutaneous solution for injection developed as a similar biological medical product (biosimilar) to the reference medicinal product Neulasta.

The finished product formulation is identical to reference medicinal product Neulasta in terms of the active substance and excipients.

The suitability of the formulation is supported by real-time, accelerated and stress stability data. In addition, the applicant has performed an excipients range establishment study varying excipient concentrations under different conditions. Based on the results, the finished product in PFS with studied excipient concentration was found to be the most stable formulation.

The manufacturing process is standard for this type of product and consists of thawing active substance, preparation and filtration of formulation buffer, preparation of formulated bulk, bioburden reduction filtration, sterile filtration, filling, stoppering, visual inspection and storage of naked PFS, labelling, plunger rod insertion, needle safety guard assembly and secondary packaging. Clinical and PPQ batches are manufactured. No substantial changes were made to the manufacturing process during transition from clinical to PPQ's proposed commercial process.

Assessment of parameters was performed using FMEA (failure mode effect analysis) tool to define potential critical process parameters (pCPPs) impacting on quality attributes, based on process knowledge. pCPPs were further classified into CPPs and KPPs based on data from process characterization studies, providing justification. Additional characterisation data has been provided for active substance thawing time. Set points and NORs for the process parameters are defined based on the characterization data. The NORs appear to be set equal (but not greater) to the PARs defined during the process characterization studies. If any non-compliance/failures occur, then it should be handled through deviation management. The finished product filtration process parameters were validated using filter validation studies.

The product is available in pre-filled syringes (PFS). Each PFS contains 6 mg of pegfilgrastim in 0.6 mL solution for injection. The finished product does not include overage.

The components of the primary packaging material have been properly described and comply with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. The finished product primary packaging materials consist of 1.0 mL USP Type I glass pre-fillable syringe with an elastomeric plunger stopper FluroTec fluoropolymer barrier film coating, affixed with a staked needle capped with an elastomeric needle shield (inner cap) and a polypropylene rigid needle shield (outer cap). After filling with the medicinal product and stoppering, the prefilled syringes are assembled with a plunger rod and needle safety guard (ready-to-use).

Extractables and leachables studies were performed using appropriate analytical methods. The choice of the solvents is justified. Seven known extractable components and two unknown extractable components were identified in extractable study. PFS with placebo solution was then analysed for leachable at real time and accelerated conditions. No compounds were detected in the leachable study until the time point of 36 months.

Device functionality has been assessed with multiple tests including activation, gliding force, break loose force and triggering test with multiple finished product batches of different ages. The data presented demonstrate functionality of PFS up to 36 months.

All excipients are well known pharmaceutical ingredients, and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation.

2.3.3.2. Manufacture of the product and process controls

The manufacturing process has been validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate.

Name, address, and responsibilities of the manufacturers involved in the manufacture, in-process and quality control and stability testing of FP are listed. Valid GMP certificate for CuraTeQ has been submitted during the procedure.

Batch formula, including list of all components, was provided per one PFS and per a theoretical batch size which is covered by performed media fill. One FP batch is manufactured from a single AS batch, with acceptable batch numbering system.

The finished product is manufactured by a standard process for this type of product, covering preparation and filtration of formulation buffer, thawing of AS; preparation of formulated bulk, formulated bulk first and second filtration, filling and stoppering; visual inspection, storage and labelling of PFS; plunger rod fixation; needle guard/safety device assembly; and carton packaging. The operational parameters and performance parameters along with a description of each manufacturing step are provided. All filled PFSs are 100% visually inspected by qualified personnel and defective PFS are rejected. The PFS is then stored at 2-8°C in the cold rooms until labelling and secondary packaging.

Performance controls are defined as IPCs and IPTs. All limits and acceptance criteria were defined based on trending values observed from clinical and PPQ batches.

Media fill runs were performed to qualify the aseptic filling process. Results from three initial three consecutive batches and one latest periodic verification run were provided.

Data on sterile filter validation are provided and include studies for filter extractables (including patient safety evaluation of extractables), bacterial retention and bacterial challenge, filter adsorption and bubble point ratio determination. 0.22 µm filters are used during the FP manufacturing process.

The performance qualification of PFS filling machine was carried out with fill volume verification and container closure integrity. During fill volume verification, all the speeds set for commercial manufacturing process and required deliverable volume were verified.

The Notified Body Opinion was submitted, and it confirms the conformity of the device part with all relevant GSPRs set out in Annex I of Regulation (EU) 2017/745.

Process validation was performed on three consecutive PPQ batches. The finished product release specifications, process parameters (CPP, KPP) and performance parameters (IPC, IPT) met the predefined acceptance criteria, without reported deviations. The same process and equipment as for the batches intended for the commercial manufacturing was used at the intended manufacturing facility. Process parameters (CPPs/KPPs) are defined with their operating ranges. The manufacturing process duration for the finished product PPQ batches was provided, which is a continuous manufacturing (no hold times between manufacturing steps). Additionally thawing parameters of AS have been characterized and found acceptable.

An objection was raised in regards of GMP compliance of the performed AS and FP process validation as it was not clear that the PPQ manufacturing process in 2021 was not affected by the critical deficiencies observed in the March 2023 on-site GMP inspection. The GMP re-inspection in April 2024 had zero observations related to the PPQ batch data and the facility related observations were adequately addressed using a comprehensive CAPA approach and verified by the inspectors. GMP certificate has been submitted.

Shipping validation conducted with three finished product batches showed that shipping conditions do not adversely impact product quality, device functionality or integrity of packaging components.

2.3.3.3. Product specification

The release specifications for the finished product include tests for appearance, general attributes (protein concentration, pH, osmolality, visible particles, sub-visible particles, extractable volume), identity (peptide mapping by RP-HPLC), potency (cell based assay), purity (size heterogeneity by Size Exclusion Chromatography, structural heterogeneity by Reversed Phase Chromatography, size heterogeneity by non-reduced Capillary Electrophoresis and Charge Heterogeneity by Cation exchange chromatography), microbial purity (sterility, BET by Gel Clot Method, Container closure integrity by Dye Ingress), excipients (Polysorbate 20 estimation by HPLC) and PFS Functionality testing (break loose force, glide force, activation force of needle guard and triggering test) were introduced. References to in-house methods or Ph. Eur. are included.

The analytical methods used have been adequately described. Analytical procedures used the FP release and stability testing are either in-house, already described in the section S.4.2 or Ph. Eur. compendial reference was provided. It is clarified that for the BET (Ph.Eur. 2.6.14, USP <85>) the gel clot method A is used. In addition, the Low Endotoxin Recovery (LER) study has been conducted and no interference of polysorbate on the endotoxin recovery was detected.

Justification of FP specifications from PPQ and post PPQ batches is included. The acceptance criteria are justified based on clinical batches data and will be reviewed as part of continuous process verification.

Container closure integrity testing (CCIT) verification has been performed. Polysorbate 20 estimation by HPLC with ELS detector method is appropriately validated.

The method validation at the CuraTeQ site (India) is not completed, with pending method transfer of Size Heterogeneity by Capillary Electrophoresis (non-reduced) due to the differences in the CE-SDS equipment used at Vela Labs GmbH. The applicant is recommended to complete method transfer for all non-compendial analytical method before releasing FP on EU market (quality recommendation 3).

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on three BP14 FP batches using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

Batch data are presented for eight clinical batches, three PPQ batches and three batches for transport validation All acceptance criteria were met and no significant changes between batches were observed in any

of the quality attributes. The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

Reference standards are described and characterised. The proposed procedures for re-qualification and qualification of new reference standards are acceptable.

2.3.3.4. Stability of the product

The proposed a shelf-life for finished product of 36 months when stored at 5 ± 3°C protected from light is supported by data. This claim is based on the provided data of three clinical batches placed under long-term (5 ± 3°C), accelerated (25 ± 2°C / 60 ± 5% RH) and stressed conditions (40 ± 2°C / 75 ± 5% RH) in line with ICH guidelines. In addition, stability data are provided for one pivotal batch and three PPQ batches. Stability data for the long-term real-time conditions are available. In addition, six-month stability data from accelerated conditions are available. All results long term stability were within specifications with no visible trends.

Photostability studies were conducted to determine the impact of photo stress on the finished product. Results show that when the finished product was exposed to the UV and visible light stress (0.5X and 1X ICH cycle) there was a significant difference in protein content, charge variants and purity. Results demonstrate that the FP is sensitive to light and must be stored protected from light.

Temperature excursion stability demonstrated that the finished product can be shipped at recommended storage conditions (5 ± 3°C). However, the finished product is stable when exposed to -20 ± 5°C or 25 ± 2°C for up to 72 hours. In line with the SmPC, the FP can be exposed to room temperature for a maximum of 72 hours and the FP container should be kept in the outer carton to protect BP14 FP from light.

The post-approval stability protocol and stability commitment were provided. One batch of the finished product will be placed on stability study each year.

In accordance with EU GMP guidelines, any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

2.3.3.5. Adventitious agents

No raw materials or excipients of biological origin with risk of TSE/BSE transmission are employed in the manufacture of active substance and finished product. The active substance is expressed in *E. coli* which does not support replication of mammalian viruses. The *E. coli* derived MCB was tested for the presence of bacteriophages which may replicate in *E.coli*.

2.3.3.6. Biosimilarity

Dyrupeg (in dossier also referred to as BP14) is developed as a proposed biosimilar medicinal product to the EU authorized reference medicinal product Neulasta, 6 mg, solution for injection in pre-filled syringe (Amgen Europe B.V., EU/1/02/227/004). Dyrupeg and Neulasta are formulated using the same excipients with

identical qualitative and quantitative composition. Neulasta sourced from EU market is acceptable as reference medicinal product.

The target product profile (TPP) was established based on the reference product's SmPC, labelling and the applicant's own analyses (TPP concerns indication, dosage form, strength, concentration, excipients, mechanism of action, etc.).

Based on the TPP, the applicant defines two sets of Critical Quality Attributes (CQAs) – 'obligatory' and 'risk-assessed'. The criticality assessment of quality attributes was performed with reference to ICH Guidelines Q8 (R2) and Q9, based on two factors: Impact (potential impact on efficacy, PK/PD, immunogenicity, safety) and Uncertainty. The only quality attributes subjected to risk ranking are the finished product variants (charge, size, structural variants and PEGylation variants). Some attributes were not included in the biosimilarity assessment, since they are controlled through either IPCs or specifications such as process-related impurities, composition, leachables, contaminants, and device functionality.

Depending on the calculated criticality score, the tier ranking of quality attributes is in general acceptable, and the proposed statistical evaluation strategy is considered suitable.

The selection of orthogonal methods employed is adequate for establishing an analytical similarity. The side-by-side analytical testing included methods for identity (including confirmation of PEGylation site and primary protein sequence), purity, content, product related variants and impurities (including size and charged variants), post-translation modifications, protein higher order structures and biological activity (including potency by bioassay, binding assay and immunogenicity properties by *in vitro* analysis). Most of analytical methods were in-house validated procedures used for in-process, release and stability testing of AS and/or FP.

The quantitative comparison between Dyrupeg (9 batches) and Neulasta RMP (9 batches) included analytical testing with high criticality ranking, purity and product variants. In addition, methods for extended characterization included three or six batches of Dyrupeg and Neulasta RMP, depending on pre-defined data evaluation strategy. Thirteen independent batches were included in bioassay analysis, which is considered sufficient to evaluate similarity between biosimilar and RMP.

Batches used in comparative stability studies:

Three batches of BP14 (biosimilar) and three batches of RMP (Neulasta) have been selected for each of the forced-degradation studies: high temperature, light, low pH, high pH, oxidation and mechanical stress. A total of eight Neulasta batches was used in the various force-degradation studies, supporting the batch selection based on age and availability.

Analytical similarity: results

Protein concentration: BP14 batch result is slightly above the RMP range. The result itself is not considered to preclude a positive similarity conclusion.

Primary Structure: The similarity of BP14 primary structure to that of the RMP has been determined in terms of: molecular mass, peptide mass fingerprint, N-terminal Sequence (where a BP14 critical intermediate rhGCSF (UF/FD-II) has been used for analysis), non-canonical amino acids, PEGylation site, isoelectric point, and Identity (by immunoblotting).

Intact Mass has been determined by ESI-MS and MALDI-TOF (pre- and post-PEGylaton).

The amino acid sequence is considered confirmed by the Glu-C/ RP-UPLC peptide mapping, UV-214, with a 100 % coverage and overlaying peptides.

N-Terminal Sequence by RP-UPLC (Glu C Digest, MS and MS/MS): The identity of Neulasta and BP14 N-terminal peptide sequences is confirmed.

The amounts of non-canonical amino acids (norvaline and norelucine) is determined to be highly similar between the BP14 and Neulasta.

The PEGylation site has been confirmed to be at the N-terminus (alpha-amino group of Met1). The PEGylation site has been shown to be identical for the tested and the reference products.

Isoelectric point has been found to be exactly similar in all the tested batches of the reference and proposed biosimilar products. Identity has been also confirmed by immunoblotting.

Extinction coefficient of the tested product has been found similar to the reference product using the Edelhoch method and evaluated using the 3-sigma approach. It is also independently determined via Amino Acid analysis.

Free cysteine content (Ellman Assay) is found to be similar for both tested products, although for BP14 the values are slightly more variable than for Neulasta.

Molecule heterogeneity:

The analytical similarity claim is generally supported by SEC-HPLC, CE-SDS, SDS-PAGE-nr, SV-AUC, MFI and RP-HPLC data.

It is noted that the scatter plots of the SEC-HPLC data indicate that there is a significant difference in the % HMW2 peak areas. In Neulasta, the HMW2 peak is almost absent, save for one batch, while in BP14 the HMW2 peak areas reach as high as 0.7 %, with five batches failing the 3-sigma acceptance criterion. Moreover, the total HMW is, in a number of cases, higher for BP14 than Neulasta, with one batch also failing the acceptance criteria in this regard. This is despite the fact that the tested finish product batches are by and large of later manufacturing dates. However, as the HMW2 values for both Neulasta and BP14 are frequently below the LOQ (or even below LOD) and the content of this group of impurities is rather low, no objection is raised, and the data is considered deemed to support the similarity claim.

Although the analytical ultracentrifugation data show significant variability in both % monomer and %HMW, this will not be raised as an issue given the generally low repeatability of the method itself.

MFI Evaluation: The sub-visible particle counts as determined by MFI for all particles (as well as for protein particles separately) show in general similar or lower subvisible particulate content of the test product than of the RMP and therefore favourable to the similarity claim.

Charge heterogeneity/CEX-HPLC: BP14 results evaluation only distinguishes between Main peak and Pre-peak % area. However, the peak area is invariably above 99%, which is 1–2% greater purity than that of Neulasta batches. Therefore, although the BP14 results are outside the 3-sigma window, the direction is towards higher purity and similarity claim is supported.

Secondary and tertiary structure

Far-UV CD: Both the reference and tested products display consistent secondary structures, predominantly alpha-helical, with zero percent of beta-sheet.

Near-UV CD: The profiles are highly similar and maximum wavelengths are nearly identical for all batches of the tested and the reference product

FTIR: Although the percentages of turns, beta sheets and other structures generally satisfied the established criteria, the proportion of alpha-helical secondary structure determined by FTIR is for all proposed biosimilar product batches either borderline or below the criterion. The applicant nowhere discusses the failure to meet these criteria, however, as the reported values are the result of model-based structure prediction, the slight differences are not an obstacle to biosimilarity.

DSC: The thermograms suggest that that both the reference and the tested finished product batches display similar transition behaviour and melting temperatures. The thermograms display observable "sloping" especially to the right of the transition peak which might suggest the presence of another process apart from the melting itself. However, given the consistency of the results and similarity of the thermograms between Neulasta and BP14 the data seem to support the similarity claim and no discussion is requested.

UV-Vis Spectra: Visually assessed, the UV-Vis spectra demonstrate high similarity of the reference and tested product batches.

Tertiary structure determined by intrinsic and extrinsic fluorescence supports the biosimilarity claim adequately, based on the overall shapes of the emission spectra and positions of the emission maxima.

SEC-MALS: The values of hydrodynamic radius for BP14 batches are just at the upper limit of the acceptance criterion and furthermore, the spread of the values is clearly clustered to the extremes of the acceptance range. However, the hydrodynamic radius does not corelate with molar mass in the measured sample and the values are still within the similarity acceptance range, so no objection is raised.

The position of disulfide bonds has been confirmed by Non-reduced peptide mapping LC-MS analysis.

Functional characterisation

Potency by mNSF-60 Proliferation Assay:

Neulasta (12) and BP14 (10) batches were used for potency analysis. The two products are highly similar and the BP14 potency values are well within the established acceptance criteria. Additionally, the selected batches showed similarity between the representative dose-response curves.

Note that despite the combined use of AS and FP, none of the AS batches was the source batch for a FP batch used in this analysis.

Binding Activity by GCSF-R Binding by SPR:

An SPR based receptor binding assay has been used to measure the binding kinetics and affinities of Neulasta (9) and BP14 (9) batches to evaluate their similarity, meeting the established criterion and without abnormal behaviour.

STAT3 Phosphorylation:

Endogenous levels of Signal Transducer and Activator of Transcription (STAT3) can be detected by a labelled dye sandwich FRET assay when STAT3 is phosphorylated at Tyr705. The presented data satisfy the pre-set min-max criteria for similarity. This is discussed by the applicant as expected, as it has been previously shown that STAT3 phosphorylation in response to cytokines such as IFN-γ and IL-10 treatment increased proportionally to the increasing dose, followed by decrease at higher concentrations.

Comparative stability:

Stress degradation studies involving oxidative stress, photo stress, exposure to low and high pH, and mechanical stress were performed. The analytical similarity exercise is found to be adequate in terms of the proposed strategy of testing, selection of proposed biosimilar and reference medicinal products, panel of methods, statistical evaluation, and results themselves. Differences between the reference medicinal product and proposed biosimilar are sufficiently justified.

The data from each of the stress degradation studies supports the biosimilarity claim, with the following exceptions and caveats:

- At low pH conditions, the tested product is shown to decrease slightly faster than Neulasta in % Main peak with a corresponding faster increase in %HMW. BP14 batches exhibited a higher rate of degradation than EU-approved Neulasta; however, the % relative potencies for the same samples are comparable and no new species were observed after 15 days at low pH stress conditions. This indicates that the differences in HMW percentages didn't impact the relative potencies of the two products. Given the small difference in the observed degradation rates, this is acceptable.
- Under heat and mechanical stress conditions, BP14 increases in % Basic forms faster than the reference product. The higher degradation rate caused a slight increase (~1%) in basic species, which had no impact on the potency, and no new species were observed. Based on these results, it can be concluded that BP14 is comparable to EU-approved Neulasta.
- The difference in the slope values for the %post-peak area by RP-HPLC in the photo stress study is shown by the applicant to be statistically significant ($p < 0.05$). BP14 batches exhibited a higher rate of degradation for post-peak compared to EU-approved Neulasta; however, the % relative potencies for the same samples are comparable after photo stress conditions. It is therefore accepted that the observed difference does not impact the similarity conclusion.

A summary of significance values (presumably p-values) of the determined for the comparisons of slopes of BP14 and Neulasta degradation profiles is provided in Table 1. Although the applicant identifies 21 significantly different slopes, only three of these point to faster degradation of the proposed biosimilar (%HMW in low pH, %Basic in thermal stability study, and %post-peak by RP-HPLC in photo stress study). These differences are discussed and shown not to impact potency quality and safety of the proposed biosimilar.

Table 1: Summary and conclusion on analytical similarity between BP14 and EU-approved Neulasta

Product Characteristic	Product Quality Attribute	Conclusion and Key Findings	Supports Similarity
Protein Content/Dose	Protein Concentration	BP14 batches are similar to the Neulasta® batches for protein concentration.	✓
Primary Structure	Amino acid composition	The relative molar ratios for BP14 and the Neulasta® batches are comparable.	✓
	Molecular Mass (Intact)	The primary structure (intact mass, amino acid sequence, including the N-terminal sequence and the site of	✓
			✓
			✓

Product Characteristic	Product Quality Attribute	Conclusion and Key Findings	Supports Similarity
	Peptide Mapping – UPLC-MS/MS	PEG) are similar between BP14 and Neulasta® batches.	✓
			✓
	N-Terminal Sequence (BP14 Critical Intermediate; rhGCSF)		✓
	Non-canonical Amino Acids		✓
	Site of PEGylation		✓
			✓
	pI	Similar pI of the main peak observed in BP14 and Neulasta® are comparable.	✓
	Identity	The visual profile assessment (band profiles) between both products was found to be similar.	✓
	Extinction Coefficient	The extinction coefficient of BP14 batches and Neulasta® batches are similar.	✓
	Free Cysteine	The free cysteine levels are found to be similar in both products.	✓
Size Heterogeneity	Monomer, Aggregates, High Molecular Weight species	BP14 and Neulasta® are similar for monomer and aggregate content by SE-HPLC and AUC.	✓
		Qualitative profiles are highly similar, and molecular weight band distribution is similar among BP14 and Neulasta® batches.	✓
	Sub-visible Particles	Sub-visible particle sizes of <10 µm and < 25 µm were lower in number for BP14 compared to Neulasta®.	✓
	Monomer, Di-PEGylated species, Des-PEGylated species, Low Molecular Weight species	A slight difference is observed in the proportion of the impurity levels in BP14 and Neulasta® batches. The difference observed in the impurities level is shown not to have any impact on product stability.	✓
		Qualitative profiles and molecular weight band distribution is similar among BP14 and Neulasta® batches.	✓

Product Characteristic	Product Quality Attribute	Conclusion and Key Findings	Supports Similarity
Structural Heterogeneity	Des-PEGylated species, Di-PEGylated species, Reduced species	The overall purity was found to be higher in BP14 batches compared to Neulasta® batches. The observed difference is not expected to impact product quality negatively.	✓
Charge Heterogeneity	Acidic isoforms, Basic isoforms, Neutral isoform (Main Peak)	A similar proportion of acidic variants was observed between both products. The purity of both products is similar to each other.	✓
Post Translational Modification (PTM)	Oxidation and Deamidation	The oxidation and deamidation are found to be similar in BP14 and Neulasta® batches.	✓
Higher Order Structure	Secondary and Tertiary Structure	The higher-order structures of BP14 and Neulasta® is similar.	✓
	Molar Mass, Hydrodynamic Radii, Polydispersity	The size variant profiles in SEC-MALS are found to be similar in BP14 and Neulasta® batches.	✓
	Di-Sulfide Bond Assignment	The MS data and peptide mass profiling data indicate the presence of same disulphide bond in BP14 and Neulasta® batches.	✓
Functional Characterization	Potency	The relative potency of BP14 and Neulasta® batches are found to be similar, and Equivalence testing criteria are met.	✓
	Binding Activity	Relative binding affinities of all the BP14 batches fall within the quality ranges of Neulasta®.	✓
	Signalling	The fold change of phospho-STAT3 is comparable between BP14 and Neulasta® batches.	✓
Immunogenicity	Innate Immune Response	TLR activating components are absent in the BP14 drug product, indicating no potential to induce innate immunity	✓
	Adaptive Immune Response	Neulasta® and BP14 batches showed no evidence of any increased immunogenicity risk.	✓
PEG Moiety	mPEG (Released)	The Released PEG mass analyzed by MALDI-TOF and 1 D 1H-NMR are found to be similar in BP14 and Neulasta® batches.	✓
		The residual m-PEG levels observed in BP14 and Neulasta® batches are found to be less than LOQ.	✓

2.3.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

A major GMP objection was raised due to the unavailability of a valid GMP compliance certificate before the marketing authorisation approval for the site CuraTeQ Biologics Private Limited (Telangana, India), with the manufacturing and quality control activities for both the active substance and the finished product in scope. The applicant provided a valid certificate of GMP compliance covering the scope of defined manufacturing and quality control activities thereby resolving the major objection.

The overall strategy for establishing analytical similarity is considered appropriate with regard to the extent of analytical exercise, choice of the reference medicinal product, selection of batches and approach to statistical evaluation. The results of the analytical similarity exercise demonstrate the high similarity of Neulasta and BP14.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product. These points are put forward and agreed as recommendations for future quality development. To conclude, three recommendations related to Module 3 remain.

2.3.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.3.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

Number	Description
1	The applicant is recommended to proceed with the provided plan to develop and implement an endotoxin assay based on recombinant Factor C.
2	Since the maximum amount of HCP found in PPQ batches is 5 ng/ml, and no data are available for batches used in clinical trials and for the newly developed process-specific method, the applicant should describe the action to be taken in case of results out of trend but below 50 ng/mg are seen.
3	The applicant should provide completed method transfer for all analytical methods including Size Heterogeneity by Capillary Electrophoresis (non-reduced). It should be noted that without completed method transfer the FP cannot be released to EU market.

2.4. Non-clinical aspects

2.4.1. Pharmacology

In vitro pharmacodynamic studies like receptor binding assays (by Surface Plasmon Resonance, SPR) and functional assays (mNFS-60 cell proliferation assay, phospho-STAT3 signalling assay) were conducted by the applicant to demonstrate similar biological activity between BP14 and Neulasta-EU. The reader is referred to Quality Assessment report for more details.

Neither *in vitro* secondary pharmacology, *in vivo* safety pharmacology studies nor pharmacodynamic drug interactions studies were performed. This is in accordance with regulatory requirements for biosimilars.

No *in vivo* studies were conducted by the applicant.

2.4.1.1. Primary pharmacodynamic studies

SPR-based assays measured the binding affinity to the G-CSF receptor, with BP14's relative binding affinity ranging from 89% to 110%, within the quality range of 84%-111% obtained with Neulasta, demonstrating similarity in receptor binding.

Using cell-based proliferation assays in a murine myeloblastic cell line (mNFS-60), BP14 and Neulasta batches showed relative potencies of 99 ± 8.5% and 97 ± 7.3%, respectively, indicating comparable proliferation activity. Additionally, phospho-STAT3 signalling activity assays confirmed comparable activation profiles between BP14 and Neulasta lots, further supporting their pharmacodynamic similarity.

2.4.1.2. Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were conducted, in accordance with the relevant EMA Guidelines for similar biological medicinal products.

2.4.1.3. Safety pharmacology programme

No safety pharmacodynamic studies were conducted, in accordance with the relevant EMA Guidelines for similar biological medicinal products.

2.4.1.4. Pharmacodynamic drug interactions

Not applicable.

2.4.2. Pharmacokinetics

No pharmacokinetic studies were performed, in accordance with the EMA Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1) and the annexure Draft Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (rG-CSF) (EMEA/CHMP/BMWP/31329/2005 Rev1).

2.4.3. Toxicology

No *in vivo* toxicology studies were performed, in accordance with EMA Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1), the annexure Draft Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (EMEA/CHMP/BMWP/31329/2005 Rev1) and the CHMP response to the Scientific Advice to the BP14 (Pegfilgrastim) development program (EMA/CHMP/SAWP/546617/2019).

The applicant conducted three *in vitro* immunogenicity studies. These qualitative tests comparatively assessed the immunogenicity of BP14 against the reference medicinal product Neulasta. Method validation of these studies was not provided and in general, these studies are not routinely conducted for biosimilarity or (immuno)toxicity assessment.

No reproduction toxicology, mutagenicity and carcinogenicity were submitted as these are not routinely required for non-clinical testing of similar biological medicinal products containing recombinant G-CSF as active substance.

2.4.4. Ecotoxicity/environmental risk assessment

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, pegfilgrastim is not expected to pose a risk to the environment.

2.4.5. Discussion on non-clinical aspects

No *in vivo* pharmacology studies were conducted. The applicant´s position to omit in vivo data in comparative exercise is appreciated and in line with Draft Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor as well as the scientific advice provided in 2019 (EMA/CHMP/SAWP/546617/2019).

To support the waiver of these studies, analytical similarity studies and supporting *in vitro* studies, including receptor binding assays and functional assays, were conducted to compare the biological activity of BP14 and Neulasta-EU.

Using cell-based proliferation assays in a murine myeloblastic cell line (mNFS-60), BP14 and Neulasta batches showed comparable proliferation activity. SPR-based assays demonstrated similarity in binding affinity of BP14 and Neulasta to the G-CSF receptor. Additionally, phospho-STAT3 signalling activity assays confirmed comparable activation profiles between BP14 and Neulasta, further supporting their pharmacodynamic similarity.

No *in vitro* secondary pharmacology, safety pharmacology, pharmacokinetic, or pharmacodynamic drug interactions studies were conducted, in accordance with regulatory requirements for biosimilars.

The applicant conducted three *in vitro* immunogenicity comparative studies of BP14 and Neulasta. In all three studies only 3 batches for BP14 and Neulasta were tested which limits a predictive value to a clinical situation.

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, pegfilgrastim is not expected to pose a risk to the environment.

2.4.6. Conclusion on the non-clinical aspects

The primary *in vitro* pharmacodynamic studies demonstrate similarity between BP14 and Neulasta, i.e. comparable proliferation potencies, binding affinity to G-CSF receptor, and activation profiles. In addition, the applicant also compared *in vitro* the similarity of immunogenicity of BP14 and Neulasta.

2.5. Clinical aspects

2.5.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Protocol	Design	Objective(s)	Treatment	Status
BP14-101 (PK/PD similarity)	Phase 1, Randomized, Single-Dose, Double-Blind, Two-Sequence, Two-Period Crossover Study to compare Pharmacokinetics, Pharmacodynamics, Immunogenicity, and Safety of BP14 (Pegfilgrastim) with EU-Neulasta® in Healthy Male Adult Subjects N=124 (62 subjects for Sequence AB and 62 subjects for Sequence BA)	**Primary Objective**: The primary objective is to compare the PK (C_{max} and AUC_{0-t}) and PD (ANC C_{max} and ANC AUC_{0-t}) of BP14 (Pegfilgrastim) with EU-Neulasta® in healthy adult male subjects. **Acceptance Criteria:** PD: 95% CI of ANC C_{max} and ANC AUC_{0-t} to be within 90 – 111%. PK: 90% CI of C_{max} and AUC_{0-t} to be within 80 – 125%. **Secondary Objectives:** The secondary objectives are to assess the safety, tolerability, and immunogenicity and to further characterize and compare the PK ($AUC_{0-\infty}$, $t_{1/2}$), PD (CD34+ E_{max}, CD34+ AUC_{0-t}) of BP14, and EU-Neulasta® in healthy adult male subjects.	Subjects received a single dose of 6 mg subcutaneous (SC) injection of either BP14 or Neulasta® on Day 1 of each treatment period.	Completed
BP14-102 (PK/PD similarity)	Phase 1, Randomized, Double-blind, Two-period, Two-treatment, Two-sequence, Crossover, Balanced, Single-dose study to compare Pharmacokinetic, Pharmacodynamic, Immunogenicity, and Safety in Healthy Adult Male Subjects. N=184 (92 subjects for Sequence AB and 92 subjects for Sequence BA)	**Primary objective**: The primary objective is to compare and evaluate the pharmacokinetics (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) of BP14 (Pegfilgrastim) 6 mg/0.6 mL solution for Injection with that of EU-Neulasta® (Pegfilgrastim) 6 mg/0.6 mL solution for Injection administered through subcutaneous route in healthy adult male subjects. **Acceptance Criteria:** PK: 90% CI of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ to be within 80 – 125%. **Secondary Objectives:** The secondary objectives are to assess the safety, tolerability, and immunogenicity and to further characterize and compare the PK (T_{max}, K_{el}, AUC_%Extrap_obs and $t_{1/2}$), PD (ANC E_{max}, ANC AUC_{0-t}) of BP14, and EU-Neulasta® in healthy adult male subjects.	Subjects received a single dose 6 mg subcutaneous (SC) injection of either BP14 (Pegfilgrastim) or Neulasta® on Day 1 of each treatment period.	Completed

2.5.2. Clinical pharmacology

2.5.2.1. Pharmacokinetics

Bioanalytical methods

BP14-101 study

Human serum pegfilgrastim concentrations were determined over the range from 0.20 ng/mL to 8.00 ng/mL using commercially available ELISA kit (Human G-CSF Duo set kit, R&D Systems). Calibration standards were prepared using BP14 and suitability of the one-assay approach was demonstrated during the method development and validation.

The performance of the assay was demonstrated in the validation. The acceptance criteria were met for all parameters except for selectivity at LLOQ QC level and poor intra-run accuracy at high QC levels in two runs out of 10 runs. The factors which might have led to the higher intra-run bias and variability in response, especially at the upper end of the analytical range, and measures which were taken were summarised. Overall validation and in-study intra- and inter-run accuracy and precision is considered acceptable. Total % ISR samples passed was 91.4%, indicating that the methods generated reproducible results.

No validation tests for PK assay tolerance to anti-drug antibodies (ADA) was submitted. As pharmacokinetic equivalence was not demonstrated in the BP14-101 study, the applicant excluded subjects with the presence of ADA in the second period. Statistical analysis of primary PK parameters in ADA-negative subjects was performed. Again, bioequivalence criteria were not met and intrasubject variability remained high. In addition, the ADA-positive subjects were evaluated for potential effect of ADA on the PK and PD profile. The PK parameters (AUC(0-t) and Cmax) were not affected by the presence of ADAs in all subjects based on the study report. The presence of ADA was therefore eliminated as a primary source of variability in the study.

BP14-102 study

A validated ELISA method based on a commercially available ELISA kit (R&D Systems) was developed and validated. The assay used a single calibrator (BP14) for the analysis of clinical samples from subjects treated with either BP14 or Neulasta (one-assay approach). The validated range was from 200.000 pg/mL to 6400.000 pg/mL. Bioanalytical similarity was demonstrated. The assay was validated for precision and accuracy, total error, selectivity in normal, lipemic and haemolysed human serum, specificity, dilution linearity, prozone effect and stability. Method performance in the study was demonstrated by back-calculated calibration standards, inter-batch precision and accuracy of QC samples, and ISR. Study samples were analysed without exceeding validated short-term, long-term or freeze-thaw stability periods. The assay was suitable for the determination of pegfilgrastim levels after BP14 or Neulasta administration.

Bioequivalence

Study BP14-101

Study title: A Randomized, Single-Dose, Double-Blind, Two-Sequence, Two-Period Crossover Study to compare Pharmacokinetics, Pharmacodynamics, Immunogenicity and Safety of BP14 (pegfilgrastim) with EU-approved Neulasta in Healthy Male Adult Subjects

Study design

Study BP14-101 was a randomized, double-blind, comparative single-dose, 2-period crossover study to compare PK, PD, immunogenicity, and safety of BP14 with Neulasta in healthy male subjects.

BP14-101 was conducted under a two-sequence, two-period crossover design. A first group of 6 sentinel subjects (3 subjects receiving the test product BP14 and 3 subjects receiving EU-approved Neulasta) were dosed first to establish the safety profile (e.g., onset of serious allergic reactions, including anaphylaxis) prior to dosing the rest of the study population. Dosing of the remaining subjects commenced after a minimum of

24 hours after dosing the sentinel subjects. Subjects received a single dose of 6 mg subcutaneous (SC) injection of either BP14 or Neulasta on Day 1 of each treatment.

Primary objectives

- To compare the PK and PD of BP14 (pegfilgrastim) with EU-approved Neulasta

Secondary objectives

- To compare the PK of BP14 (pegfilgrastim) with EU-approved Neulasta
- To compare CD34+ cell response between BP14 and EU-approved Neulasta
- To explore the potential immunogenicity of BP14 and EU-approved Neulasta
- To assess and compare the safety and tolerability of BP14 and EU-approved Neulasta

Dose and mode of administration

Subjects received a single dose of 6 mg subcutaneous (SC) injection of either BP14 or Neulasta on Day 1 of each treatment period via a single prefilled syringe on intact, non-irritated skin on outer area of the abdomen.

Sampling schedule

PK samples were taken pre-dose (i.e., between 5 and 45 minutes prior to dosing), 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 (Day 1), 24, 28, 32, 36, 40 (Day 2), 48 (Day 3), 60, 72 (Day 4), 96 (Day 5), 120 (Day 6), 144 (Day 7), 168 (Day 8), 192 (Day 9), 216 (Day 10), 240 (Day 11), 264 (Day 12), 312 (Day 14) and 480 (Day 21) hours post-dose of both treatment periods.

The Treatment Period 1 was followed by a washout period of at least 6 weeks (42 days), effectively resulting in at least 9 weeks washout period between the 2 doses of investigational medicinal products (IMPs).

Protocol deviations

All randomized subjects in the study had at least one protocol deviation, the majority of which were considered minor.

One subject had a major protocol deviation (positive test for drug abuse during the study) and was discontinued from the study prior to Treatment Period 2.

All 124 (100%) subjects had protocol deviations related to procedures/tests that were considered to be minor. A total of 14/124 (11.3%) subjects had minor protocol deviations related to visit schedule, 2/124 (1.6%) subjects had other minor protocol deviation and 1/124 (0.8%) subject had minor protocol deviation related to informed consent.

Prior and concomitant medication

A total of 27/124 (21.8%) subjects (13/62 [21.0%] subjects in treatment sequence AB and 14/62 [22.6%] subjects in treatment sequence BA) were reported to have received at least one prior medication. The prior medications used by at least 2 subjects (≥ 1%) included vitamins (not otherwise specified) (9/124 [7.3%] subjects), paracetamol, cetirizine hydrochloride, and vitamin D (not otherwise specified) (3/124 [2.4%] subjects each), and zinc, salbutamol, ibuprofen, creatine, fish oil, magnesium, and ascorbic acid (2/124 [1.6%] subjects each).

The applicant confirmed that 105/124 (84.7%) of the subjects received concomitant medication during the study, being the most frequently used paracetamol (99/124 [79.8%] subjects) and ibuprofen (51/124 [41.1%] subjects) prescribed by investigators for the treatment of TEAEs (headache, backpain, bone pain & myalgia) attributed to the study drugs. In addition, these painkiller medications were used only for one or two days. In fact, headache, backpain, bone pain & myalgia are very common or common adverse reactions reported in the SmPC of Neulasta.

The use of ibuprofen was noted to be higher in subjects randomized to treatment sequence AB (51.5%) than in subjects randomized to treatment sequence BA (30.5%).

There was no major imbalance in the use of any other prior or concomitant medications across treatment sequences.

No prohibited concomitant medication was taken by any subject during the study.

In conclusion, the reported prior and concomitant medications are not considered to have significant effect on the study results.

Sample size determination

Power analysis for sample size calculation was performed based on log-transformed PK data for achieving a 90% power for establishment of bioequivalence (biosimilarity) between BP14 and the Neulasta-EU using Two One-Sided Equivalence Tests for Ratio of Two Log-Normal Means at the 5% level of significance. As a result, a total of 110 subjects (55 subjects per sequence) were required for establishment of bioequivalence (biosimilarity) between BP14 and the Neulasta-EU with a 90% power. A total of 124 subjects were recruited to account for a possible drop-out rate of 10%.

The sample size estimates were based on the following assumptions:

- Intra-subject covariance (CV) of 45%
- 90% Confidence Interval (CI)
- Equivalence Range 0.80 - 1.25
- Expected Ratio of Means of 95% to 105%
- 90% Power for equivalence

Above sample size calculations were performed using validated nQuery (ver 8.1.20) software.

Population(s) studied

A total of 291 subjects were screened for the study and 124 subjects were randomized, with 62 subjects randomized to each of the treatment sequences AB and BA. Overall, 113/124 (91.1%) subjects completed both treatment periods. For treatment sequence AB, all 62 (100%) subjects completed Treatment Period 1

and 55/62 (88.7%) subjects completed Treatment Period 2. For treatment sequence BA, 61/62 (98.4%) subjects completed Treatment Period 1 and 58/62 (93.5%) subjects completed Treatment Period 2.

Overall, 1/124 (0.8%) subject in treatment sequence AB discontinued study due to protocol deviation and another 1/124 (0.8%) subject due to AE, 2/124 (1.6%) subjects (1 subject in each treatment sequence) were lost to follow-up, and 7/124 (5.6%) subjects (4 subjects in treatment sequence AB and 3 subjects in treatment sequence BA) discontinued from study due to withdrawal of consent.

A total of 167 subjects (57.4%) were considered screen failures, of whom 114 subjects did not meet the inclusion/exclusion criteria and 53 subjects were considered screen failures due to other reasons (commonly due to withdrawal by subject).

The PK Analysis Set was defined as all subjects who were randomized, received IMP and completed PK sampling in both periods without a major protocol violation with relevant impact on PK data.

Table 2.Summary of Analysis Population (Randomized Analysis Set)

Categories	Sequence AB		Sequence BA		Overall (N=124)
	Period 1 n (%)	Period 2 n (%)	Period 1 n (%)	Period 2 n (%)	n (%)
Randomized Analysis Set	62 (50.0)		62 (50.0)		124 (100%)
Safety Analysis Set	62 (50.0)	55 (44.4)	62 (50.0)	58 (46.8)	124 (100%)
PK Analysis Set	55 (44.4)	55 (44.4)	58 (46.8)	58 (46.8)	113 (91.1)
PD Analysis Set	55 (44.4)	55 (44.4)	58 (46.8)	58 (46.8)	113 (91.1)

Table 3. Summary of Demographic Characteristics (Safety Analysis Set)

Characteristics	Sequence AB (N=62) n (%)	Sequence BA (N=62) n (%)	Overall (N=124) n (%)
Age (Years)			
N	62	62	124
Mean	29.5	30.3	29.9
SD	8.80	9.24	9.00
Median	28.0	28.0	28.0
Min, max	18, 54	18, 55	18, 55
Sex			
Male	62 (100)	62 (100)	124 (100)
Race			
American Indian or Alaska Native	0	1 (1.6)	1 (0.8)
Asian	10 (16.1)	10 (16.1)	20 (16.1)
Black or African American	1 (1.6)	3 (4.8)	4 (3.2)
Native Hawaiian or Other Pacific Islander	2 (3.2)	1 (1.6)	3 (2.4)
White	47 (75.8)	45 (72.6)	92 (74.2)
Other	2 (3.2)	2 (3.2)	4 (3.2)
Ethnicity			
Hispanic or Latino	8 (12.9)	7 (11.3)	15 (12.1)
Not Hispanic or Latino	50 (80.6)	46 (74.2)	96 (77.4)
Not Stated	3 (4.8)	6 (9.7)	9 (7.3)
Unknown	1 (1.6)	3 (4.8)	4 (3.2)

Weight (kg)			
N	62	62	124
Mean	79.46	79.57	79.51
SD	13.196	10.162	11.729
Median	80.05	79.70	80.05
Min, max	50.0, 109.9	59.1, 102.1	50.0, 109.9
Height (cm)			
N	62	62	124
Mean	178.7	178.1	178.4
SD	7.11	7.10	7.08
Median	179.0	177.0	178.0
Min, max	163, 200	163, 196	163, 200
BMI (kg/m^2)			
N	62	62	124
Mean	24.83	25.07	24.95
SD	3.547	2.873	3.217
Median	24.90	25.05	24.90
Min, max	18.5, 31.7	19.5, 32.0	18.5, 32.0

Randomization and blinding

The treatment sequence assignment code was prepared at the start of the study and kept in a secured location (i.e., study site pharmacy) that was locked at all times.

In order to maintain the double-blind nature of the study, only the randomization statistician, study centre pharmacy team, administering nurses/trained personnel (who did not participate in the study as staff), and designated Clinical Monitor(s) were unblinded to the treatment codes. All other study-related individuals, including the subjects, ancillary study centre staff, other Clinical Monitor(s), Investigator, Sponsor, and CRO staff, remained blinded to the treatments.

A sealed envelope that contained the IMP assignment for each subject (master randomization list) was provided to the Investigator. The sealed envelope was retained by the Investigator (or representative) in a secured area.

On evening of Day -1 or on Day 1, subjects were assigned a unique (randomization) number in ascending numerical order at the study site.

In the event of an emergency, the Investigator had the sole responsibility for determining if unblinding of a subject's IMP assignment was warranted. Subject safety was always the first consideration in making such a determination. If the Investigator decided that unblinding was warranted, the Investigator made every effort to contact the Sponsor prior to unblinding a subject's treatment assignment unless this delayed emergency treatment of the subject. If a subject's IMP assignment was unblinded, the Sponsor was notified within 24 hours after breaking the blind.

Once the study was complete, all envelopes (sealed and opened) were inventoried and returned to the Sponsor. Investigators were strongly discouraged from requesting the blind to be broken for an individual subject, unless there was a subject safety issue that required the Investigator to know which treatment was given to the subject. If the blind was broken, it could be broken for only the subject in question.

The Sponsor and CRO were notified immediately if a subject and/or the Investigator was unblinded during the course of the study and pertinent information regarding the circumstances of unblinding of a subject's treatment code was documented in the subject's source documents and eCRF.

Pharmacokinetic variables

The primary PK endpoints of the study were:

- $AUC_{(0-t)}$: AUC of the drug up to the last measurable concentration
- C_{max}: Maximum concentration of the drug in the serum

The secondary PK endpoints of the study were:

- $t_{1/2}$: Terminal elimination half-life of the drug
- AUC_{0-inf}: AUC of the drug extrapolated to infinite time

Statistical methods

Pharmacokinetics analysis

To compare the PK of BP14 with EU-approved Neulasta, a linear mixed effects model was fitted separately on natural log-transformed area under the time curve concentration ($AUC_{(0-t)}$) and C_{max}. The model contained terms for sequence, period and treatment as fixed effects, and term for subject nested within sequence as random effect. Within the framework of this model, the difference between least squares means of BP14 and Neulasta-EU was exponentiated to obtain ratio of adjusted geometric means. Similarly, the 90% CI for the difference in least squares means obtained in natural logarithm scale was exponentiated to obtain 90% CI for ratio of geometric means.

A comparability range of 80 - 125% was considered for assessment of clinical biosimilarity using above 90% CI for ratio of geometric means.

Pharmacodynamics analysis

The primary PD endpoints ANC AUC(0-t) and ANC Emax were analysed using analysis of covariance (ANCOVA). The ANCOVA model contained terms for sequence, period and treatment as fixed effects, the term for subject nested within sequence as random effect and baseline ANC (pre-dose ANC in each period) as a covariate. Within the framework of this model, 95% CI for ratio of adjusted least squares means of BP14 and Neulasta-EU were obtained.

A comparability range of 90% to 110% were considered for assessment of clinical biosimilarity using above 95% CI for ratio of means.

Changes Following Study Unblinding and Post-hoc Analyses

Post-hoc analyses were conducted to explore potential confounding factors affecting the demonstration of bioequivalence between BP14 and Neulasta:

- Effect of Potential Outliers: To evaluate the impact of potential outliers identified through studentized residuals methodology with cut-off value beyond ± 3
- Summary of Serum Pharmacokinetics Parameters $AUC_{(0-t)}$ and C_{max} following the exclusion of subjects identified as outliers based on studentized residuals
- Listing of studentized residuals for the Pharmacokinetics Parameters $AUC_{(0-t)}$ and C_{max}
- Boxplot of studentized residuals of PK parameters $AUC_{(0-t)}$ and C_{max} by period

- Effect of ADA: To evaluate the impact of ADA on the primary PK parameters, $AUC_{(0-t)}$ and C_{max}, using a linear mixed effects model using the subset of subjects that are ADA negative.
- Period Effect: To evaluate the period effect on the primary PK parameters, $AUC_{(0-t)}$ and C_{max}, first using a linear mixed effects model
- Treatment difference: To evaluate the treatment difference of BP14 with Neulasta in each period
- Frequencies for ADA and NAb results.

Equivalence range (ER) and confidence level of confidence interval (CI) for primary PK parameters (ER: (80%, 125%), 90% CI) and for primary PD parameters (ER: (90%, 111%), 95% CI) were considered as acceptable.

Several post-hoc analyses were also performed but these were considered only supportive.

Pharmacokinetic results

Following a single 6 mg SC administration of BP14 or EU-approved Neulasta, serum concentrations of pegfilgrastim exhibited high variability across the majority of the concentration time profile in both periods. In Period 1, during the absorption phase, the variability in serum concentrations (geometric mean CV%) ranged from 124.7% to 226.8% for BP14 and 123.0% to 223.3% for Neulasta. After the maximum serum concentrations were reached (18 hours for BP14 and 16 hours for Neulasta), variability remained high until 96 hours post dose for both products. During this timeframe, observed serum concentration variability ranged from 130.6% to 243.4% for BP14 and 128.8% to 231.6% for Neulasta. After 96 hours post-dose, the variability in serum concentrations dropped precipitously, with geometric mean CV% ranging from 9.4% to 57.2% and 28.2% to 85.6% for BP14 and Neulasta, respectively.

Similar variability was observed in Period 2. During the absorption phase in Period 2, the variability in serum concentrations (geometric mean CV%) ranged from 101.7% to 224.1% for BP14 and 123.6% to 229.3% for Neulasta. After the maximum serum concentrations were reached (18 hours for BP14 and 16 hours for Neulasta), variability remained high until 96 hours post dose for both products. During this timeframe, observed serum concentration variability ranged from 125.6% to 184.2% for BP14 and 135.1% to 320.0% for Neulasta. After 96 hours post-dose, the variability in serum concentrations dropped precipitously, with geometric mean CV% ranging from 11.4% to 90.8% and 1.7% to 61.3% for BP14 and Neulasta, respectively.

Visual review of the individual overlaid serum concentration time-profiles identified three potential outliers, with serum BP14 and Neulasta concentrations roughly 9-fold higher than the other subjects in Period 1 and roughly 9-fold lower in Period 2. The observed variability in plasma concentrations may be driven by these subjects.

Figure 2. Arithmetic Mean (+SD) Serum Concentration Time Profiles for BP14 and Neulasta in Linear Scale (PK Analysis Set) (0 – 96 hours)

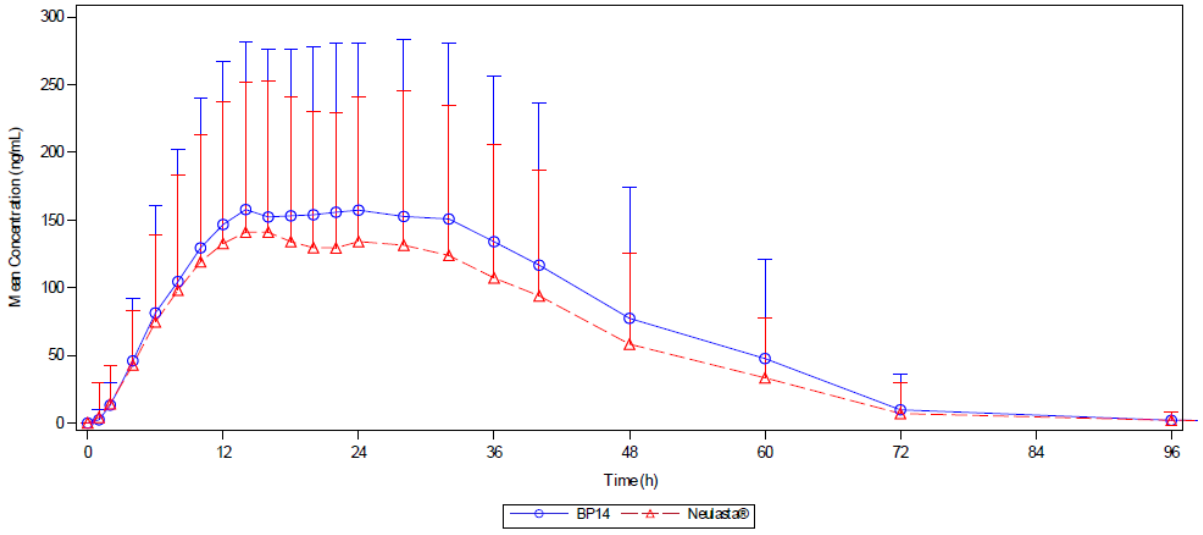


Figure 3. Arithmetic Mean Serum Concentration Time Profiles for BP14 and Neulasta in Semilogarithmic Scale (PK Analysis Set) (0 – 96 hours)

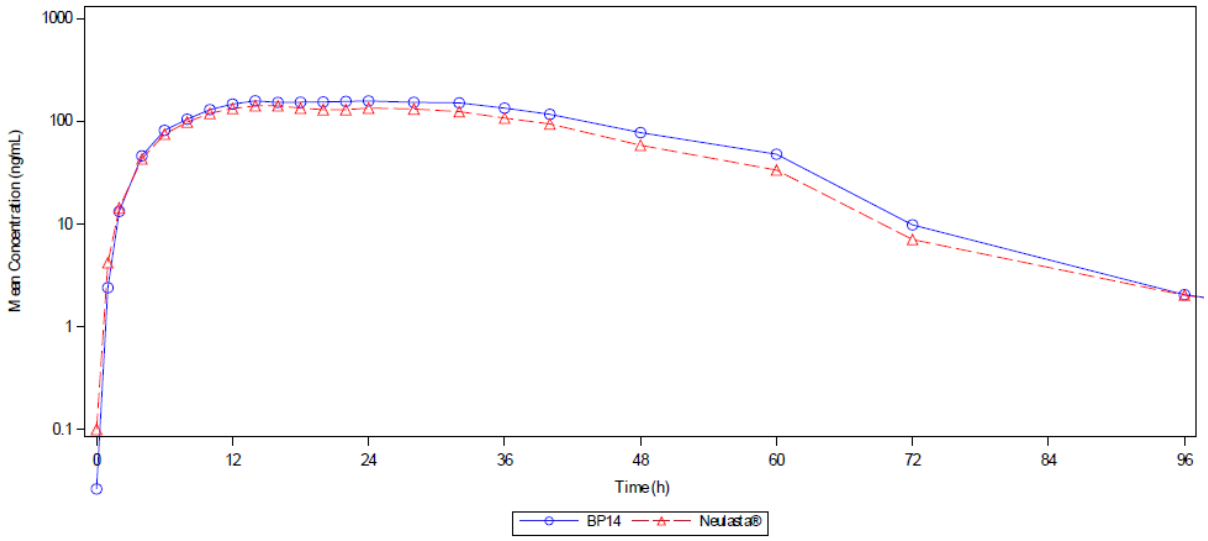


Figure 4. Arithmetic Mean (+SD) Serum Concentration Time Profiles for BP14 and Neulasta in Linear Scale (PK Analysis Set) (0 – 480 hours)

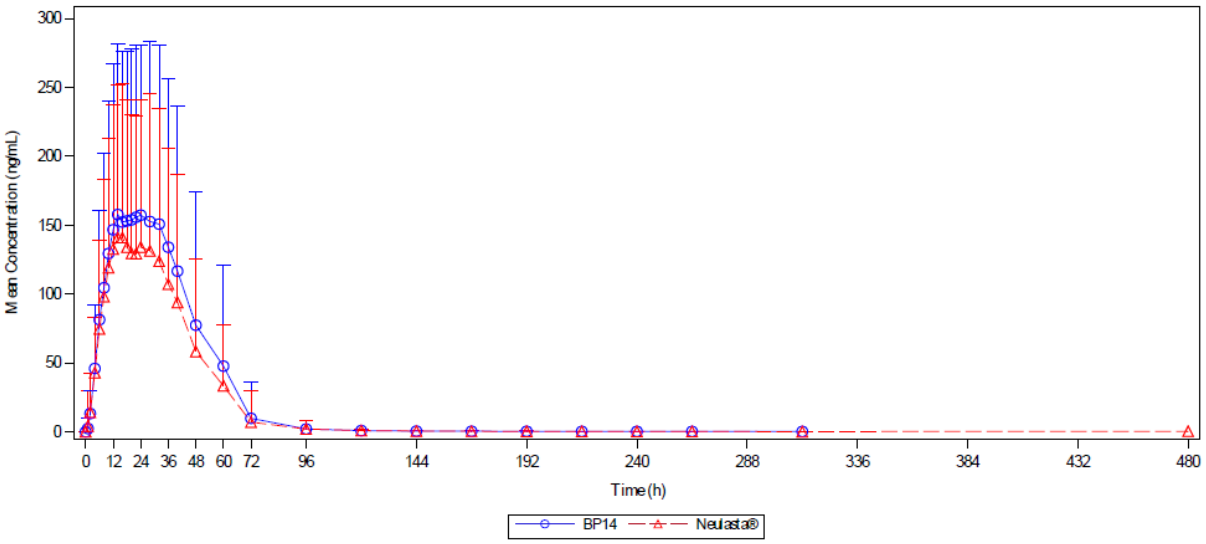
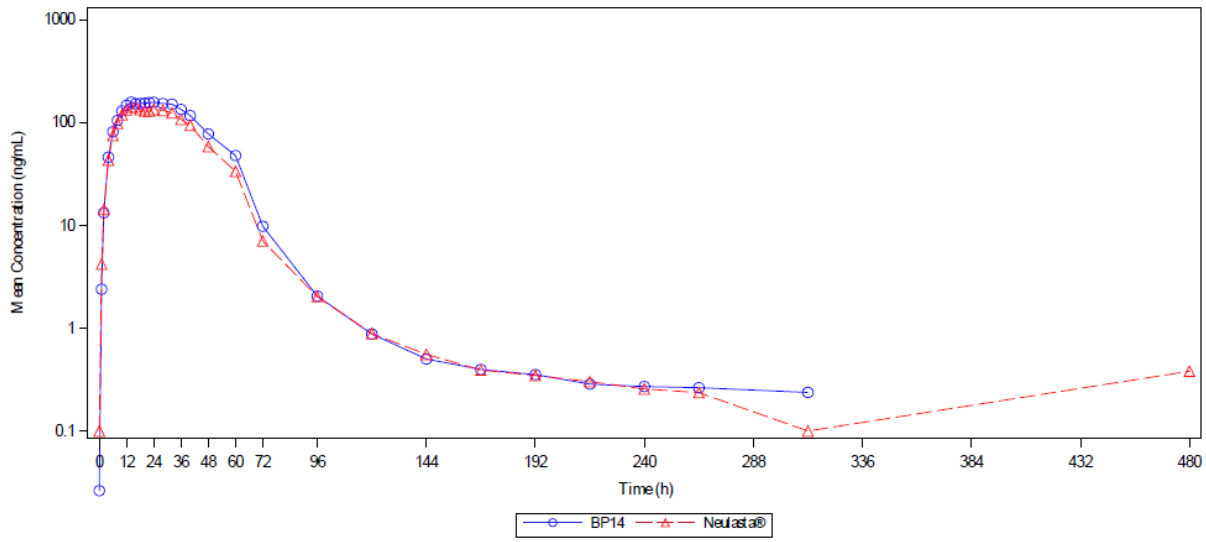


Figure 5. Arithmetic Mean Serum Concentration Time Profiles for BP14 and Neulasta in Semilogarithmic Scale (PK Analysis Set) (0 – 480 hours)



Primary pharmacokinetic analysis

Estimates of lambda z related parameters, specifically $AUC_{(0\text{-inf})}$ and $t_{1/2}$, were not deemed reliable for two subjects because the terminal elimination phase was not well characterized.

Statistical analysis to assess the bioequivalence of BP14 with Neulasta for the PK Analysis Set did not pass the bioequivalence criteria range (0.800 – 1.250). The GMR (90% CI) for C_{max} and $AUC_{(0\text{-t})}$ were 1.158 (1.037, 1.293) and 1.215 (1.081, 1.366), respectively. The GMR estimates for both C_{max} and $AUC_{(0\text{-t})}$ were higher than 1.00, suggesting that the bioavailability of BP14 following a single SC administration may be greater than Neulasta.

The upper 90% CI for each parameter was greater than 1.25 suggesting that the differences between BP14 and Neulasta are statistically significant. The intrasubject variability (CV%) was high for both C_{max} and $AUC_{(0-t)}$ and was greater than 50%; the intrasubject variability was 53.3% for C_{max} and 57.1% $AUC_{(0-t)}$. The estimates of $t_{1/2}$ for either product were similar, suggesting that the increase in peak serum pegfilgrastim concentrations following administration of BP14 compared with Neulasta did not have any impact on this parameter.

Table 4. Statistical Analysis to Assess Bioequivalence of BP14 with Neulasta (PK Analysis Set)

	BP14			Neulasta®			Ratio: BP14 / Neulasta®		
	N[1]	GM	90% CI	N[1]	GM	90% CI	GMR	90% CI	Intra Subject CV%
C_{max} (ng/mL)	113	134.173	(116.283, 154.816)	113	115.830	(100.386, 133.651)	1.158	(1.037, 1.293)	53.3
$AUC_{(0-t)}$ (h*ng/mL)	113	4722.573	(4062.788, 5489.506)	113	3886.343	(3343.386, 4517.474)	1.215	(1.081, 1.366)	57.1

A linear mixed effects model with sequence, period and treatment as fixed effects and subject nested within sequence as random effect, after logarithmic transformation of the data was used for the statistical analysis. [1] N: number of observations used in the model. Abbreviations: GM = geometric mean; GMR = geometric mean ratio; CI = confidence interval; AUC = Area under curve; C_{max} = Maximum observed concentration; CV = coefficient of variation

Impact of Antidrug Antibodies (ADA) on Primary PK Analysis

Using only subjects that were ADA negative, statistical analysis of the primary PK parameters yielded GMR (90% CI) of 1.124 (1.006, 1.256) for C_{max} and 1.174 (1.044, 1.320) for $AUC_{(0-t)}$, demonstrating that in this subset of subjects the bioequivalence of BP14 with Neulasta did not pass the bioequivalence criteria range of (0.800 – 1.250). While slight decreases in the GMR for both C_{max} and $AUC_{(0-t)}$ were observed using only ADA-negative subjects, intrasubject variability remained high, with intrasubject CV% of 49.4% and 52.7% for C_{max} and $AUC_{(0-t)}$, respectively, eliminating the presence of ADA as a primary source for variability in this study and further evidence of highly variable drug product (HVDP) status for pegfilgrastim.

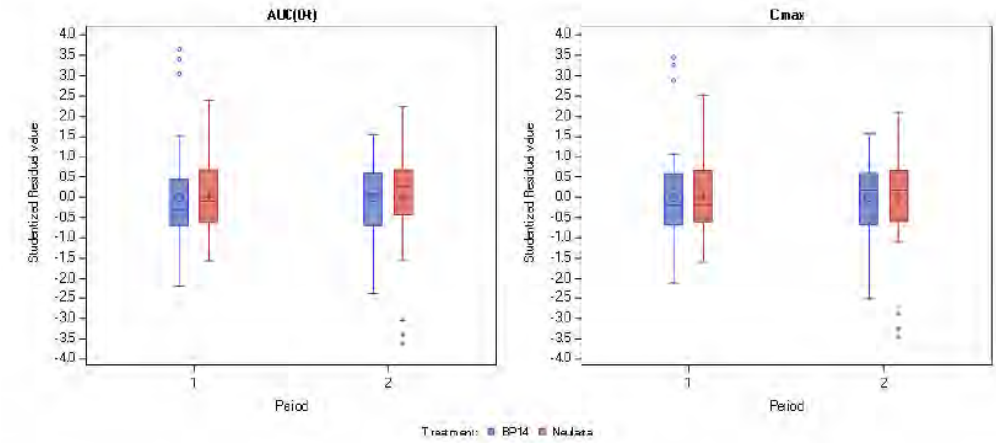
Identification of Potential Pharmacokinetic Outliers

Due to the high variability observed in the study data for both BP14 and Neulasta (intrasubject CV >50%), an outlier analysis was performed using the studentized residuals to identify potential outlier subjects. Any subjects with residual values plotted outside the upper and lower whiskers, namely residual values above 3 or below -3 in the boxplot, were considered outlier subjects. Any outlier subjects identified were then excluded from the statistical analysis to evaluate their influence on the primary PK analysis results.

Boxplots of the studentized residuals to identify potential outliers are presented in Figure 6.

Based on the criteria for outliers of residual values above 3 or below -3, in Period 1, two subjects were identified as outliers for C_{max} and three subjects) were identified as outliers for $AUC_{(0-t)}$. These subjects had much greater than average exposure in period 1, with residuals greater than 3. In period 2, these same subjects were identified as outliers, however, in this period they had much lower-than-average exposure, with residuals lower than -3.

Figure 6. Boxplot of Studentized Residuals for BP14 and Neulasta - $AUC_{(0-t)}$ and C_{max} (PK Analysis Set)



Impact of Identified Pharmacokinetic Outliers on Primary PK Analysis

Statistical analysis of the primary PK endpoints for the PK analysis set with the outliers excluded is presented in Table 5.

Following a single SC administration of BP14 or Neulasta and the exclusion of the identified outliers, geometric mean (gCV%) C_{max} values were 131.0 ng/mL (109.4%) and 118.4 ng/mL (116.3%). Geometric mean (gCV%) $AUC_{(0-t)}$ estimates were 4600 h*ng/mL (120.7%) and 4067 h*ng/mL (119.0%) for BP14 and Neulasta, respectively.

The GMR (90% CI) for C_{max} and $AUC_{(0-t)}$ were 1.108 (1.003, 1.225) and 1.131 (1.023, 1.249), demonstrating bioequivalence between BP14 and Neulasta following exclusion of the 3 identified outlier subjects. Removal of two subjects decreased the intrasubject variability from 53.3% to 47.3% for C_{max} and removal of three subjects decreased the intrasubject variability from 57.1% to 46.8% for $AUC_{(0-t)}$. Removal of the outliers identified using the method of studentized residuals resulted in slight decreases in the GMR for both C_{max} and $AUC_{(0-t)}$ and slight decreases in the intrasubject CV%. The intrasubject CV% remained greater than 46%, indicating that the presence of outliers is not adequate to account for the high intrasubject variability, further supporting classification of pegfilgrastim as a HVDP.

Table 5. Statistical analysis to assess Bioequivalence of BP14 with Neulasta following exclusion of subjects identified as outliers (PK Analysis Set)

	BP14			Neulasta®			Ratio: BP14 / Neulasta®		
	N[1]	GM	90% CI	N[1]	GM	90% CI	GMR	90% CI	Intra Subject CV%
Cmax (ng/mL)	111	131.332	(113.792, 151.576)	111	118.520	(102.691, 136.789)	1.108	(1.003, 1.225)	47.3
AUC(0-t) (h*ng/mL)	110	4614.650	(3972.317, 5360.849)	110	4081.856	(3513.685, 4741.902)	1.131	(1.023, 1.249)	46.8

Three subjects from AUC analysis and Two subjects from C_{max} analysis were excluded based on Studentized Residuals methodology with the cut-off value of +/-3. Studentized residuals are derived from the general linear regression model with sequence, period and treatment as fixed effects and with logarithm transformation of the dependent variable data.
A linear mixed effects model with sequence, period and treatment as fixed effects and subject nested within sequence as random effect, after logarithmic transformation of the data was used for the statistical analysis. [1] N: number of observations used in the model.
Abbreviations: GM = geometric mean; GMR = geometric mean ratio; CI = confidence interval; AUC = Area under curve; C_{max} = Maximum observed concentration; CV = coefficient of variation
Subjects were excluded based on Studentized Residuals methodology.

Pharmacokinetic conclusions

Based on PK analysis set, bioequivalence (BE) was not concluded for both primary PK parameters C_{max} and $AUC_{(0-t)}$ as corresponding 90% CI for treatment ratio BP14/Neulasta was not within the equivalence range (80%, 125%). The 90% CI for C_{max} was (103.70%, 129.30%) and for $AUC_{(0-t)}$ was (108.10%, 136.60%). Intra-subject coefficient of variation (ISCV) was 53.3% for C_{max} and 57.1% for $AUC_{(0-t)}$.

Also, it should be noted that the GMR estimates for both Cmax and $AUC_{(0-t)}$ were greater than 1.00 (1.158 for Cmax and 1.215 for $AUC_{(0-t)}$) showing that the bioavailability of BP14 was higher compared to the reference product Neulasta. The non-equivalence showed for the confidence intervals for $AUC_{(0-t)}$ and Cmax is not primarily based on high variability observed but more likely on higher bioavailability of the tested product.

For the secondary PK parameter $AUC_{(0-\infty),}$ the ratio with 90% CI was 1.200 with (1.073, 1.342). Two subjects were identified as outlying subjects and after exclusion of these subjects, the ratio with 90% CI was 1.142 with (1.034, 1.261). BE was not concluded for the secondary PK parameter $AUC_{(0-\infty)}$.

Pharmacokinetic equivalence was therefore not demonstrated in the study.

To identify the issue of non-equivalence the applicant performed several post hoc analyses to evaluate the data:

If solely antidrug antibody (ADA) negative subjects from PK analysis set were considered, the corresponding 90% CI for treatment ratio BP14/Neulasta both for C_{max} and $AUC_{(0-t)}$ was again not within ER (80%, 125%). In this case, 90% CI for C_{max} was (100.60%, 125.60%) and for $AUC_{(0-t)}$ was (104.40%, 132.00%). ISCV was 49.4% for C_{max} and 52.7% for $AUC_{(0-t)}$.

Further, period effect was evaluated both for C_{max} and $AUC_{(0-t)}$ through reached significance level (p-value) obtained from corresponding linear mixed-effects model. If p-value was less than 0.05 then the effect was considered as statistically significant on 5% significance level. However, using 5% significance level, period effect was not statistically significant both for C_{max} and $AUC_{(0-t)}$.

Similarly, statistical significance of treatment effect was evaluated based on data from period 1 only and for data from period 2 only, respectively, for C_{max} and $AUC_{(0-t)}$. Using 5% significance level, treatment effect was not concluded to be statistically significant.

The applicant also performed identification of subjects with outlying observations of PK parameters. Criterion was that corresponding studentized residual was in absolute value above 3. Two subjects were identified for C_{max} and three subjects for $AUC_{(0-t)}$. For all identified subjects, outlying observation was both in period 1 and in period 2, i.e. 4 outlying observations were for C_{max} and 6 outlying observations were from $AUC_{(0-t)}$. After exclusion of 2 outlying subjects for C_{max} and 3 outlying subjects for $AUC_{(0-t)}$, respectively, BE was concluded with respect to ER (80%, 125%) for treatment ratio BP14/Neulasta. In this case, 90% CI for C_{max} was (100.30%, 122.50%) and 90% CI for $AUC_{(0-t)}$ was (102.30%, 124.90%). ISCV was 47.3% for C_{max} and 46.8% for $AUC_{(0-t)}$.

In conclusion, bioequivalence was not shown in the BP14-101 study as the confidence intervals for both primary PK parameters were outside predefined limits 80-125%. Also point estimates for both parameters were higher for test product (1.158 for Cmax and 1.215 for AUC(0-t)). The equivalence was not concluded also for only ADA negative subjects. The applicant conducted another bioequivalence study BP14-102 and a meta-analysis of both Phase 1 PK/PD trials. Please see the assessment below.

Study BP14-102

Title: Single dose study to compare pharmacokinetic, pharmacodynamic, immunogenicity and safety of BP14 (Pegfilgrastim) 6 mg/0.6 mL solution for injection and Neulasta (Pegfilgrastim) 6 mg/0.6 mL solution for injection in healthy adult male subjects.

Study design

Study BP14-102 was a double blind, randomized, two-period, two-treatment, two-sequence, crossover, balanced, single dose study to compare pharmacokinetic, pharmacodynamic, immunogenicity, and safety in healthy adult male subjects.
Subjects were housed in the clinic from at least 11 hours prior to dosing and until at least 48 hours post dose in each study period.

Primary objectives

To compare and evaluate the PK and PD of BP14 (6 mg/0.6 mL solution for Injection) and Neulasta(6 mg/0.6 mL solution for Injection) administered through subcutaneous route in healthy, adult, male subjects.

Secondary objectives

- To assess the immunogenicity of the investigational products

- To monitor the safety and tolerability of the investigational products

Dose and mode of administration

A single dose (6 mg) of either test product BP14 or Neulasta was administered to the subject's right upper quadrant (RUQ) or left upper quadrant (LUQ) of the subject's abdomen in a supine posture in each study period.

Sampling schedule

For PK and PD, blood samples were taken pre-dose (0 hour) and at 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 32, 40, 48, 60,
72 (Day 3), 96 (Day 4), 120 (Day 5), 144 (Day 6), 168 (Day 7), 216 (Day 09), 264 (Day
11), 312 (Day 13) and 480 (Day 20) hours post-dose.
For immunogenicity, blood samples were taken pre-dose (0 hour), at 24 (Day 1) and 480 (Day 20) hours post-dose.
Between doses there was washout period of 42 days.

Protocol deviations

Sampling deviations occurred in 42/184 (22.83%) subjects, safety assessment deviations occurred in 4/184 (2.17%) subjects, requirements and restrictions deviations occurred in 40/184 (21.74%) subjects, sample handling and storage deviations occurred in 1/184 (0.54%) subjects and sample shipment deviations occurred in 101/184 (54.89%) subjects. No major protocol deviations occurred during the study.

The investigator judged the reported deviations were unlikely to have affected the results and conclusions of the study and safety of the subjects was not considered to be at risk. The reported deviations had no impact on study outcome and integrity.

Prior and concomitant medication

In the interests of subject safety and acceptable standards of medical care, the principal investigator or sub investigator were permitted to prescribe treatment(s) at their discretion, the details of which were recorded on the adverse event forms.

Otherwise, no concomitant medications were allowed during confinement and throughout the study except those permitted by principal investigator or sub investigator due to adverse event.

The reported prior and concomitant medications are not considered to have significant effect on the study results.

Sample size determination

The sample size estimation using two one-sided tests method assumed:

- T/R ratio = 95.00% - 105.26%,
- intra-subject C.V (%) ~ 57%,
- significance level = 5%,
- power = 90%,
- BE limits = 80% - 125%.

Based on the above estimates, a sample size of 166 subjects were to be sufficient to establish bioequivalence with adequate power. Considering dropouts and withdrawals, 184 subjects were required.

Population(s) studied

184 subjects were planned in the study. 184 and 175 subjects were dosed in Period 1 and 2 respectively. A total of 175 subjects completed the study. Serum samples of 184 (175 completed and 09 discontinued) subjects were assayed for Pegfilgrastim. Serum concentration data of 175 completed subjects were used in pharmacokinetic, pharmacodynamic and statistical evaluation for Pegfilgrastim.

The study was conducted on Asian male subjects and all subjects were selected based on the absence of any clinically significant findings on medical history, medication history, family medical history, vital signs and well-being, comprehensive physical examination, ECG, chest X-ray recordings (within past six months), ultrasonography abdomen and clinical laboratory evaluations performed within 28 days of initial study dosing. The Investigator evaluated all the laboratory values individually. All were determined to be non-reactive, normal, negative or not clinically significant for those subjects enrolled in the study.

Table 6. Summary of mean demographic data (±SD)

	Subjects Dosed (N=184)		
	All (N = 184)	Sequence AB (N = 92)	Sequence BA (N = 92)
Height (cm)	166.9 (± 5.6)	166.8 (± 5.5)	166.9 (± 5.7)
Weight (kg)	66.1 (± 9.4)	66.1 (± 9.4)	66.1 (± 9.4)
BMI (kg/m²)	23.7 (± 3.1)	23.8 (± 3.1)	23.7 (± 3.2)
Age (Years)	36 (± 8)	36 (± 8)	36 (± 8)

Pharmacokinetic variables

The primary PK endpoints of the study were:
- $AUC_{(0-t)}$: AUC of the drug up to the last measurable concentration
- C_{max}: Maximum concentration of the drug in the serum

The secondary PK endpoints of the study were:
- $t_{1/2}$: Terminal elimination half-life of the drug
- AUC_{0-inf}: AUC of the drug extrapolated to infinite time

Statistical methods

The alternative hypothesis of bioequivalence (H1: kL <=μT - μR <=kU), and the null hypothesis of inequivalence (Ho: μT – μR>=U or μT - μR) was expressed as the following two separate one-sided hypotheses:

Ho A: μT - μR <kL vs. H1 A: kL <=μT – μR
Ho B: μT - μR >kU vs. H1 B: μT - μR <=kU

where μT and μR represent the average bioavailability on a log scale for the test (BP14) and reference (Neulasta) products respectively and [kL, kU] defines the bioequivalence range.

Statistical analysis was performed using SAS statistical software (Version: 9.4; SAS Institute Inc, USA).

Pharmacokinetics analysis

Descriptive statistics (arithmetic mean, geometric mean, standard deviation, coefficient of variance, median, maximum and minimum) for all applicable pharmacokinetic parameters were calculated.

Ln-transformed data of Cmax and AUCt were evaluated statistically using the PROC GLM from SAS for difference due to treatment, period, sequence and subject(sequence) as fixed effects in analysis of variance (ANOVA) model. Treatment and period were to be tested using mean square error and sequence was to be tested using subject(sequence) as the error term at 5% level of significance.

Bioequivalence criteria were that the 90% CI of the relative mean (geometric least square mean) of BP14 to Neulasta for Ln-transformed Pharmacokinetic parameters Cmax and AUCt was within 80% to 125% .

Potency correction was done if the measured drug content of the lots of BP14 and Neulasta used in the study (expressed as percent of label claim) were not within 5% of each other. In this case, the results with potency correction were also presented and the conclusion of bioequivalence was based on both potency corrected and uncorrected data.

Pharmacodynamics analysis

Descriptive statistics (arithmetic mean, geometric mean, standard deviation, coefficient of variance, median, maximum and minimum) for all applicable pharmacodynamic parameters were calculated.

Ln-transformed data of Amax and AUECt were evaluated statistically using the PROC GLM from SAS for difference due to treatment, period, sequence and subject(sequence) as fixed effects using analysis of variance model.

Pharmacodynamic variables

The following pharmacodynamic parameters were determined from the time and concentration data. All Below Limit of Quantitation (BLQ) concentration values were set to zero before pharmacodynamic analysis. The actual time of blood collection for all samples were used for the calculation of pharmacodynamic parameters. All missing samples were disregarded from pharmacodynamic analysis.

The pharmacodynamic parameters of the study were:

- Amax: Maximum measured whole blood activity over the time span specified
- AUECt: The area under the whole blood activity versus time effect curve was calculated using the linear trapezoidal rule from the zero time point to the last quantifiable concentration.

Pharmacokinetic results

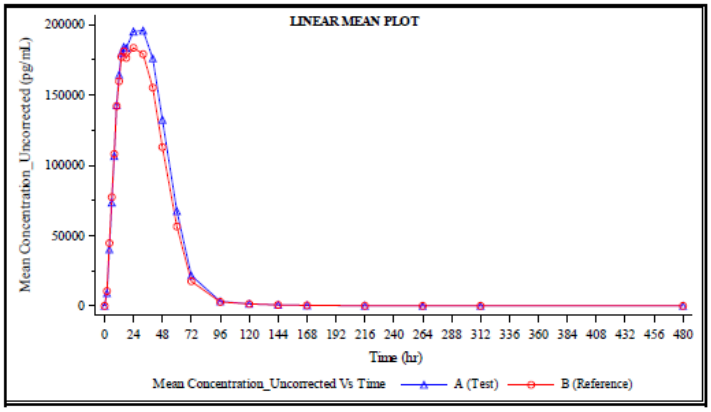
Primary pharmacokinetic analysis

Table 7. Summary of statistical analysis for serum potency - Uncorrected pharmacokinetic data of pegfilgrastim

PARAMETER	Unit	REFERENCE LEAST SQUARE MEANS Ln DATA	TEST LEAST SQUARE MEANS Ln DATA	REFERENCE GEOMETRIC MEANS	TEST GEOMETRIC MEANS
Cmax	(pg/mL)	11.982	12.046	159872.466	170349.573
AUCt	(pg/mL)*(hr)	15.643	15.732	6218523.477	6795005.565

PARAMETER	Unit	INTRA-SUBJECT CV(%)	RATIO OF GEOMETRIC MEANS	90% CONFIDENCE INTERVAL	Bioequivalence
Cmax	(pg/mL)	43.950	106.55%	(98.92%; 114.78%)	Yes
AUCt	(pg/mL)*(hr)	47.273	109.27%	(100.92%; 118.31%)	Yes

Figure 7. Linear and semi-logarithmic mean plots of pegfilgrastim for serum PK concentration data



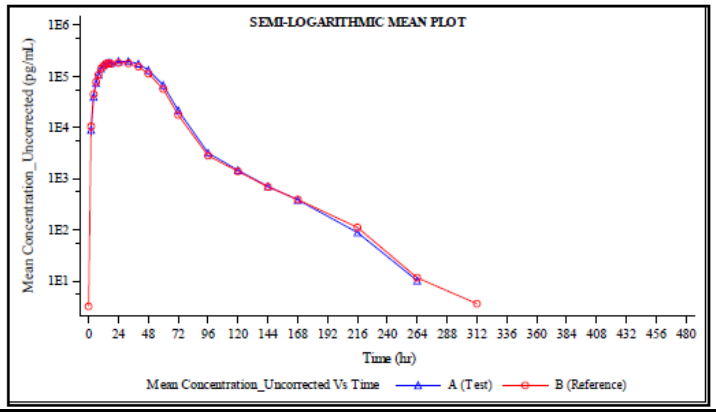


Table 8. Summary of statistical analysis for serum potency - Corrected pharmacokinetic data of pegfilgrastim

PARAMETER	Unit	REFERENCE LEAST SQUARE MEANS Ln DATA	TEST LEAST SQUARE MEANS Ln DATA	REFERENCE GEOMETRIC MEANS	TEST GEOMETRIC MEANS
Cmax	(pg/mL)	12.006	12.014	163709.405	165068.736
AUCt	(pg/mL)*(hr)	15.667	15.700	6367768.040	6584360.392

PARAMETER	Unit	INTRA-SUBJECT CV(%)	RATIO OF GEOMETRIC MEANS	90% CONFIDENCE INTERVAL
Cmax	(pg/mL)	43.950	100.83%	(93.61%; 108.61%)
AUCt	(pg/mL)*(hr)	47.273	103.40%	(95.50%; 111.95%)

Impact of Antidrug Antibodies (ADA) on Primary PK Analysis

Table 9. Summary of statistical analysis after excluding anti-peg ADA positive subjects for potency – Uncorrected pegfilgrastim

PARAMETER	Unit	REFERENCE LEAST SQUARE MEANS Ln DATA	TEST LEAST SQUARE MEANS Ln DATA	REFERENCE GEOMETRIC MEANS	TEST GEOMETRIC MEANS
Cmax	(pg/mL)	12.040	12.056	169348.476	172151.472
AUCt	(pg/mL)*(hr)	15.704	15.742	6612515.044	6867883.769

PARAMETER	Unit	INTRA-SUBJECT CV(%)	RATIO OF GEOMETRIC MEANS	90% CONFIDENCE INTERVAL	Bioequivalence
Cmax	(pg/mL)	41.884	101.66%	(94.04%;109.88%)	Yes
AUCt	(pg/mL)*(hr)	44.894	103.86%	(95.59%;112.85%)	Yes

The primary analysis based on PK data without potency correction is considered appropriate. CI for both primary parameters C_{max} (98.92% to 114.78%) and AUC_t (100.92% to 118.31%) were within the predefined acceptance range 80-125% and bioequivalence was concluded.

The applicant also performed additional analysis with potency corrected PK data for the difference in protein content between test and reference batch. This analysis also supported bioequivalence with GMs closer to 100%.

To investigate the effect of ADA on PK, the applicant performed analysis excluding ADA positive subject, which confirmed bioequivalence of the products tested. The impact of ADA on PK was minimal.

Bioequivalence was shown for both primary parameters in this study.

Meta-analysis of studies BP14-101 and BP14-102

Primary PK parameters of two Phase 1 PK/PD trials were pooled and presented per the methodology defined in the literature (Gattu S, 2021).

Table 10. Meta-analysis results for both Phase 1 PK/PD trials

PK Parameters		Study 1 (BP14-101)		Study 2 (BP14-102)		Pooled results	
		BP14	Neulasta®	BP14	Neulasta®	BP14	Neulasta®
AUC_{0-inf} (ng • h/mL) n=288	LS Geo Mean	4792.829	3993.624	7045.946	6452.612	5779.229	5101.958
	95 % CI Geo Mean	4016.722, 5718.895	3346.933, 4765.269	6214.631, 7988.464	5691.301, 7315.761	5205.466, 6416.234	4595.434, 5664.312
	GMR (90% CI)	1.200 (1.074: 1.341)		1.092 (1.009: 1.181)		**1.133 (1.062: 1.208)**	
	Intra CV%	53.551		46.976		49.609	
AUC_{0-last} (ng • h/mL) n=288	LS Geo Mean	4722.573	3886.343	7022.777	6433.253	5725.323	5028.762
	95% CI Geo Mean	3945.506, 5652.684	3246.871, 4651.758	6191.102, 7966.174	5671.393, 7297.456	5151.528, 6363.029	4524.777, 5588.883
	GMR (90%CI)	1.215 (1.082: 1.365)		1.092 (1.009: 1.181)		**1.139 (1.066: 1.217)**	
	Intra CV%	57.134		47.214		51.292	
C_{max} (ng/mL) n=288	LS Geo Mean	134.173	115.83	175.373	164.831	152.695	138.81
	95%CI Geo Mean	113.089, 159.189	97.628, 137.426	156.497, 196.526	147.089, 184.713	138.482, 168.367	125.889, 153.057
	GMR (90%CI)	1.158 (1.038: 1.292)		1.064 (0.988: 1.146)		**1.100 (1.034: 1.171)**	
	Intra CV%	53.254		44.178		47.871	

The 90% CI of pooled results meet the standard bioequivalence limits of 0.80 to 1.25 proving the robustness of data.

Estimated summary test to reference treatment ratio (summary ratio) with corresponding 90% CI was: 1.100 (1.034, 1.171) for Cmax, 1.139 (1.066, 1.217) for AUC0-t and 1.133 (1.062, 1.208) for AUC0-∞.

Based on these results, BE was concluded as the summary ratio with corresponding 90% CI for the PK parameters Cmax, AUC0-t and AUC0-∞ were within standard BE range (0.8, 1.25).

Inequivalence observed in study BP14-101 can be the consequence of underestimated value of intra-subject coefficient of variation ISCV which was 45%. Basic number of 110 subjects derived in study BP14-101 for ISCV 45% leads to statistical power lower than 80% to conclude BE with respect to standard BE range (0.8, 1.25) if more precise value of ISCV based on results for PK parameters Cmax and AUC0-t is used. After increase of ISCV to 57% for sample size calculation in study BP14-102, BE is concluded.

Meta-analysis of failed BE study BP14-101 and non-failed BE study BP14-102 concluded BE as values of ratio and corresponding 90% CI for the PK parameters Cmax, AUC0-t and AUC0-∞ in study BP-102 are more

shifted to 1 (100%) and the upper limit of 90% CI is less than the upper BE limit 1.25 compared to inequivalent results for the PK parameters from study BP-101.

2.5.2.2. Pharmacodynamics

Bioanalytical methods

BP14-101 study

ANC was determined using an XN10 & XN20-Series multi-parameter automated haematology analyser from Sysmex. White cell count (WCC) was determined by a fluorescent flowcytometry technology using a semiconductor laser. The samples were transported within a couple of hours of collection from the near Q-Pharm clinical site. Accreditation certificate by NATA/RCPA was attached. The installation qualification was performed by the Sysmex supplier. Analysers were serviced 6 monthly where calibration was also checked. Tri-level controls were run twice daily.

BD Stem Cell Enumeration Kit was used to quantify CD34+ hematopoietic and progenitor cells. This quasi-quantitative cell flow cytometry method was demonstrated to be accurate and reproducible. Stability was demonstrated for CD34+ in human whole blood (EDTA) samples and solutions under varying conditions of storage. Study samples were stored at -80°C without exceeding long-term (1281 days in polypropylene 2 mL cryo vials at -80°C) and short-term stability. Low, mid, and high control samples were analysed within each run, most of them having passed expected range. There were no protocol and/or significant SOP deviations.

BP14-102 study

ANC in whole blood K2EDTA samples were determined on XN-550 automated haematology analyser from Sysmex using flow cytometry method using semiconductor laser principle. Study samples were stored at 2 - 8°C within three days. The Sample Analysis Report was provided. SOP for complete blood count with differential count was attached to the report. There was no deviation during the study. Cytometer was calibrated every 6 months. Calibration certificate valid in time of ANC determination in the BP14-102 study was provided. All Quality Control Samples were within the acceptable limits for ANC. Incurred samples reanalysis confirmed reproducibility of the method. ANC 100 % data output was attached to the bioanalytical report.

Mechanism of action

Pegfilgrastim is polyethyleneglycol-(PEG)-ylated recombinant human granulocyte-colony stimulating factor (rhGCSF) that acts on haematopoietic cells by binding to specific cell surface receptors stimulating their proliferation and differentiation. The GCSF, is an endogenous hematopoietic growth factor that, selectively stimulates granulopoietic cells of the neutrophil lineage.

Primary and Secondary pharmacology

Study BP14-101

For details on study design see section 2.5.2.1 Pharmacokinetics.

The PD Analysis Set was defined as subjects who were randomized, received IMP and completed PD sampling in both periods without a major protocol violation with relevant impact on PD data.

Study endpoints

The primary PD endpoints of the study were:

- $AUC_{(0-t)}$: AUC of the ANC up to the last measurable concentration
- E_{max}: Maximum change from baseline for ANC

The secondary PD endpoints of the study were

- $AUC_{(0-t)}$: AUC of the absolute CD34+ cell count up to the last measurable concentration
- E_{max}: Maximum change from baseline for absolute CD34+ cell count

Pharmacodynamic results

Absolute neutrophil count (ANC) and CD34+ cell count

Geometric mean (gCV%) ANC $AUC_{(0-t)}$ was 3791 h×10^9/L (26.0%) and 3700 h×10^9/L (29.1%) for BP14 and Neulasta, respectively. Geometric mean ANC E_{max} values were 29.15×10^9/L (26.0%) and 28.30×10^9/L (27.5%) for BP14 and Neulasta, respectively.

The GMR (95% CI) for the ratio of BP14:Neulasta for ANC $AUC_{(0-t)}$ and ANC E_{max} were 1.024 (0.984, 1.065) and 1.033 (0.997, 1.070), demonstrating bioequivalence for these endpoints, as the 95% CI's were within the pre-defined bioequivalence criteria of 0.90 – 1.11.

Geometric mean (gCV%) CD34+ $AUC_{(0-t)}$ was 3997 h×cells/µL (47.3%) and 3780 h×cells/µL (51.8%) for BP14 and Neulasta, respectively. Geometric mean (gCV%) CD34+ E_{max} was 37.18×cells/µL (61.9%) and 33.50×cells/µL (64.7%) for BP14 and Neulasta, respectively.

The GMR (95% CI) for the ratio of BP14:Neulasta for CD34+ $AUC_{(0-t)}$ and CD34+ E_{max} were 1.053 (0.996, 1.113) and 1.105 (1.009, 1.211), indicating a slight increase in the CD34+ endpoints with the administration of BP14 compared with Neulasta.

Figure 8. Arithmetic Mean (± SD) of ANC Serum Concentrations (109/L) Time Data for BP14 and Neulasta - Linear Scale and Semilogarithmic Scale (PD Analysis Set)

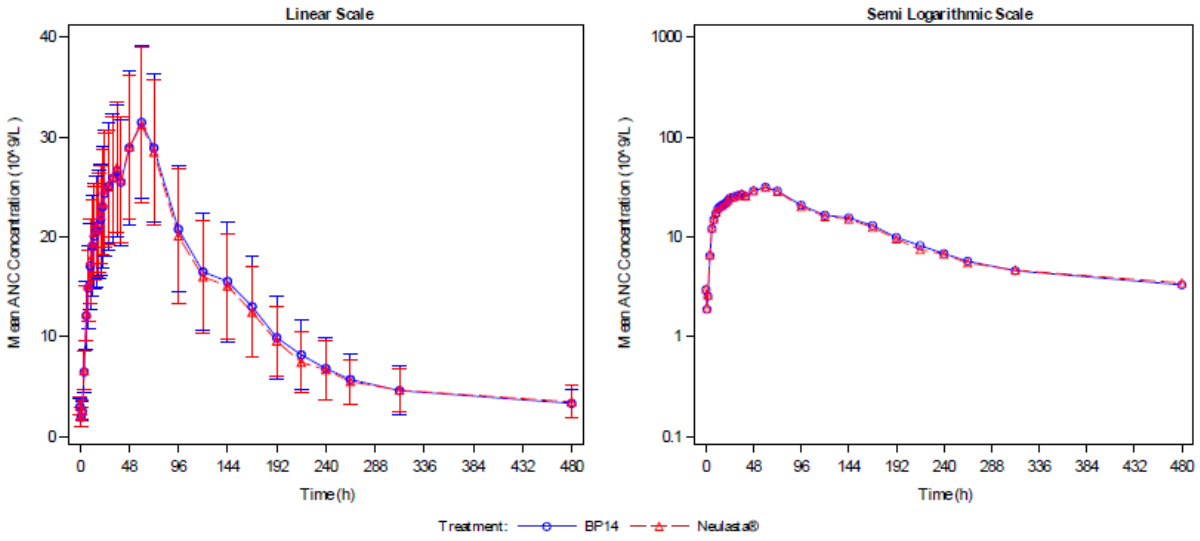


Figure 9. Arithmetic Mean (± SD) of CD34+ Serum Concentrations (cells/µL) Time Data for BP14 and Neulasta - Linear Scale and Semilogarithmic Scale (PD Analysis Set)

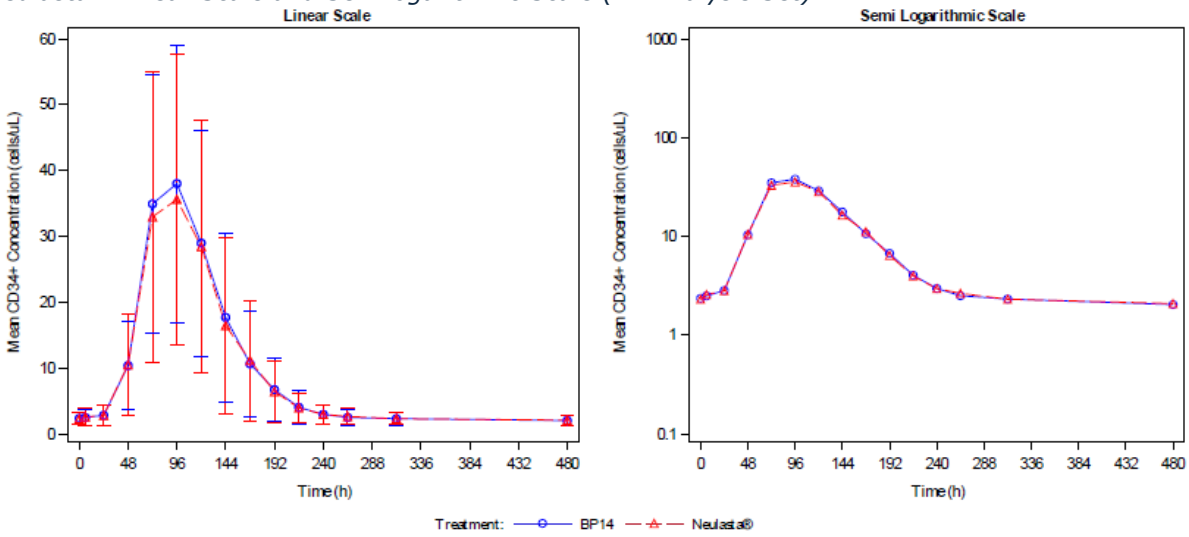


Table 11. Statistical Analysis to Assess Bioequivalence of BP14 with Neulasta (PD Analysis Set)

	BP14			Neulasta®			Ratio: BP14 / Neulasta®		
	N[1]	GM	95% CI	N[1]	GM	95% CI	GMR	95% CI	Intra Subject CV%
ANC $AUC_{(0-t)}$ ($h\times10^9/L$)	113	3789.315	(3603.352, 3984.875)	113	3701.918	(3520.243, 3892.969)	1.024	(0.984, 1.065)	15.0
ANC E_{max} ($10^9/L$)	113	29.189	(27.824, 30.621)	113	28.259	(26.938, 29.646)	1.033	(0.997, 1.070)	13.6
CD34+ $AUC_{(0-t)}$ ($h\times$ cells/µL)	102	3982.537	(3675.605, 4315.099)	102	3782.425	(3490.913, 4098.280)	1.053	(0.996, 1.113)	20.2
CD34+ E_{max} (cells/µL)	102	37.053	(33.410, 41.093)	102	33.531	(30.234, 37.187)	1.105	(1.009, 1.211)	33.7

An analysis of covariance (ANCOVA) with sequence, period and treatment as fixed effects, subject nested within sequence as random effect, and ANC (pre-dose ANC in each period) as a covariate, after logarithmic transformation of the data, was used for the statistical analysis.
[1] N: number of observations used in the model.
Abbreviations: GM = geometric mean; GMR = geometric mean ratio; CI = confidence interval; PD = pharmacodynamic; ANC = absolute neutrophil count; AUC = area under the curve; E_{max} = maximum change from baseline; CV = Coefficient of variation.

Based on PD analysis set, BE was concluded for ANC E_{max} and ANC $AUC_{(0-t)}$ with respect to ER given by (90%, 111%). In this case, corresponding 95% CI was (99.70%, 107.00%) for ANC E_{max} and (98.40%, 106.50%) for ANC $AUC_{(0-t)}$. ISCV was 13.6% for ANC E_{max} and 15.0% for ANC $AUC_{(0-t)}$.

Regarding PD parameters CD34+ E_{max} and CD34+ $AUC_{(0-t)}$, BE was not concluded for ER (90%, 111%). In this case, corresponding 95% CI was (100.90%, 121.10%) for CD34+ E_{max} and (99.60%, 111.30%) for CD34+ $AUC_{(0-t)}$. Higher levels of CD34+ for test product might corelate with higher exposure observed, also questioning biosimilarity of BP14 and Neulasta. Since CD34+ PD parameters were considered as secondary, inequivalence has less relevance in the BE assessment. ISCV was 33.7% for CD34+ E_{max} and 20.2% for CD34+ $AUC_{(0-t)}$.

Study BP14-102

For details on study design see section 2.5.2.1 Pharmacokinetics.

Pharmacodynamics analysis

Descriptive statistics (arithmetic mean, geometric mean, standard deviation, coefficient of variance, median, maximum and minimum) for all applicable pharmacodynamic parameters were calculated.

Ln-transformed data of Amax and AUECt were evaluated statistically using the PROC GLM from SAS for difference due to treatment, period, sequence and subject(sequence) as fixed effects using analysis of variance model.

Pharmacodynamic variables

Pharmacodynamic parameters were determined from the time and concentration data using SAS statistical software (Version: 9.4; SAS Institute Inc, USA). All Below Limit of Quantitation (BLQ) concentration values were set to zero before pharmacodynamic analysis. The actual time of blood collection for all samples collected were used for the calculation of pharmacodynamic parameters. All missing samples were disregarded from pharmacodynamic analysis.

The pharmacodynamic parameters of the study were:

- Amax: Maximum measured whole blood activity over the time span specified

- AUECt: The area under the whole blood activity versus time effect curve was calculated using the linear trapezoidal rule from the zero time point to the last quantifiable concentration.

Pharmacodynamic Results

Table 12. Descriptive statistics of pharmacodynamic parameters for test (BP14) and reference (Neulasta) treatment for whole blood data of pegfilgrastim (n=175)

Treatment=A

Variable	N	Mean	Std Dev	Coeff of Variation	Minimum	Maximum	Median	Geometric Mean
Amax	175	35280.629	8703.709	24.670	17130.000	62980.000	33990.000	34265.131
AUECt	175	5531027.641	1273412.227	23.023	2872770.000	10515530.000	5389733.600	5391919.377

Treatment=B

Variable	N	Mean	Std Dev	Coeff of Variation	Minimum	Maximum	Median	Geometric Mean
Amax	175	35186.114	8679.922	24.669	15380.000	65100.000	34470.000	34170.337
AUECt	175	5484181.117	1304336.299	23.784	2472204.100	11516438.900	5428772.400	5337493.110

Source: Appendix 16.2.6 (16.2.6.10)
Note:- Treatment A = Test (BP14) and B = Reference (Neulasta®)

Table 13. Summary of statistical analysis for pharmacodynamic parameters for test (BP14) and reference (Neulasta) treatment for pegfilgrastim

PARAMETER	Unit	REFERENCE LEAST SQUARE MEANS Ln DATA	TEST LEAST SQUARE MEANS Ln DATA	REFERENCE GEOMETRIC MEANS	TEST GEOMETRIC MEANS	INTRA-SUBJECT CV(%)
Amax	(/µL)	10.439	10.442	34160.837	34271.603	8.550
AUECt	(/µL)*(hr)	15.490	15.501	5336067.859	5392470.497	8.101

PARAMETER	Unit	RATIO OF GEOMETRIC MEANS	90% CONFIDENCE INTERVAL	95% CONFIDENCE INTERVAL
Amax	(/µL)	100.32%	(98.82%; 101.85%)	(98.53%; 102.15%)
AUECt	(/µL)*(hr)	101.06%	(99.62%; 102.51%)	(99.35%; 102.80%)

Based on PD analysis set, bioequivalence was concluded for ANC Amax and ANC AUC(0-t) with respect to ER given by (90%, 111%). In this case, corresponding 95% CI was (98.53%, 102.15%) for ANC Amax and (99.62%, 102.51%) for ANC AUC(0-t).

CD34+ cell counts (CD34+) is recommended as secondary endpoint but was not evaluated in this study. Considering bioequivalence was shown for primary PK and PD endpoints this issue was not further pursued.

2.5.3. Discussion on clinical pharmacology

The PK and PD properties of BP14 were compared with EU-approved Neulasta following single dose administration in healthy subjects in the two Phase I studies BP14-101 and BP14-102.

The pharmacokinetic equivalence was not shown in study BP14-101 as the confidence intervals for both primary PK parameters were outside the predefined limits of 80-125%. The 90% CI for C_{max} was (103.70%, 129.30%) and for $AUC_{(0-t)}$ was (108.10%, 136.60%). Also, the GMR estimates for both primary parameters of BP14 were greater than 1.00 (1.158 for C_{max} and 1.215 for $AUC_{(0-t)}$), indicating higher bioavailability of BP14 compared to the reference product Neulasta.

To identify the issue of non-equivalence in study BP14-101, the applicant performed several post hoc analyses. Only the exclusion of observations with high value of studentized residuals (above 3 in absolute value) led to BE of BP14 to Neulasta for the pharmacokinetic parameters C_{max} and $AUC_{(0-t)}$. However, such exclusion can only be performed when firmly supported by other aspects (e.g. bioanalytical or clinical) which are "independent" of statistical analysis. Exclusion based purely on mathematical criterion made as post-hoc analysis and driven by observed data was not endorsed and only results of the primary analysis were considered valid for bioequivalence conclusions.

The applicant also provided reference to the draft Guideline on similar biological medicinal products containing rG-CSF (EMEA/CHMP/BMWP/31329/2005 Rev 1) which states in section 5.1 that ER for PK

parameters could be extended up to (66%, 150%) but the point estimate of ratio should be also taken into account when assessing BE. However, the applicant's proposal to broader equivalence margins was not considered substantiated and not agreed. Additionally, standard confidence intervals for BE assessment were recommended during scientific advice and were predefined for statistical analysis in the study protocol.

Study BP14-102 demonstrated bioequivalence between BP14 and EU-approved Neulasta as the confidence intervals for both primary PK parameters C_{max} (98.92% to 114.78%) and AUC_t (100.92% to 118.31%) were within the predefined acceptance range 80-125%.

The primary analysis in the study BP14-102 was based on PK data without potency correction which is considered appropriate. The applicant also performed additional analysis with potency corrected PK data for the difference in protein content between test and reference batch. This analysis also supported bioequivalence with GMRs closer to 100%. To investigate the effect of ADA on PK, the applicant performed analysis excluding ADA positive subject, which confirmed bioequivalence of the products tested.

The applicant also performed a meta-analysis of studies BP14-101 and BP14-102 showing that the 90% CI of pooled results for the primary PK parameters were within the standard ER (80%, 125%). Pharmacokinetic equivalence between BP14 and Neulasta was confirmed and these results support the conclusion on bioequivalence from the main PK study BP14-102.

From a pharmacodynamic perspective, BP14 and Neulasta are equivalent with respect to the primary parameters ANC E_{max} and $AUC_{(0-t)}$ as shown in studies BP14-101 and BP14-102. Corresponding 95% CI were (99.70%, 107.00%) and (98.53%, 102.15%) for ANC E_{max} and (98.40%, 106.50%) and (99.62%, 102.51%) for ANC $AUC_{(0-t)}$ in studies BP14-101 and BP14-102, respectively.

For the secondary parameters CD34+ E_{max} and $AUC_{(0-t)}$, bioequivalence between BP14 and Neulasta was not concluded in study BP14-101, as these parameters were not in the specified equivalence range (corresponding 95% CI was (100.90%, 121.10%) for CD34+ E_{max} and (99.60%, 111.30%) for CD34+ $AUC_{(0-t)}$). Higher levels of CD34+ for the test product might corelate with higher exposure observed in the study BP14-101. Nevertheless, as these were secondary parameters, impact on bioequivalence is not considered critical.

2.5.4. Conclusions on clinical pharmacology

Based on the submitted results, biosimilarity between BP14 and the reference product Neulasta can be concluded from PK and PD perspectives.

2.5.5. Clinical efficacy

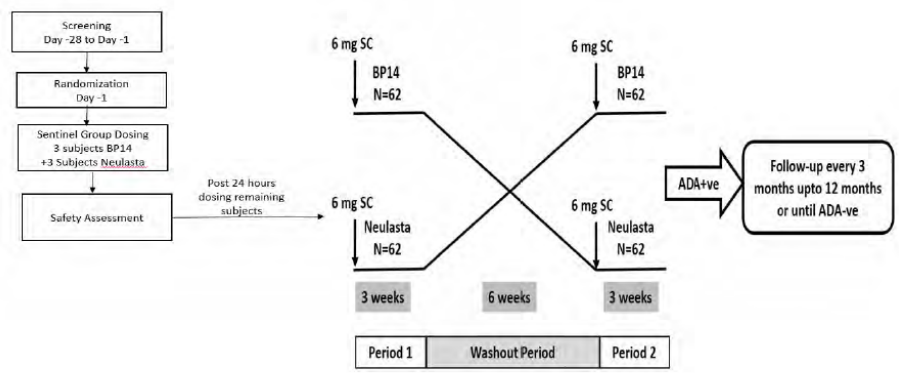
No clinical efficacy studies were conducted/submitted by the applicant.

2.5.6. Clinical safety

BP14-101 is a randomized, double-blind, comparative single-dose, 2-period crossover study to compare PK, PD, immunogenicity, and safety of BP14 with Neulasta in healthy male subjects.

Immunogenicity and comparative assessment of the safety and tolerability of BP14 and EU-Neulasta were secondary objectives of the study.

Figure 10. BP14-101 study design



Abbreviations: ADA=Anti-drug antibodies; SC=Subcutaneous

BP14-102 is a double blind, randomized, two-period, two-treatment, two-sequence, crossover, balanced, single dose study to compare pharmacokinetic, pharmacodynamic, immunogenicity, and safety in healthy adult male subjects. The study was conducted with 184 subjects (randomized in a 1:1 ratio). Subjects were randomized to one of the two sequences: either AB or BA (A – test product BP14, B – reference product EU-Neulasta).

Table 14. Schedule of assessments

Day	Proposed time relative to dosing	Details of events	Applicable to
-28	Within 28 days prior to dosing	Informed consent document presentation for screening and Screening of volunteers	P- 1
-1	When volunteers report to facility	Attendance	Each period
	Prior to Check-in	Study specific Informed consent document presentation	P- 1
		Body weight measurement	Each period
		Urine scans for drugs of abuse and alcohol test	
		Physical examination (Clinical examination), vital signs recording & well-being assessments and Injection site assessment	

Day	Proposed time relative to dosing	Details of events	Applicable to
		Compliance check	
		Total WBC count, SGPT, SGOT, Serum creatinine measurement	
		Inclusion Criteria and Exclusion Criteria assessment	P- 1
	At least 11 hours prior to dosing	Subjects check-in	Each period
-1, 0, 1 & 2	Check-in to 48 hours post dose	Housing duration	
-1	After check-in	Dinner	
0	Prior to dosing	Pre-dose vital signs recording and well-being assessments	
		Cannulation	
	0 hour	Dosing	
	At least 04 hours post-dose	Posture Restriction - Supine position	
0 to 07, 09, 11, 13 & 20	At pre-dose (0.0 hour) and at 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 24.0, 32.0, 40.0, 48.0, 60.0, 72.0 (Day 3), 96.0 (Day 4), 120.0 (Day 5), 144.0 (Day 6), 168.0 (Day 7), 216.0 (Day 09), 264.0 (Day 11), 312.0 (Day 13) and 480.0 (Day 20) hours post dose	Collection of 3.5 mL each venous blood samples for Pharmacokinetic assessment in labelled gel clot activator tubes Compliance assessment and well-being assessments will be performed during ambulatory samples	
0, 1 & 20	At pre-dose (0.0 hour) and at 24.0 (Day 1) and **480.0 (Day 20)** hours post dose	Collection of 5.0 mL each venous blood samples for Immunogenicity assessment in labelled gel clot activator tubes Compliance assessment and well-being assessments will be performed during ambulatory samples	
0 to 07, 09, 11, 13 & 20	At pre-dose (0.0 hour) and at 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 24.0, 32.0, 40.0, 48.0, 60.0, 72.0 (Day 3), 96.0 (Day 4), 120.0 (Day 5), 144.0 (Day 6), 168.0 (Day 7), 216.0 (Day 09), 264.0 (Day 11), 312.0 (Day 13) and 480.0 (Day 20) hours post dose	Collection of 2.0 mL each venous blood samples for Pharmacodynamic assessment of absolute neutrophil count in labelled K_2EDTA-vacutainers Compliance assessment and well-being assessments will be performed during ambulatory samples	

Day	Proposed time relative to dosing	Details of events	Applicable to
0 &1	At 1.0 & 2.0 (± 30 minutes) hours post dose and at 4.0, 10.0, 23.0 & 35.0 hours (± 40 minutes) post dose	Post-dose vital signs recording and well-being assessments	Each period
	Meals will be provided at appropriate times	Meals like lunch, snacks, dinner and breakfast	
0 & 1	At 1.0, 2.0, 4.0, 10.0, 23.0 and 35.0 hours (± 40 minutes) post dose	Injection site assessment	
1 & 2	At about 16.0, 32.0 and 48.0 hours post dose	Well-being assessments	
2	Prior to check-out	Physical examination (Clinical examination), vital signs recording, well-being assessments and Injection site assessment and 12-lead ECG recording	
	After 48.0 hours post dose	Check-out	
7	At 168.0 hour post dose	Total WBC count measurement	
20	At about 480.0 hours post dose	1)Physical examination (Clinical examination), vital signs recording, well-being assessments and 12-lead ECG recording 2) Collection of blood for end study laboratory assessment	P-2 (End study)

2.5.6.1. Patient exposure

BP14-101 Study

Table 15. Exposure of subjects to investigation products (BP14-101 Study)

BP14		Neulasta®	
Treatment Period 1	62	Treatment Period 1	62
Treatment Period 2	58	Treatment Period 2	55
Total	**120**		**117**

Table 16. Summary of demographic characteristics (safety analysis set- BP14-101 Study)

Characteristics	Sequence AB (N=62) n (%)	Sequence BA (N=62) n (%)	Overall (N=124) n (%)
Age (Years)			
N	62	62	124
Mean	29.5	30.3	29.9
SD	8.80	9.24	9.00
Median	28.0	28.0	28.0
Min, max	18, 54	18, 55	18, 55
Sex			
Male	62 (100)	62 (100)	124 (100)
Race			
American Indian or Alaska Native	0	1 (1.6)	1 (0.8)
Asian	10 (16.1)	10 (16.1)	20 (16.1)
Black or African American	1 (1.6)	3 (4.8)	4 (3.2)
Native Hawaiian or Other Pacific Islander	2 (3.2)	1 (1.6)	3 (2.4)
White	47 (75.8)	45 (72.6)	92 (74.2)
Other	2 (3.2)	2 (3.2)	4 (3.2)
Ethnicity			
Hispanic or Latino	8 (12.9)	7 (11.3)	15 (12.1)
Not Hispanic or Latino	50 (80.6)	46 (74.2)	96 (77.4)
Not Stated	3 (4.8)	6 (9.7)	9 (7.3)
Unknown	1 (1.6)	3 (4.8)	4 (3.2)
Weight (kg)			
n	62	62	124
Mean	79.46	79.57	79.51
SD	13.196	10.162	11.729
Median	80.05	79.70	80.05
Min, max	50.0, 109.9	59.1, 102.1	50.0, 109.9
Height (cm)			
n	62	62	124
Mean	178.7	178.1	178.4
SD	7.11	7.10	7.08
Median	179.0	177.0	178.0
Min, max	163, 200	163, 196	163, 200
BMI (kg/m^2)			
n	62	62	124
Mean	24.83	25.07	24.95
SD	3.547	2.873	3.217
Median	24.90	25.05	24.90
Min, max	18.5, 31.7	19.5, 32.0	18.5, 32.0

Treatment A: BP14; Treatment B: Neulasta®; BMI: body mass index, calculated as weight (kg)/(height [m])^2; max: maximum; min: minimum; SD: standard deviation; n: The number of subjects in specific category; N: The number of subjects in the safety analysis set; %: Calculated using the number of subjects in the safety analysis set as the denominator (n/N*100)

Subjects were between the ages of 18 and 55 years old (median 28.0 years). The majority of subjects were White (92/124 [74.2%] subjects) and were Not Hispanic or Latino (96/124 [77.4%] subjects). Subject characteristics, including height, weight, and BMI, were generally well balanced between the treatment sequences.

A total of 27/124 (21.8%) subjects (13/62 [21.0%] subjects in treatment sequence AB and 14/62 [22.6%] subjects in treatment sequence BA) were reported to have received at least one prior medication. The prior medications used by at least 2 subjects (≥ 1%) included vitamins (not otherwise specified) (9/124 [7.3%] subjects), paracetamol, cetirizine hydrochloride, and vitamin D (not otherwise specified) (3/124 [2.4%] subjects each), and zinc, salbutamol, ibuprofen, creatine, fish oil, magnesium, and ascorbic acid (2/124 [1.6%] subjects each).

Overall, 105/124 (84.7%) subjects received concomitant medication during the study. The most frequently used concomitant medications during the study were paracetamol (99/124 [79.8%] subjects) and ibuprofen (51/124 [41.1%] subjects).

The use of ibuprofen was noted to be higher in subjects randomized to treatment sequence AB (51.5%) than in subjects randomized to treatment sequence BA (30.5%).

There was no major imbalance in the use of any other prior or concomitant medications across treatment sequences.

No prohibited concomitant medication was taken by any subject during the course of the study.

BP14-102 Study

Table 17. Extend of exposure of all dosed subjects (BP14-101 Study)

BP14 (Treatment A)		**Neulasta® (Treatment B)**	
Treatment Period 1	92	Treatment Period 1	92
Treatment Period 2	87	Treatment Period 2	88
Total	179	**Total**	180

Table 18. Summary of mean demographic data (±SD) (BP14-102 Study)

		Subjects Dosed (N=184)			Subjects Completed (N=175)		
Gender	Males	All (N = 184)	Sequence AB (N = 92)	Sequence BA (N = 92)	All (N = 175)	Sequence AB (N = 88)	Sequence BA (N = 87)
Height (cm)	Mean (±SD)	166.9 (± 5.6)	166.8 (± 5.5)	166.9 (± 5.7)	166.8 (± 5.6)	166.9 (± 5.6)	166.6 (± 5.6)
	Median	167.0	167.0	166.8	166.5	167.0	166.0
	Min	153.0	153.0	155.5	153.0	153.0	155.5
	Max	188.0	180.5	188.0	188.0	180.5	188.0
Weight (kg)	Mean (±SD)	66.1 (± 9.4)	66.1 (± 9.4)	66.1 (± 9.4)	65.7 (± 9.4)	65.8 (± 9.4)	65.6 (± 9.4)
	Median	65.0	65.6	64.3	64.4	65.4	64.2
	Min	50.2	50.5	50.2	50.2	50.5	50.2
	Max	86.2	86.2	85.0	86.2	86.2	85.0
Race	Asian	184 (100%)	92 (50%)	92 (50%)	175 (100%)	88 (50.29%)	87 (49.71%)
Ethnicity	Not Hispanic/Latino	184 (100%)	92 (50%)	92 (50%)	175 (100%)	88 (50.29%)	87 (49.71%)
BMI (kg/m^2)	Mean (±SD)	23.7 (± 3.1)	23.8 (± 3.1)	23.7 (± 3.2)	23.6 (± 3.1)	23.6 (± 3.0)	23.6 (± 3.2)
	Median	23.7	23.8	23.5	23.4	23.7	23.1
	Min	18.7	18.7	18.7	18.7	18.7	18.7
	Max	29.8	29.8	29.8	29.8	29.8	29.8
Age (Years)	Mean (±SD)	36 (± 8)	36 (± 8)	36 (± 8)	36 (± 8)	36 (± 8)	36 (± 8)
	Median	36	36	36	36	36	36
	Min	19	20	19	19	20	19
	Max	52	52	52	52	52	52

All subjects' demographic data were found normal according to the protocol.

Study was conducted on Asian subjects and all subjects were selected based on the absence of any clinically significant findings on the medical history, medication history, family medical history, vital signs and well-being, comprehensive physical examination, ECG, chest X-ray recordings (within past six months), ultrasonography abdomen and clinical laboratory evaluations performed within 28 days of initial study dosing. The Investigator evaluated all the laboratory values individually. All were determined to be non-reactive, normal, negative or not clinically significant for those subjects enrolled in the study.

No subject had medical history (except one subject, who had minor burn with burn mark on right upper limb and another subject who had appendicectomy, and both subjects were declared as clinically not significant by physician), family medical history and medication history during screening.

2.5.6.2. Adverse events

BP14-101

Adverse events (AE) were coded using the MedDRA Version 24.0. Unless specified otherwise, all AE summaries included TEAEs only and AE summary counts of AEs were the number of subjects reporting AEs and not the number of events reported.

Table 19. Overview of Adverse Events (Safety Analysis Set)

Category	BP14 (N=120) n (%) E	Neulasta® (N=117) n (%) E	Overall(N=124) n (%) E
Adverse events	113 (94.2) 220	107 (91.5) 252	120 (96.8) 472
TEAEs	113 (94.2) 218	107 (91.5) 250	120 (96.8) 468
Severity			
Mild	113 (94.2) 219	106 (90.6) 247	120 (96.8) 466
Moderate	1 (0.8) 1	5 (4.3) 5	6 (4.8) 6
Severe	0	0	0
CTCAE Toxicity grade			
Grade 1: Mild	113 (94.2) 218	106 (90.6) 248	120 (96.8) 466
Grade 2: Moderate	1 (0.8) 1	3 (2.6) 3	4 (3.2) 4
Grade 3: Severe or medically significant	1 (0.8) 1	0	1 (0.8) 1
Grade 4: Life-threatening or disabling	0	1 (0.9) 1	1 (0.8) 1
Grade 5: Death related to AE	0	0	0
Serious TEAEs			
Yes	0	0	0
No	113 (94.2) 218	107 (91.5) 250	120 (96.8) 468
Relationship to study treatment			
Probably related	51 (42.5) 58	57 (48.7) 66	78 (62.9) 124
Possibly related	73 (60.8) 104	81 (69.2) 121	102 (82.3) 225
Unlikely related	33 (27.5) 40	30 (25.6) 39	54 (43.5) 79
Not related	14 (11.7) 18	23 (19.7) 26	33 (26.6) 44
Action taken with study treatment			
Dose increased	0	0	0
Dose reduced	0	0	0
Dose not changed	90 (75.0) 164	83 (70.9) 161	115 (92.7) 325
Drug interrupted	0	0	0
Drug withdrawn	1 (0.8) 1	0	1 (0.8) 1
Unknown*	0	1 (0.9) 1	1 (0.8) 1
Not applicable	39 (32.5) 55	52 (44.4) 90	74 (59.7) 145
TEAEs outcome			
Death related to adverse event	0	0	0
Not recovered or not resolved	3 (2.5) 3	4 (3.4) 5	7 (5.6) 8
Recovered or resolved	113 (94.2) 216	106 (90.6) 245	120 (96.8) 461
Recovered or resolved with sequelae	0	0	0
Recovering or resolving	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Unknown	0	1 (0.9) 1	1 (0.8) 1

n: number of subjects reporting at least one AE in each category; N: The number of subjects in the Safety Analysis Set; E: number of events.
Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100).
All AEs were coded using MedDRA version 24. TEAEs include any AEs occurring or worsening after the first dose of study medication.; All AEs occurring in washout period are counted under Period 1.

Abbreviations: AE = adverse event; CTCAE = Common Terminology Criteria for Adverse Events; MedDRA = Medical Dictionary for Regulatory Activities; TEAE: treatment emergent adverse event
*Action taken with the study drug is "dose not changed"; Refer to Section 12.1.3

A total of 472 AEs, out of which 468 TEAEs, were reported in 120/124 (96.8%) subjects; 220 AEs reported in 113/120 (94.2%) subjects exposed to BP14 and 252 AEs reported in 107/117 (91.5%) subjects exposed to Neulasta. There were no major differences in number of TEAEs reported between the subjects exposed to the 2 IMPs.

Most TEAEs were considered to be mild and Grade 1 in severity. No TEAEs of Grade 5 severity or severe intensity were reported during the study.

No serious TEAEs or deaths were reported during the study in both treatment groups.

No action was taken with the IMP due to TEAEs in most subjects (325 events in 115/124 [92.7%] subjects; 164 events in 90/120 [75.0%] subjects exposed to BP14 and 161 events in 83/117 [70.9%] exposed to Neulasta). BP14 was permanently withdrawn due a TEAE in one subject.

A total of 461 events in 120/124 (96.8%) subjects (216 events in 113/120 [94.2%] subjects exposed to BP14 and 245 events in 106/117 [90.6%] subjects exposed to Neulasta) had resolved by the end of the study. Overall, 8 events in 7/124 (5.6%) subjects (3 events in 3/120 [2.5%] subjects exposed to BP14 and 5 events in 4/117 [3.4%] subjects exposed to Neulasta) had not resolved by the end of the study.

Table 20. Summary of Treatment-Emergent Adverse Events Reported in ⩾ 1% Subjects by Preferred Term (Safety Analysis Set)

System Organ Class/Preferred Term	BP14 (N=120) n (%)	Neulasta® (N=117) n (%)	Overall (N=124) n (%)
Cardiac disorders			
Palpitations	2 (1.7) 2	1 (0.9) 1	3 (2.4) 3
Gastrointestinal disorders			
Abdominal pain	2 (1.7) 2	4 (3.4) 4	6 (4.8) 6
Abdominal pain lower	2 (1.7) 2	1 (0.9) 1	3 (2.4) 3
Abdominal pain upper	3 (2.5) 3	1 (0.9) 1	4 (3.2) 4
Diarrhoea	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Dry mouth	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Gastrooesophageal reflux disease	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Nausea	1 (0.8) 1	2 (1.7) 2	2 (1.6) 3
Vomiting	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
General disorders and administration site conditions			
Catheter site pain	1 (0.8) 1	2 (1.7) 2	3 (2.4) 3
Chest pain	0	2 (1.7) 2	2 (1.6) 2
Fatigue	4 (3.3) 4	0	4 (3.2) 4
Injection site bruising	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Injection site erythema	0	3 (2.6) 3	3 (2.4) 3
Injection site pain	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Injection site reaction	0	4 (3.4) 4	4 (3.2) 4
Malaise	2 (1.7) 2	2 (1.7) 2	3 (2.4) 4
Non-cardiac chest pain	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Pyrexia	2 (1.7) 2	0	2 (1.6) 2
Vessel puncture site bruise	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Infections and infestations			
Upper respiratory tract infection	1 (0.8) 1	2 (1.7) 2	3 (2.4) 3

System Organ Class/Preferred Term	BP14 (N=120) n (%)	Neulasta® (N=117) n (%)	Overall (N=124) n (%)
Musculoskeletal and connective tissue disorders			
Back pain	48 (40.0) 48	45 (38.5) 47	72 (58.1) 95
Bone pain	42 (35.0) 42	44 (37.6) 44	64 (51.6) 86
Musculoskeletal chest pain	0	5 (4.3) 5	5 (4.0) 5
Musculoskeletal pain	1 (0.8) 1	1 (0.9) 1	2 (1.6) 2
Myalgia	4 (3.3) 4	3 (2.6) 3	7 (5.6) 7
Neck pain	4 (3.3) 4	1 (0.9) 1	5 (4.0) 5
Pain in extremity	3 (2.5) 3	2 (1.7) 2	5 (4.0) 5
Spinal pain	3 (2.5) 3	0	3 (2.4) 3
Nervous system disorders			
Dizziness	4 (3.3) 4	4 (3.4) 4	7 (5.6) 8
Headache	44 (36.7) 48	63 (53.8) 67	72 (58.1) 115
Lethargy	3 (2.5) 3	1 (0.9) 1	3 (2.4) 4
Respiratory, thoracic and mediastinal disorders			
Oropharyngeal pain	0	2 (1.7) 2	2 (1.6) 2
Skin and subcutaneous tissue disorders			
Night sweats	2 (1.7) 2	0	2 (1.6) 2
Rash	2 (1.7) 2	5 (4.3) 5	7 (5.6) 7
Vascular disorders			
Haematoma	2 (1.7) 2	0	2 (1.6) 2

n: number of subjects reporting at least one AE in each category; N: The number of subjects in the Safety Analysis Set; E: number of events.
Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100).
All AEs were coded using MedDRA version 24; All AEs occurring in washout period are counted under Period 1.
Treatment emergent adverse events (TEAEs) include any AEs occurring or worsening on or after the first dose of study medication.
Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities

The TEAEs that were reported ≥ 5% of subjects included:

- Headache (115 events in 72/124 [58.1%] subjects in the overall group; 48 events in 44/120 [36.7%] subjects exposed to BP14 and 67 events in 63/117 [53.8%] subjects exposed to Neulasta).
- Back pain (95 events in 72/124 [58.1%] subjects in the overall group; 48 events in 48/124 [40.0%] subjects exposed to BP14 and 47 events in 45/117 [38.5%] subjects exposed to Neulasta).
- Bone pain (86 events in 64/124 [51.6%] subjects in the overall group; 42 events in 42/120 [35.0%] subjects exposed to BP14 and 44 events in 44/117 [37.6%] subjects exposed to Neulasta).
- Dizziness (8 events in 7/124 [5.6%] subjects in the overall group; 4 events in 4/120 [3.3%] subjects exposed to BP14 and 4 events in 4/117 [3.4%] subjects exposed to Neulasta).
- Rash (7 events in 7/124 [5.6%] subjects in the overall group; 2 events in 2/120 [1.7%] subjects exposed to BP14 and 5 events in 5/117 [4.3%] subjects exposed to Neulasta).
- Myalgia (7 events in 7/124 [5.6%] subjects in the overall group; 4 events in 4/120 [3.3%] subjects exposed to BP14 and 3 events in 3/117 [2.6%] subjects exposed to Neulasta).

While the incidence of headache and rash were marginally higher in subjects exposed to Neulasta when compared to subjects exposed to BP14, there were no major imbalance in incidence of other TEAEs reported.

Toxicity and Severity of Adverse Events:

Overall, a total of 466 events in 120/124 (96.8%) subjects were considered to be of Grade 1 and mild in severity. Four TEAEs in 4/124 (3.2%) subjects were assessed to be of Grade 2 severity which included one event each of injection site erythema, non-cardiac chest pain, back pain, and headache, all in subjects exposed to Neulasta. One non-serious TEAE of increased AST in a subject exposed to BP14 was considered to be of Grade 3 toxicity and moderate severity. 21 days after BP14 administration, during the Treatment Period 1, the subject's AST was 296 U/L (normal range: 10 to 45 U/L). On the same day, the subject's ALT and bilirubin were 183 U/L (normal range: 5 to 45 U/L) and 23 µmol/L (normal range: 0 to 19 µmol/L), respectively. No treatment was reported for this event. Ten days later, during an unscheduled visit, the subject's AST and bilirubin had returned to normal at 36 U/L and 16 µmol/L, respectively and ALT had decreased to 48 U/L. On the same day, the event of increased AST was considered to be resolved. The Investigator assessed this event to be probably related to IMP. The subject proceeded to receive Neulasta during Treatment Period 2 as per schedule on 14 Oct 2020.

One non-serious TEAE of neutropenia in a subject exposed to Neulasta was considered of Grade 4 toxicity and mild severity. The subject received Neulasta during the Treatment Period 1, the post 1-hour ANC was 0.22×10^9/L (normal range: 1.8×10^9/L to 7.7×10^9/L). No treatment was reported for the event. The subsequent ANC values on the same day at 2-hour post dose and 4-hour post dose were 1.84×10^9/L and 5.71×10^9/L, respectively, which were within normal range, and the event was considered to be resolved on the same day. The Investigator assessed this event to be probably related to IMP. The subject proceeded to receive BP14 during Treatment Period 2 as per schedule.

Six TEAEs in 6/124 (4.8%) subjects were of moderate severity, including 2 events of headache, one event each of injection site erythema, non-cardiac chest pain, and hand fracture (all in subjects exposed to Neulasta) and one event of increased AST in a subject exposed to BP14.

Injection Site Reactions

A total of 12 TEAEs of injection site reactions (bruising, erythema, pain, reaction, and warmth) in 12 subjects were reported in the study; 2 TEAEs of injection site reactions were reported in 2 subjects exposed to BP14 and 10 TEAEs of injection site reactions were reported in 10 subjects exposed to Neulasta.

One TEAE each of injection site bruising and injection site pain was reported in 2 subjects exposed to BP14.

One TEAE each of injection site bruising, injection site pain, and injection site warmth, 3 TEAEs of injection site erythema, and 4 TEAEs of injection site reaction were reported in 10 subjects exposed to Neulasta.

Overall, the incidence of injection site reactions was lower in subjects exposed to BP14 when compared to subjects exposed to Neulasta.

Others

One non-serious TEAE of insomnia in a subject exposed to Neulasta was considered to be of Grade 1 toxicity and mild severity. 2 days post receipt of Neulasta during the Treatment Period 1, an AE of insomnia was reported. The subject received treatment with herbal medication (nature's own complete sleep) for the event. The event resolved 7 days later. The Investigator assessed this event to be unlikely related to IMP. Action taken with the IMP was incorrectly reported as "unknown" instead of "dose not changed". However, the subject proceeded to receive BP14 during Treatment Period 2 as per schedule.

Table 21. Comparison of Adverse Events Reported in BP14-101 Study and Neulasta SmPC

Preferred Term	BP14 (N=120), n (%)	Neulasta® (N=117), n (%)	SmPC
Palpitations	2 (1.7) 2	1 (0.9) 1	Not reported
Abdominal pain	2 (1.7) 2	4 (3.4) 4	Not reported
Abdominal pain lower	2 (1.7) 2	1 (0.9) 1	Not reported
Abdominal pain upper	3 (2.5) 3	1 (0.9) 1	Not reported
Diarrhoea	1 (0.8) 1	1 (0.9) 1	Not reported
Dry mouth	1 (0.8) 1	1 (0.9) 1	Not reported
Gastroesophageal reflux disease	1 (0.8) 1	1 (0.9) 1	Not reported
Nausea	1 (0.8) 1	2 (1.7) 2	Very common
Vomiting	1 (0.8) 1	1 (0.9) 1	Not reported
Catheter site pain	1 (0.8) 1	2 (1.7) 2	Common
Chest pain	0	2 (1.7) 2	Common
Fatigue	4 (3.3) 4	0	Not reported
Injection site bruising	1 (0.8) 1	1 (0.9) 1	Common
Injection site erythema	0	3 (2.6) 3	Common
Injection site pain	1 (0.8) 1	1 (0.9) 1	Common
Injection site reaction	0	4 (3.4) 4	Uncommon
Malaise	2 (1.7) 2	2 (1.7) 2	Not reported
Non-cardiac chest pain	1 (0.8) 1	1 (0.9) 1	Common
Pyrexia	2 (1.7) 2	0	Not reported
Vessel puncture site bruise	1 (0.8) 1	1 (0.9) 1	Not reported
Upper respiratory tract infection	1 (0.8) 1	2 (1.7) 2	Uncommon
Back pain	48 (40.0) 48	45 (38.5) 47	Very common
Bone pain	42 (35.0) 42	44 (37.6) 44	Very common
Musculoskeletal chest pain	0	5 (4.3) 5	Common
Musculoskeletal pain	1 (0.8) 1	1 (0.9) 1	Common
Myalgia	4 (3.3) 4	3 (2.6) 3	Common
Neck pain	4 (3.3) 4	1 (0.9) 1	Common
Pain in extremity	3 (2.5) 3	2 (1.7) 2	Common
Spinal pain	3 (2.5) 3	0	Common
Dizziness	4 (3.3) 4	4 (3.4) 4	Not reported
Headache	44 (36.7) 48	63 (53.8) 67	Very common
Lethargy	3 (2.5) 3	1 (0.9) 1	Not reported
Oropharyngeal pain	0	2 (1.7) 2	Not reported
Night sweats	2 (1.7) 2	0	Not reported
Rash	2 (1.7) 2	5 (4.3) 5	Reported in literature
Haematoma	2 (1.7) 2	0	Not reported

BP14-102

Table 22. Analysis of Adverse Event (Safety Analysis Set)

Category	BP14 (Treatment A) (N=179) n (%) E	Neulasta® (Treatment B) (N=180) n (%) E	Overall (N=184) n (%) E
Adverse events			
TEAEs	37 (20.67%) 39	47 (26.11%) 49	69 (37.50%) 88
Severity			
Mild	23 (12.85%) 23	22 (12.22%) 24	40 (21.74 %) 47
Moderate	16 (8.94%) 16	25 (13.89%) 25	36 (19.57%) 41
Severe	00	00	00
Serious TEAEs			
Yes	00	00	00
No	37 (20.67%) 39	47 (26.11%) 49	69 (37.50%) 88
Relationship to study treatment			
Certain related	00	00	00
Probable/Likely related	00	00	00
Possible related	28 (15.64%) 30	44 (24.44%) 44	60 (32.61%) 74
Unlikely related	09 (5.03%) 09	05 (2.78%) 05	14 (7.61%) 14
Not related	00	00	00
Action taken			
Dose increased	00	00	00
Dose reduced	00	00	00
Drug interrupted	00	00	00
Not applicable	20 (11.17%) 21	24 (13.33%) 26	44 (23.91%) 47
Unknown/Lost to follow up	00	00	00
Dose not changed	17 (9.50%) 18	21 (11.67%) 21	38 (20.65%) 39
Dose withdrawn	00	02 (1.11%) 02	02 (1.09%) 02
Outcome			
Fatal	00	00	00
Not recovered/Not resolved	00	00	00
Recovered/Resolved	37 (20.67%) 39	47 (26.11%) 49	69 (37.50%) 88
Recovered/Resolved with sequelae	00	00	00
Recovering/Resolving	00	00	00
Unknown	00	00	00

Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities; TEAE: treatment-emergent adverse event

n= number of subjects reporting at least one AE in each category; N= Number of subjects dosed for each treatment (Overall N= Number of subjects in the Safety Analysis Set); E = number of events. Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100). All AEs were coded using MedDRA version 26.0 and 26.1. TEAEs include any AEs occurring or worsening after the first dose of study medication.

A total of 88 AEs were reported by 69 subjects during the entire study. Forty-seven (47) AEs were mild in severity and 41 AEs were moderate in severity. There were no AEs reported in the study with severe in intensity. The proportion of subjects with mild TEAEs per treatment group was 12.85% for BP14 and 12.22% for Neulasta. The proportion of subjects with moderate TEAEs was 8.94% for BP14 and 13.89% for Neulasta.

The moderate TEAEs by PT that had a higher incidence (≥ 8.94% of subjects in either treatment group) were 16 events (8.94%) and 25 events (13.89%), reported in the BP14 and Neulasta group respectively.

Change of dose of study drug was not needed for 18 adverse events and action was not required for 21 adverse events following administration of BP14. Change of dose of study drug was not needed for 21 adverse events, dose of study drug was withdrawn for 2 adverse events and action was not required for 26 adverse events following administration of Neulasta.

Eighty-eight (88) adverse events were reported in the study and were Recovered/Resolved.

There were no injection site reactions reported in the study.

Table 23. Summary of Treatment-Emergent Adverse Events by System Organ Class and Preferred Term (Safety Analysis Set)

System Organ Class/Preferred Term	BP14 (Treatment A) (N=179) n (%) E	Neulasta® (Treatment B) (N=180) n (%) E	Overall (N=184) n (%) E
General disorders and administration site conditions			
Pain	12 (6.70%) 12	25 (13.89%) 25	34 (18.48%) 37
Musculoskeletal and connective tissue disorders			
Backpain	16 (8.94%) 17	15 (8.33%) 15	27 (14.67%) 32
Pain in extremity	00	01 (0.56%) 01	01 (0.54%) 01
Investigations			
Alanine Aminotransferase Increased	01 (0.56%) 01	02 (1.11%) 02	03 (1.63%) 03
Blood Bilirubin Increased	03 (1.68%) 03	02 (1.11%) 02	05 (2.72%) 05
Blood Glucose Increased	03 (1.68%) 03	02 (1.11%) 02	05 (2.72%) 05
Lymphocyte Percentage Decreased	01 (0.56%) 01	00	01 (0.54%) 01
White Blood Cell Count Decreased	01 (0.56%) 01	00	01 (0.54%) 01
Blood Creatinine Increased	00	01 (0.56%) 01	01 (0.54%) 01
Cardiac disorders			
Sinus Tachycardia	01 (0.56%) 01	00	01 (0.54%) 01
Gastrointestinal disorders			
Diarrhoea	00	01 (0.56%) 01	01 (0.54%) 01

Source: Appendix 16.2.7

Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities

n: number of subjects reporting at least one AE in each category; N: Number of subjects dosed for each treatment (Overall N= Number of subjects in the Safety Analysis Set); E: Number of events.

Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100).

All AEs were coded using MedDRA version 26.0 and 26.1.

Treatment-emergent adverse events (TEAEs) include any AEs occurring or worsening on or after the first dose of study medication.

Table 24. Summary of Treatment-Emergent Adverse Events by System Organ Class and Preferred Term by severity (Safety Analysis Set)

System Organ Class/Preferred Term	BP14 (Treatment A) (N=179) n (%) E	Neulasta® (Treatment B) (N=180) n (%) E	Overall (N=184) n (%) E
AEs severity (Mild)			
General disorders and administration site conditions			
Pain	08 (4.47%) 08	13 (7.22%) 13	21 (11.41%) 21
Musculoskeletal and connective tissue disorders			
Back-pain	05 (2.79%) 05	03 (1.67%) 03	08 (4.35%) 08
Pain in extremity	00	01 (0.56%) 01	01 (0.54%) 01
Investigations			
Blood Creatinine Increased	00	01 (0.56%) 01	01 (0.54%) 01
Alanine Aminotransferase Increased	01 (0.56%) 01	02 (1.11%) 02	03 (1.63%) 03
Blood Bilirubin Increased	03 (1.68%) 03	02 (1.11%) 02	05 (2.72%) 05
Blood Glucose Increased	03 (1.68%) 03	02 (1.11%) 02	05 (2.72%) 05
Lymphocyte Percentage Decreased	01 (0.56%) 01	00	01 (0.54%) 01
White Blood Cell Count Decreased	01 (0.56%) 01	00	01 (0.54%) 01
Cardiac disorders			
Sinus Tachycardia	01 (0.56%) 01	00	01 (0.54%) 01
AEs severity (Moderate)			
General disorders and administration site conditions			
Pain	04 (2.23%) 04	12 (6.67%) 12	16 (8.70%) 16
Musculoskeletal and connective tissue disorders			
Back-pain	12 (6.70%) 12	12 (6.67%) 12	21 (11.41%) 24
Gastrointestinal disorders			
Diarrhoea	00	01 (0.56%) 01	01 (0.54%) 01

Source: Appendix 16.2.7

Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities

n: number of subjects reporting at least one AE in each category; N: Number of subjects dosed for each treatment (Overall N= Number of subjects in the Safety Analysis Set); E: Number of events.

Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100).

All AEs were coded using MedDRA version 26.0 and 26.1.

Treatment-emergent adverse events (TEAEs) include any AEs occurring or worsening on or after the first dose of study medication.

Table 25. Overview of Adverse Events (Safety Analysis Set) – divided by the period and treatments.

Category	Period 1			Period 2		
	BP14 (Treatment A) (N=92) n (%) E	Neulasta® (Treatment B) (N=92) n (%) E	Overall N= 184 n (%)	BP14 (Treatment A) (N=87) n (%) E	Neulasta® (Treatment B) (N=88) n (%) E	Overall N= 175 n (%)
Adverse events						
TEAEs	17 (18.48%) 18	23 (25.00%) 23	40 (21.74%) 41	20 (22.99%) 21	24 (27.27%) 26	44 (25.14%) 47
Severity						
Mild	09 (9.78%) 09	09 (9.78%) 09	18 (9.78%) 18	14 (16.09%) 14	13 (14.77%) 15	27 (15.43%) 29
Moderate	09 (9.78%) 09	14 (15.22%) 14	23 (12.50%) 23	07 (8.05%) 07	11 (12.50%) 11	18 (10.29%) 18
Severe	00	00	00	00	00	00
Serious TEAEs						
Yes	00	00	00	00	00	00
No	17 (18.48%) 18	23 (25.00%) 23	40 (21.74%) 41	20 (22.99%) 21	24 (27.27%) 26	44 (25.14%) 47
Relationship to study treatment						
Certain related	00	00	00	00	00	00
Probable/Likely related	00	00	00	00	00	00
Possible related	17 (18.48%) 18	22 (23.91%) 22	39 (21.20%) 40	11 (12.64%) 12	22 (25.00 %) 22	33 (18.86%) 34
Unlikely related	00	01 (1.09%) 01	01 (0.54%) 01	09 (10.34%) 09	04 (4.55%) 04	13 (7.43%) 13
Not related	00	00	00	00	00	00
Action taken						
Dose increased	00	00	00	00	00	00
Dose reduced	00	00	00	00	00	00
Drug interrupted	00	00	00	00	00	00
Not applicable	00	00	00	20 (22.99%) 21	24 (27.27%) 26	44 (25.14%) 47
Unknown/Lost to follow up	00	00	00	00	00	00
Dose not changed	17 (18.48%) 18	21 (22.83%) 21	38 (20.65%) 39	00	00	00
Dose withdrawn	00	02 (2.17%) 02	02 (1.09%) 02	00	00	00
Outcome						
Fatal	00	00	00	00	00	00
Not recovered/Not resolved	00	00	00	00	00	00
Recovered/Resolved	17 (18.48%) 18	23 (25.00%) 23	40 (21.74%) 41	20 (22.99%) 21	24 (27.27%) 26	44 (25.14%) 47
Recovered/Resolved with sequelae	00	00	00	00	00	00
Recovering/Resolving	00	00	00	00	00	00
Unknown	00	00	00	00	00	00

Table 26. Summary of number of subjects and proportion of subjects who received concomitant medication

	Period 1			Period 2		
	Test (Number of Subject=92)	**Reference (Number of Subject=92)**	**Total (Number of Subject=184)**	**Test (Number of Subject=87)**	**Reference (Number of Subject=88)**	**Total (Number of Subject=175)**
number of subjects who received concomitant medication	09	14	23	07	11	18
proportion of subjects who received concomitant medication	9.78%	15.22%	12.5%	8.05%	12.50%	10.29%

Data from reference medicinal product (Neulasta)

Table 27. Comparison *of Adverse Events Reported in BP14-101 Study and Neulasta SmPC*

Preferred Term	BP14 (N=120), n (%)	Neulasta® (N=117), n (%)	SmPC
Palpitations	2 (1.7) 2	1 (0.9) 1	Not reported
Abdominal pain	2 (1.7) 2	4 (3.4) 4	Not reported
Abdominal pain lower	2 (1.7) 2	1 (0.9) 1	Not reported
Abdominal pain upper	3 (2.5) 3	1 (0.9) 1	Not reported
Diarrhoea	1 (0.8) 1	1 (0.9) 1	Not reported
Dry mouth	1 (0.8) 1	1 (0.9) 1	Not reported
Gastroesophageal reflux disease	1 (0.8) 1	1 (0.9) 1	Not reported
Nausea	1 (0.8) 1	2 (1.7) 2	Very common
Vomiting	1 (0.8) 1	1 (0.9) 1	Not reported
Catheter site pain	1 (0.8) 1	2 (1.7) 2	Common
Chest pain	0	2 (1.7) 2	Common
Fatigue	4 (3.3) 4	0	Not reported
Injection site bruising	1 (0.8) 1	1 (0.9) 1	Common
Injection site erythema	0	3 (2.6) 3	Common
Injection site pain	1 (0.8) 1	1 (0.9) 1	Common
Injection site reaction	0	4 (3.4) 4	Uncommon
Malaise	2 (1.7) 2	2 (1.7) 2	Not reported
Non-cardiac chest pain	1 (0.8) 1	1 (0.9) 1	Common
Pyrexia	2 (1.7) 2	0	Not reported
Vessel puncture site bruise	1 (0.8) 1	1 (0.9) 1	Not reported
Upper respiratory tract infection	1 (0.8) 1	2 (1.7) 2	Uncommon

Preferred Term	BP14 (N=120), n (%)	Neulasta® (N=117), n (%)	SmPC
Back pain	48 (40.0) 48	45 (38.5) 47	Very common
Bone pain	42 (35.0) 42	44 (37.6) 44	Very common
Musculoskeletal chest pain	0	5 (4.3) 5	Common
Musculoskeletal pain	1 (0.8) 1	1 (0.9) 1	Common
Myalgia	4 (3.3) 4	3 (2.6) 3	Common
Neck pain	4 (3.3) 4	1 (0.9) 1	Common
Pain in extremity	3 (2.5) 3	2 (1.7) 2	Common
Spinal pain	3 (2.5) 3	0	Common
Dizziness	4 (3.3) 4	4 (3.4) 4	Not reported
Headache	44 (36.7) 48	63 (53.8) 67	Very common
Lethargy	3 (2.5) 3	1 (0.9) 1	Not reported
Oropharyngeal pain	0	2 (1.7) 2	Not reported
Night sweats	2 (1.7) 2	0	Not reported
Rash	2 (1.7) 2	5 (4.3) 5	Reported in literature
Haematoma	2 (1.7) 2	0	Not reported

Table 28. Comparison of adverse events reported in BP14-102 study and Neulasta SmPC

System Organ Class/Preferred Term	BP14 (N=179) n (%), E	Neulasta® (N=180) n (%), E	SmPC
General disorders and administration site conditions			
Pain	12 (6.70%), 12	25 (13.89%), 25	Common
Musculoskeletal and connective tissue disorders			
Back pain	16 (8.94%), 17	15 (8.33%), 15	Common
Pain in extremity	00	01 (0.56%), 01	Common
Investigations			
Alanine Aminotransferase Increased	01 (0.56%), 01	02 (1.11%), 02	Uncommon
Blood Bilirubin Increased	03 (1.68%), 03	02 (1.11%), 02	Not reported
Blood Glucose Increased	03 (1.68%), 03	02 (1.11%), 02	Not reported
Lymphocyte Percentage Decreased	01 (0.56%), 01	00	Not reported
White Blood Cell Count Decreased	01 (0.56%), 01	00	Common
Blood Creatinine Increased	00	01 (0.56%),01	Not reported
Cardiac disorders			
Sinus Tachycardia	01 (0.56%), 01	00	Not reported
Gastrointestinal disorders			
Diarrhoea	00	01 (0.56%), 01	Not reported
Source: Appendix 16.2.7			

Adverse drug reactions

BP14-101

Among the TEAEs that were considered to be probably or possibly related to IMP, the most frequently reported TEAEs (reported in ≥ 5% of overall subjects) included:

- Headache: 11 events in 11/124 (8.9%) subjects (6 events in 6/120 [5.0%] subjects exposed to BP14 and 5 events in 5/117 [4.3%] subjects exposed to Neulasta) were considered probably related and 88 events in 63/124 (50.8%) subjects (37 events in 35/120 [29.2%] subjects exposed to BP14 and 51 events in 49/117 [41.9%] subjects exposed to Neulasta) were considered possibly related.
- Bone pain: 83 events in 61/124 (49.2%) subjects (42 events in 42/120 [35.0%] subjects exposed to BP14 and 41 events in 41/117 [35.0%] subjects exposed to Neulasta) were considered probably related.
- Backpain: 12 events in 12/124 (9.7%) subjects (5 events in 5/120 [4.2%] subjects exposed to BP14 and 7 events in 7/117 [6.0%] subjects exposed to Neulasta) were considered probably related and 72 events in 59/124 (47.6%) subjects (37 events in 37/120 [30.8%] subjects exposed to BP14 and 35 events in 34/117 [29.1%] subjects exposed to Neulasta) were considered possibly related.

While the incidence of IMP-related headache was marginally higher in subjects exposed to Neulasta when compared to subjects exposed to BP14, there was no major imbalance in incidence of other IMP-related TEAEs.

BP14-102

Table 29. Summary of Treatment-Emergent Adverse Events by System Organ Class and Preferred Term by relationship with investigational product (Safety Analysis Set)

System Organ Class/Preferred Term	BP14 (Treatment A) (N=179) n (%)	Neulasta® (Treatment B) (N=180) n (%)	Overall (N=184) n (%)
AEs related (Possible)			
General disorders and administration site conditions			
Pain	12 (6.70%) 12	25 (13.89%) 25	34 (18.48%) 37
Musculoskeletal and connective tissue disorders			
Back-pain	16 (8.94%) 17	15 (8.33%) 15	27 (14.67%) 32
Musculoskeletal and connective tissue disorders			
Pain in extremity	00	01 (0.56%) 01	01 (0.54%) 01
Investigations			
Blood Creatinine Increased	00	01 (0.56%) 01	01 (0.54%) 01
Alanine Aminotransferase Increased	01 (0.56%) 01	02 (1.11%) 02	03 (1.63%) 03
AEs related (Unlikely)			
Cardiac disorders			
Sinus Tachycardia	01 (0.56%) 01	00	01 (0.54%) 01
Gastrointestinal disorders			
Diarrhoea	00	01 (0.56%) 01	01 (0.54%) 01
Investigations			
Blood Bilirubin Increased	03 (1.68%) 03	02 (1.11%) 02	05 (2.72%) 05
Blood Glucose Increased	03 (1.68%) 03	02 (1.11%) 02	05 (2.72%) 05
Lymphocyte Percentage Decreased	01 (0.56%) 01	00	01 (0.54%) 01
White Blood Cell Count Decreased	01 (0.56%) 01	00	01 (0.54%) 01

Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities

n: number of subjects reporting at least one AE in each category; N: Number of subjects dosed for each treatment (Overall N= Number of subjects in the Safety Analysis Set); E: Number of events.

Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100).

All AEs were coded using MedDRA version 26.0 and 26.1.

Treatment-emergent adverse events (TEAEs) include any AEs occurring or worsening on or after the first dose of study medication.

Thirty (30) AEs were considered as possibly related and 9 AEs were considered as unlikely related following administration of BP14. Forty-four (44) AEs were considered as possibly related and 5 AEs were considered as unlikely related following administration of Neulasta.

Overall, the proportion of subjects with TEAEs assessed by the Investigator as related to study treatment was higher in the Neulasta treatment group (26.11%) compared with the BP14 treatment group (20.67%). Treatment related AEs most frequently reported were musculoskeletal and connective tissue disorders (8.94% in BP14 treatment group) and general disorders and administration site conditions (13.89% in Neulasta treatment group).

2.5.6.3. Serious adverse event/deaths/other significant events

BP14-101

There were no deaths or SAEs reported during the study.

Adverse Events of Special Interest were not established by the applicant.

No TEAEs of severe intensity were reported during the study.

BP14-102

There were no deaths or SAEs reported during the study.

Adverse Events of Special Interest were not established by the applicant.

Two significant adverse events were reported which led to discontinuation from the study.

One subject discontinued due to AE of Diarrhoea, moderate in severity. The relationship of AE with Neulasta was declared as unlikely related. The event was resolved completely.

Another subject discontinued due to AE of Blood Creatinine Increased, mild in severity. The relationship of AE with Neulasta was declared as possibly related. The event was resolved completely.

2.5.6.4. Laboratory findings

BP14-101 Study

No relevant trends were identified in vital signs values over time. None of the abnormal vital signs were considered clinically significant. None of the abnormal ECG results were considered clinically significant in the opinion of the Investigator and no relevant trends were identified in ECG results over time.

None of the abnormal physical examination results were considered clinically significant in the opinion of the Investigator except for a subject (treatment sequence AB) who had muscular neck pain on the left side on full rotation on Day 21 of Treatment Period 1. A mild and Grade 1 TEAE of post traumatic neck syndrome (verbatim term: whiplash) was reported which was considered not related to the IMP.

BP14-102 Study

The safety related clinical laboratory evaluations (haematology, biochemistry, urinalysis, immunological tests with additional tests [Iron, Transferrin saturation, Total iron-binding capacity (TIBC), Ferritin]) were carried out on the study subjects during the time of screening. Laboratory parameters (haematology and biochemistry) were reassessed at the end of the study (except subjects who did not report to the facility for end of study procedures, so they were declared as lost to follow-up).

No clinically significant abnormality (difference from base line) was observed in vital signs measurements in subjects who received BP14 and Neulasta. No TEAEs related to vital signs abnormalities were recorded in any subject in both treatments.

Physical examination (clinical examination) was conducted by qualified medical designate at the time of check-in, prior to check-out of each study period and during the visit for the last study sample and were found clinically acceptable.

Subjects were advised to report any AE during the study and were specifically asked for these by trained study personnel in a non-leading manner at the time of physical examination (clinical examinations), during vital signs recording, at about 16, 32 and 48 hours post dose and during ambulatory visits in each period. Two subjects in Period 1, during well-being assessment at 35 hours post dose vital sign recording, were unwell due to adverse event. The necessary treatment of adverse event was performed by the investigator or physician and recorded.

12-lead ECG was recorded at the time of check-out (for all dosed subjects) in each period and at the end of the study. The ECG was repeated for one subject at the time of check-out of Period 2 as per investigator/physician discretion and documented as an adverse event for period 2.

Total WBC count, SGPT, SGOT and serum creatinine were measured prior to check-in of each period. Only subjects with normal limit or clinically non-significant limit of these tests were dosed in each period (except one subject who had Blood Creatinine Increased which was documented as an adverse event and discontinued from the study).

Total WBC count was measured at 168 (Day 7) hours post-dose in each period (except 2 subjects whose total WBC count were not measured in Period 1, for deviation). In the safety assessment test, total WBC count was found normal and clinically non-significant in the study.

Injection site assessment was performed prior to check-in and at 1, 2, 4, 10, 23 and 35 hours (± 40 minutes) post dose and at the time of check-out (only for dosed subjects) in each period and were found normal.

2.5.6.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.5.6.6. Safety in special populations

Not applicable.

2.5.6.7. Immunological events

BP14-101 Study

A standard multi-tiered approach comprising screening, confirmatory, titer, and neutralizing antibody assay was used to assess the immunogenicity of BP14 and EU-Neulasta.

In the BP14-101 study, human anti-pegfilgrastim raised against Neulasta was used as a positive control in anti-pegfilgrastim assay and NAb assay while surrogate anti-PEG mouse monoclonal IgM antibody was used in anti-PEG assay. Surrogate mouse antibodies were used as a positive control in all assays in the BP14-102 study.

Screening and confirmatory cut-points were established statistically during the validation using about 50 healthy individual human serum samples and the same correction factors for floating cut point calculation were used throughout the study samples analysis. The targeted false positive rates of 5% for the screening assay and 1% for the confirmatory assay were used.

The ADA and NAb assays were validated for sensitivity, precision, hook effect, drug tolerance, selectivity, specificity, stability and analytical comparability. Acceptance criterium of precision $\leq$ 20% CV was applied. Study samples were analysed within demonstrated short-term and freeze/thaw stability.

In the BP14-101 study anti-Peg antibodies were tested only in ADA positive samples while in the BP14-102 study all immunogenicity samples were tested for anti-Peg antibodies and pre-existing anti-Peg antibodies could be detected.

Anti-drug antibodies were assessed during the pre-dose (between 5 and 45 minutes prior to dosing) on Day 1, and on Days 14 and 21 for both treatment periods. Any ADA-positive subject was followed up every 3 months till 12 months or until subject was ADA-negative, whichever came first.

Out of 124 randomized subjects, 17 (13.7%) were confirmed positive for anti-pegfilgrastim antibodies, with antibodies in 12 subjects emerging from the period -1 BP14 treatment arm and five subjects from the EU-Neulasta treatment arm. Based on the characterization results, the majority of the confirmed positive subjects, 15 out of 17 subjects (88.2%), were specific to the PEG domain only, and 2 out of 17 subjects (11.7%) were found to contain ADAs against both PEG and Filgrastim domains. The highest antibody titer was observed to be 358 and 316 in subjects who received EU-Neulasta and BP14, respectively, in period 1. Two of the confirmed ADA-positive samples emerging from BP14 treatment showed neutralizing activity. A total of 3 subjects (2 treated with BP14 and 1 with EU-Neulasta during treatment period 1) showed persistent ADAs till the end of the study with a time dependent decrease in antibody titers. Anti-GCSF antibodies detected in 2 subjects did not persist till the end of the study. The subjects with positive ADAs were examined for the potential impact of ADA on the PK and PD profile. The PK parameters (AUC0-t and Cmax) were not impacted by the presence of ADAs in all the subjects.

Table 30. Frequencies for ADA assay data (Safety Analysis Set)

Category	Sequence AB (N=62) n (%)	Sequence BA (N=62) n (%)
ADA screening assay		
Number of samples tested	347	358
Number of reactive pre-dose samples[1]	4 (1.2)	4 (1.1)
Number of reactive post-dose samples[1]	44 (12.7)	35 (9.8)
ADA confirmatory assay		
Number of subjects with ≥ 1 confirmed ADA positive sample (BP14 as competing inhibitor)[2]	12 (19.4)	5 (8.1)
Number of subjects with ≥ 1 confirmed ADA positive sample (PEG only as competing inhibitor)[2]	10 (16.1)	5 (8.1)
Number of subjects with ≥ 1 confirmed ADA positive sample (Filgrastim only as competing inhibitor)[2]	2 (3.2)	0
Number of subjects [BP14 (+ve), PEG (+ve) and Filgrastim (+ve)]	2 (3.2)	0
Neutralizing antibody assay		
Number of samples that tested positive in the NAb assay	2	0
Number of subjects with ≥ 1 NAb positive sample[2]	2 (3.2)	0

ADA: Anti-drug antibodies; NAb: Neutralizing antibody.
n: Number of subjects; +ve: Positive.
1: Percentages are calculated based on the "Number of samples tested."
2: Percentages are calculated based on "N" count.

BP14-102 Study

Immunogenicity testing was performed as a single assay format, using BP14 for inhibition. The inhibitory comparability using both BP14 and Neulasta was demonstrated. The precision of screening assays using BP14 and Neulasta was also comparable. A standard multi-tiered approach was employed including a screening, confirmation and titer assay. In confirmation assay filgrastim (GCSF) and pegfilgrastim (Peg-GCSF) were used as a control. Confirmatory assay was followed by functional assay of neutralizing capacity of antibodies. All immunogenicity samples were tested for anti-PEG antibodies by the separate assay. All assays (screening, confirmatory, titer, and NAb) were validated for the intended use.

Screening and confirmatory cut-points were established for anti Peg-GCSF and anti-Peg assays based on data from about 50 drug naïve serum samples. In NAb assay, 30 drug naïve serum samples were used. The correction factor for screening assay 1.129 was calculated. This correction factor was used to generate plate specific cut points after multiplying with plate specific negative control. There were 1075 immunogenicity samples analysed for ADA in the BP14-102 study, out of them 18 were screened positive and 8 confirmed positive. It means that in-study baseline samples yielded a False Positive Rate (FPR) likely lower than 1%. To avoid false negative results in the Peg-GCSF ADA assay the applicant revised the in-study screening cut point, calculated a correction factor of 1.1001, and applied it to determine putative positive samples instead of the original correction factor of 1.129 determined in the validation. Additional eight samples were identified as putative positive, out of them two samples were confirmed positive but with not reportable titer. Although the FPR was still below 2%, the applicant considered that no true positive samples were missed due to false negative results and that information on the immunogenicity of the products is not biased.

Neutralizing capacity of the ADA was tested by cell-based NAb assays. The NAb assays were validated for sensitivity, precision, drug tolerance, selectivity, specificity, stability and analytical comparability. Acceptance criterium of precision ≤ 20% CV was applied. The applicant was asked to discuss whether the negative results for NAb in the BP14-102 study may in fact be a false negative, given that the antibody levels were likely low (the maximum titer used in the anti Peg-GCSF assay was 2) and the sensitivity of the NAb method was relatively high (699 ng/mL) compared to the sensitivity of the anti-Peg GCSF essay (8.79 ng/mL). In addition, drug tolerance appeared to be problematic in one sample (Period 2, time point 24 h) because the residual drug level was approximately 115 ng/mL, but drug tolerance for the NAb assay was determined to be only 40 ng/mL for HPC and 5 ng/mL for LPC. The applicant justified that cell-based assays are inherently more complex with higher variation, inferior sensitivity, robustness and drug tolerance as compared to non-cell-based assays. Efforts were made to obtain the most relevant results: single assay suitability was verified, three-tiered approach was used, sensitivity and drug tolerance were increased as much as possible. Immunogenicity of pegfilgrastim was expected to be low in general and neutralizing activity of antibodies is not usually detected.

Immunogenicity assessment was listed by treatment group and number/percentage of subjects positive for ADA. Blood samples were collected at pre-dose (0 hour) and at 24 (Day 1) and 480 (Day 20) hours after administration of each dose.

For ADA assessment, a total of 15 subjects (3 subjects to Peg-GCSF, 5 subjects to GCSF and 7 subjects to Peg) had samples that were confirmed positive for anti-drug antibody for visit 1 (0 hr), visit 2 (24 hr) and visit 3 (480 hr) in sequence treatment AB. A total of 9 subjects (2 subjects to GCSF and 7 subjects to Peg) had samples that were confirmed positive for anti-drug antibody for visit 1 (0 hr), visit 2 (24 hr) and visit 3 (480 hr) in sequence treatment BA.

Out of the 8 anti-Peg-GCSF antibody positive samples, none of the subjects receiving BP14 or Neulasta had sample positive for Nab.

Table 31. Frequencies for ADA assay data (Safety Analysis Set)

Category	Sequence AB (N=88) n (%)	Sequence BA (N=87) n (%)
Peg-GCSF_ADA screening assay		
Number of samples tested	536	539
Number of reactive pre-dose samples[1]	17 (3.2%)	11 (2.0%)
Number of reactive post-dose samples[1]	28 (5.2%)	25 (4.6%)
Peg-GCSF_ADA confirmatory assay		
Number of subjects with ≥ 1 confirmed ADA positive sample (BP14 as competing inhibitor)[2]	03 (3.4%)	00 (0.0%)
Number of subjects with ≥ 1 confirmed ADA positive sample (Filgrastim only as competing inhibitor)[2]	05 (5.7%)	02 (2.3%)
Peg _ADA confirmatory assay		
Number of subjects with ≥ 1 confirmed ADA positive sample (PEG only as competing inhibitor)[2]	07 (8.0%)	07 (8.0%)
Number of subjects [BP14 (+ve), PEG (+ve) and Filgrastim (+ve)]	15 (17.0%)	09 (10.3%)
Neutralizing antibody assay	03 (0.6%)	05 (0.9%)
Number of samples tested positive in NAb assay	00 (0.0%)	00 (0.0%)
Number of subjects with ≥ 1 NAb positive sample[2]	00 (0.0%)	00 (0.0%)
ADA: Anti-drug antibodies; NAb: Neutralizing antibody. n: Number of subjects; +ve: Positive. 1: Percentages are calculated based on "Number of samples tested". 2: Percentages are calculated based on "N" count.		

Table 32. Immunogenicity assessment by treatment group Number/percentage of subjects positive and negative for AntiPeg-ADA

		Treatment	
Visit (Hour)	Results	Test (N=179) n (%)	Reference (N=180) n (%)
Visit-1 (0.0)	Positive	10 (5.59%)	7 (3.89%)
	Negative	169 (94.41%)	173 (96.11%)
Visit-2 (24.0)	Positive	4 (2.23%)	4 (2.22%)
	Negative	175 (97.77%)	176 (97.78%)
Visit-3 (480.0)	Positive	4 (2.23%)	6 (3.33%)
	Negative	174 (97.21%)	173 (96.11%)
	Missing Samples	1 (0.56%)	1 (0.56%)

Table 33. Immunogenicity assessment by treatment and period group Number/percentage of subjects positive and negative for AntiPeg-ADA

		Treatment			
		Test (N=179) n (%)		Reference (N=180) n (%)	
Hour	Results	Period I (N=92)	Period II (N=87)	Period I (N=92)	Period II (N=88)
Visit-1 (0.0)	Positive	5 (2.79%)	5 (2.79%)	3 (1.67%)	4 (2.22%)
	Negative	87 (48.60%)	82 (45.81%)	89 (49.44%)	84 (46.67%)
Visit-2 (24.0)	Positive	1 (0.56%)	3 (1.68%)	2 (1.11%)	2 (1.11%)
	Negative	91 (50.84%)	84 (46.93%)	90 (50.00%)	86 (47.78%)
Visit-3 (480.0)	Positive	3 (1.68%)	1 (0.56%)	4 (2.22%)	2 (1.11%)
	Negative	88 (49.16%)	86 (48.04%)	87 (48.33%)	86 (47.78%)
	Missing Samples	1 (0.56%)	0 (0.00%)	1 (0.56%)	0 (0.00%)

Table 34. Immunogenicity assessment by treatment group Number/percentage of subjects positive and negative for Peg-GCSF-ADA

		Treatment	
Visit (Hour)	Results	Test (N=179) n (%)	Reference(N=180) n (%)
Visit-1 (0.0)	Positive	0 (0.00%)	1 (0.56%)
	Negative	179 (100.00%)	179 (99.44%)
Visit-2 (24.0)	Negative	179 (100.00%)	180 (100.00%)
Visit-3 (480.0)	Positive	3 (1.68%)	0 (0.00%)
	Negative	175 (97.77%)	179 (99.44%)
	Missing Samples	1 (0.56%)	1 (0.56%)

Table 35. Immunogenicity assessment by treatment and period group Number/percentage of subjects positive and negative for Peg-GCSF-ADA

		Treatment			
		Test (N=179) n (%)		Reference (N=180) n (%)	
Hour	Results	Period I (N=92)	Period II (N=87)	Period I (N=92)	Period II (N=88)
Visit-1 (0.0)	Positive	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.56%)
	Negative	92 (51.40%)	87 (48.60%)	92 (51.11%)	87 (48.33%)
Visit-2 (24.0)	Negative	92 (51.40%)	87 (48.60%)	92 (51.11%)	88 (48.89%)
Visit-3 (480.0)	Positive	3 (1.68%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Negative	88 (49.16%)	87 (48.60%)	91 (50.56%)	88 (48.89%)
	Missing Samples	1 (0.56%)	0 (0.00%)	1 (0.56%)	0 (0.00%)

Table 36. Immunogenicity assessment by treatment group Number/percentage of subjects positive and negative for GCSF-ADA

		Treatment	
Visit (Hour)	Results	Test (N=179) n (%)	Reference(N=180) n (%)
Visit-1 (0.0)	Positive	0 (0.00%)	1 (0.56%)
	Negative	179 (100.00%)	179 (99.44%)
Visit-2 (24.0)	Positive	0 (0.00%)	1 (0.56%)
	Negative	179 (100.00%)	179 (99.44%)
Visit-3 (480.0)	Positive	3 (1.68%)	3 (1.67%)
	Negative	175 (97.77%)	176 (97.78%)
	Missing Samples	1 (0.56%)	1 (0.56%)

Table 37. Immunogenicity assessment by treatment and period group Number/percentage of subjects positive and negative for GCSF-ADA

		Treatment			
		Test (N=179) n (%)		Reference (N=180) n (%)	
Hour	Results	Period I (N=92)	Period II (N=87)	Period I (N=92)	Period II (N=88)
Visit-1 (0.0)	Positive	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.56%)
	Negative	92 (51.40%)	87 (48.60%)	92 (51.11%)	87 (48.33%)
Visit-2 (24.0)	Positive	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.56%)
	Negative	92 (51.40%)	87 (48.60%)	92 (51.11%)	87 (48.33%)
Visit-3 (480.0)	Positive	3 (1.68%)	0 (0.00%)	2 (1.11%)	1 (0.56%)
	Negative	88 (49.16%)	87 (48.60%)	89 (49.44%)	87 (48.33%)
	Missing Samples	1 (0.56%)	0 (0.00%)	1 (0.56%)	0 (0.00%)

Table 38. Adverse Event Profiles of Treatment-Induced ADA/NAb Positive Subjects to those of ADA/NAb-Negative Subjects Periodwise (Safety Analysis Set)

		Period 1			Period 2		
		BP14 (Treatment A) (N=92) n (%) E	Neulasta® (Treatment B) (N=92) n (%) E	Overall N= 184 n (%)	BP14 (Treatment A) (N=87) n (%) E	Neulasta® (Treatment B) (N=88) n (%) E	Overall N= 175 n (%)
For Peg-GCSF ADA							
No. of AEs for Treatment-induced Peg-GCSF ADA positive/negative subjects (0.0 hour)	Positive	00	00	00	00	00	00
	Negative	00	01 (1.09%) 01	01 (0.54%) 01	00	00	00

No. of AEs for Treatment-induced Peg-GCSF ADA positive/negative subjects [(24.0 hour (Day-1)]	Positive	00	00	00	00	00	00
	Negative	17 (18.48%) 18	20 (21.74) 20	37 (20.11%) 38	12 (13.79%) 12	20 (22.73%) 20	32 (18.29%) 32
No. of AEs for Treatment-induced Peg-GCSF ADA positive/negative subjects [(480.0 hour (Day-20)]	Positive	00	00	00	00	00	00
	Negative	00	02 (2.17%) 02	02 (2.17%) 02	09 (10.34%) 09	06 (6.82%) 06	15 (8.57%) 15
For GCSF ADA							
No. of AEs for Treatment-induced GCSF ADA positive/negative subjects (0.0 hour)	Positive	00	00	00	00	00	00
	Negative	00	01 (1.09%) 01	01 (0.54%) 01	00	00	00
No. of AEs for Treatment-induced GCSF ADA positive/negative subjects [(24.0 hour (Day-1)]	Positive	00	00	00	00	00	00
	Negative	17 (18.48%) 18	20 (21.74) 20	37 (20.11%) 38	12 (13.79%) 12	20 (22.73%) 20	32 (18.29%) 32
No. of AEs for Treatment-induced GCSF ADA positive/negative subjects [(480.0 hour (Day-20)]	Positive	00	00	00	00	00	00
	Negative	00	02 (2.17%) 02	02 (2.17%) 02	09 (10.34%) 09	06 (6.82%) 06	15 (8.57%) 15
For Anti-Peg ADA							
No. of AEs for Treatment-induced Anti-Peg ADA positive/negative subjects (0.0 hour)	Positive	00	00	00	00	00	00
	Negative	00	01 (1.09%) 01	01 (0.54%) 01	00	00	00
No. of AEs for Treatment-induced Anti-Peg ADA positive/negative subjects [(24.0 hour (Day-1)]	Positive	01 (1.09%) 01	00	01 (0.54%) 01	00	00	00
	Negative	16 (17.39%) 17	20 (21.74%) 20	36 (39.13%) 37	12 (13.79%) 12	20 (22.73%) 20	32 (18.29%) 32
No. of AEs for Treatment-induced Anti-Peg ADA positive/negative subjects [(480.0 hour (Day-20)]	Positive	00	00	00	00	00	00
	Negative	00	02 (2.17%) 02	02 (2.17%) 02	09 (10.34%) 09	06 (6.82%) 06	15 (8.57%) 15

For Anti-Peg GCSF NAB							
No. of AEs for Treatment-induced Anti-Peg GCSF NAB negative subjects (0.0 hour)	Negative	00	01 (1.09%) 01	01 (0.54%) 01	00	00	00
No. of AEs for Treatment-induced Anti-Peg GCSF NAB negative subjects [(24.0 hour (Day-1)]	Negative	17 (18.48%) 18	20 (21.74) 20	37 (20.11%) 38	12 (13.79%) 12	20 (22.73%) 20	32 (18.29%) 32
No. of AEs for Treatment-induced Anti-Peg GCSF NAB negative subjects [(480.0 hour (Day-20)]	Negative	00	02 (2.17%) 02	02 (2.17%) 02	09 (10.34%) 09	06 (6.82%) 06	15 (8.57%) 15

n= number of subjects reporting at least one AE in each category; N= Number of subjects dosed for each treatment; E = number of events. Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100).

Source: Appendix 16.2.6 & 16.2.7

2.5.6.8. Safety related to drug-drug interactions and other interactions

Not applicable.

2.5.6.9. Discontinuation due to adverse events

BP14-101 Study

In BP14-101 study, 1/124 (0.8%) subject in treatment sequence AB discontinued study due to protocol deviation and AE, 2/124 (1.6%) subjects (1 subject in each treatment sequence) were lost to follow-up, and 7/124 (5.6%) subjects (4 subjects in treatment sequence AB and 3 subjects in treatment sequence BA) discontinued from study due to withdrawal of consent.

A male subject was randomized to treatment sequence AB (A = 6 mg BP14; B = 6 mg Neulasta). The subject received the dose of BP14 in Treatment Period 1. The subject's medical history included left wrist fracture, open reduction internal fixation for left wrist fracture, pneumonia, and recurrent otitis media. On the same day, the subject experienced a TEAE of peri orbital swelling. The event was assessed to be mild and Grade 1 severity. The event was considered to be probably related to the IMP (BP14) and the subject was withdrawn from the IMP and the study. Although the AE of peri-orbital swelling was not considered serious, the subject was discontinued from the study due to concerns of a possible early allergic reaction to the administered IMP which could put him at risk if dosed in the second period. No treatment was reported for the event. The event of peri-orbital swelling resolved on the same day of onset.

BP14-102 Study

In Period 1, 2/184 (1.09%) subjects in treatment sequence AB discontinued study due to protocol deviation, 2/184 (1.09%) subjects due to AE and 5/184 (2.72%) subjects due to withdrawal of consent.

1 subject discontinued due to adverse event of Diarrhoea, which was moderate in severity. The relationship of adverse event with Treatment B was declared as unlikely related. The event was resolved completely.

1 subject discontinued due to adverse event of Blood Creatinine Increased, which was mild in severity. The relationship of adverse event with Treatment B was declared as possibly related. The event was resolved completely.

2.5.6.10. Post marketing experience

Not applicable.

2.5.7. Discussion on clinical safety

Data collection

The safety assessment of BP14 is based on studies BP14-101 and BP14-102; phase I randomized, single-dose, double-blind, two-sequence, two-period crossover studies to compare immunogenicity and safety (secondary objectives) of BP14 with EU- Neulasta in healthy male adult subjects. In these studies, subjects received a single dose of 6 mg SC injection of either BP14 or Neulasta on Day 1 of each treatment period. This is in accordance with requirements given in the Guideline on similar biological medicinal products containing biotechnology-derived protein as active substance: non-clinical and clinical issues (EMA/CHMP/BMWP/42832/2005 Rev1; 18-December-2014).

Patient exposure

124 subjects were exposed in study BP14-101. In total, 114 from 124 randomized subjects (91.9%) completed both treatment periods. The main reason for discontinuation was consent withdrawal (7 subjects, 5.6%).

184 subjects were exposed in study BP14-102. In total, 175 from 184 randomized subjects (95.1%) completed both treatment periods. The main reason for discontinuation was consent withdrawal (5 subjects, 2.7%).

The number of subjects exposed to the study drug is considered sufficient to support safety assessment.

The total study duration for each subject in study BP14-101 was approximately 84 days (excluding the 28-day Screening Period). This included 21 days for Treatment Period 1, a washout period of 42 days (6 weeks) between the 2 treatment periods and 21 days of Treatment Period 2. Approximate study duration for subjects in study BP14-102 was 63 days because of a shorter washout period (21 days). Data collection in such single-dose studies is considered reliable and sufficient for establishment of safety for Dyrupeg taking into account a well-known safety profile of this active substance (pegfilgrastim) and its nature, i.e. biosimilar.

Only healthy males were enrolled in the two studies. The applicant justified the absence of a cancer patients-dedicated comparative efficacy trial based on the similarity demonstrated in physicochemical, functional, pharmacokinetic and pharmacodynamic comparisons. Absolute neutrophil count, the primary pharmacodynamic endpoint in study BP14-101, served as a sensitive surrogate for clinical efficacy outcomes such as incidence of neutropenic fever, duration of severe neutropenia, infections, or infection-related hospitalizations. The applicants' choice of population is considered acceptable. In study BP14-101 the subjects were of diverse race with the majority of subjects White (74.2%), whereas in study BP14-102 all subjects were Asian. Other demographic characteristics of the enrolled population such as age or BMI were comparable between the treatment sequences.

With respect to the concomitant medication received during the study, the use of ibuprofen in study BP14-101 occurred in higher rate in the treatment sequence AB (51.5%) compared to the treatment sequence BA (30.5%). Period-wise, the use of ibuprofen was higher in the BP14 arm only during Period 1 (21/62 subjects vs 11/62 subjects), while in Period 2 (12/58 vs 21/55), the number of subjects was reversed. Most of the subjects did not use ibuprofen for more than two days. None of the subjects enrolled had any reported TEAEs or abnormal lab investigation findings connected with the administration of concomitant medication. Therefore, the uneven use of ibuprofen is not expected to compromise the interpretation of the safety results.

Adverse events

Provided data was not pooled.

<u>BP14-101</u>

A total of 472 AEs, out of which 468 TEAEs, were reported in 120/124 (96.8%) subjects. Slightly higher incidence of AEs/TEAEs was reported in the BP14 group (220 AEs reported in 113/120 [94.2%] subjects exposed to BP14 and 252 AEs reported in 107/117 [91.5%] subjects exposed to Neulasta).

Severity

Most TEAEs (96.8%) were considered to be Grade 1-mild with higher incidence in the BP14 group (219 events in 113/120 [94.2%] subjects exposed to BP14 and 247 events in 106/117 [90.6%] subjects exposed to Neulasta).

In the BP14 group, 1 case of Grade 2-moderate backpain was observed. In the Neulasta group, the 3 cases assessed as Grade 2 included injection site erythema, non-cardiac chest pain, and headache.

One grade 3 severity case was non-serious TEAE of increased AST in a subject exposed to BP14. The Investigator assessed this event to be probably related to IMP. Transient elevations of AST or ALT are listed as ADRs in SmPC section 4.8.

One grade 4 severity case included non-serious TEAE of neutropenia in a subject exposed to Neulasta. The Investigator assessed this event to be probably related to IMP.

No TEAEs of severe intensity or deaths were reported during the study.

Frequency

The incidence of injection site reactions was lower in subjects exposed to BP14 when compared to subjects exposed to Neulasta.

Most commonly reported PTs were headache, back pain, dizziness, rash, and myalgia. The reported AEs, having regard the concrete terms, were generally balanced for both treatment groups. A similar observation was identified for those AEs which were assessed as probably or possibly related to study drug.

The following AEs were observed with higher incidence in BP14 group: palpitations, abdominal pain lower, myalgia, pain in extremity. However, the difference was only seen in 1 subject, thus this imbalance is not considered clinically significant and was not pursued further.

According to the provided Summary of Treatment-Emergent Adverse Events Reported in ≥ 1% Subjects, the following AEs were reported with higher incidence in the BP14 group compared to Neulasta (more than 2 subjects-difference): fatigue, abnormal pain upper, pyrexia, back pain, neck pain, spinal pain, lethargy, night sweats, haematoma. Considering the low incidence, and the majority of observed cases with recovered outcomes with only one discontinuation in BP14 group, there are no safety concerns, and the two groups are considered comparable from the observed AEs-safety point of view.

Relationship to treatment

A total of 124 events in 78/124 (62.9%) were considered to be probably related to study drug. Slightly lower incidence of probably related AEs was observed in the BP14 group and the same trend was observed on cases assessed as possibly related.

For most subjects, no action was taken with the IMP due to TEAEs. The IMP (BP14) was permanently withdrawn due a TEAE (probably related periorbital swelling, mild) in one subject.

Outcome

A total of 461 events in 120/124 (96.8%) subjects (216 events in 113/120 [94.2%] subjects exposed to BP14 and 245 events in 106/117 [90.6%] subjects exposed to Neulasta) had resolved by the end of the study. Overall, 8 events in 7/124 (5.6%) subjects (3 events in 3/120 [2.5%] subjects exposed to BP14 and 5 events in 4/117 [3.4%] subjects exposed to Neulasta) had not resolved by the end of the study. Following request, the applicant presented TEAEs by treatment, period, SOC/PT severity, grades, causality, action taken and outcomes. TEAEs that occurred with an incidence of greater than 5% were largely balanced,

although there was a slightly higher incidence of Headache and Injection site erythema in the Neulasta Period 2 group which was considered by the applicant as likely a chance imbalance. Overall, the safety profile of BP14 and Neulasta is considered similar.

BP14-102

Severity

A total of 88 AEs were reported by 69 subjects during the entire study, 47 mild in severity and 41 moderate in severity. The majority of mild AEs were coded as pain, with lower incidence in BP14 group. The majority of moderate AEs were coded as backpain, with similar incidence between both groups and pain, with lower incidence in BP14 group. There were no AEs severe in intensity reported in the study.

Frequency

Lower incidence of AEs was observed in the BP14 group. The most often observed AEs were pain (18.48 %) and back pain (14.67 %). Pain was reported with lower frequency in BP14 group compared to Neulasta (6.70 % vs 13.89 %, accordingly). Backpain was reported with slightly higher incidence in BP14 group (8.94 % vs 8.33 %) and is therefore listed ADR with frequency common (SmPC section 4.8). Headache, which was commonly reported in study BP14-101 was not observed.

Discrepancy frequencies obtained in study BP14-101 and study BP14-102 were justified by the applicant based on to cultural differences contributing to different levels of pain tolerance in the subject population. Study BP14-101 was performed in Australia and BP14-102 was performed in India. This justification is acceptable. No further clinically relevant differences in AEs were noted, as only small number of individual cases was observed.

Relationship to treatment

The majority of cases were assessed as possible related (in total 32.61%) to study drug. Lower incidence was observed in BP14 group. No case with certain, probable causality was observed.

Possibly related AEs to BP14 were coded as pain, backpain and alanine aminotransferase increased. While pain and alanine aminotransferase increased were observed with lower incidence in BP14 group, back pain had slightly higher incidence in the BP14 group. Transient elevations in LFTs for ALT or AST are listed ADRs for the reference medicinal product (uncommon frequency). AEs of sinus tachycardia, blood bilirubin increased, blood glucose increased, lymphocyte percentage decreased, white blood cell count decreased were evaluated as unlikely related by the applicant.

Outcome

Change of dose of study drug was not needed for 18 adverse events and action was not required for 21 adverse events following administration BP14.

Change of dose of study drug was not needed for 21 adverse events and dose of study drug was withdrawn for 2 adverse events and action was not required for 26 adverse events following administration of Neulasta.

All adverse events reported in the study were recovered/resolved. There were no injection site reactions reported and no fatal case in the study.

Deaths, SAES, AESI

There were no deaths or SAEs reported during the studies BP14-101 and BP14-102. Adverse Events of Special Interest were not established by the applicant. No TEAEs of severe intensity were reported during

study BP14-101. Two significant adverse events were reported during the conduct of the study BP14-102 in Neulasta treatment arm which led to discontinuation.

Summaries of observed AEs divided by the period and treatments were provided and no clinically significant trends were noted.

Discontinuation due to adverse events

Only 1 and 2 subjects discontinued from study BP14-101 and BP14-102 due to AE respectively, and therefore the observations in study BP14-101 and BP14-102 did not provide any new safety findings or concerns in association with pegfilgrastim.

Immunogenicity

BP14-101 - ADAs persisting till the end of the study were reported for 3 subjects (2 subjects exposed to BP14 and 1 subject exposed to Neulasta). 2 subjects were found negative at the last follow-up visit approximately 12 months after Period 1. The third subject (exposed to BP14) was still positive at the last follow-up visit with a titre value < 26.7. The adverse events observed for the subject were evaluated to co-relate the impact of persistent ADA on safety and efficacy. All observed AEs resolved. AEs which were considered related to the study drug (headache, low back pain, and generalized bone pain) are in line with pegfilgrastim known safety profile. No safety concern was identified with regard to subjects with ADAs persisting till the end of study.

The number of subjects ADA-positive were more than twice higher following treatment with BP14 (12 subjects) as compared to those treated with EU Neulasta (5 subjects). The percentage of ADA reactive post-dose samples seemed higher in the subjects receiving the investigational products in sequence AB (12,7%) than in sequence BA (9,8%). The applicant explained that the incidence of ADA positive subject ranged between 2.6% to 10.7% and that, as reported in the literature the difference in the ADA incidence could be attributed to the non-neutralizing PEG-reactive ADAs, that are reportedly present in healthy subjects, a phenomenon that may be explained by the use of other pegylated drugs, cosmetics, and food by general population. This hypothesis is not proven but the measured differences in ADA according to the sequence of administration of the drugs seem to be of low clinical significance. 15 out of 17 subjects (88.2%) were specific to PEG domain only, 2 out of 17 subjects (11.7%) were found to contain ADAs against both PEG and Filgrastim domains. Two of the confirmed ADA positive samples emerging from BP14 treatment showed neutralizing activity.

A total of 12 subjects (2 subjects exposed to BP14 and 10 subjects exposed to Neulasta) reported 12 TEAEs of injection site reactions. The applicant did not comment on the impact of the observed immunogenicity outcomes on the safety of the investigated products. The provided data showed that the same TEAEs (back pain, bone pain, headache) were reported most frequently in both groups (ADA-negative and ADA-positive). The incidence was generally higher in period 1 in all TEAEs and also in both groups. The incidence of headache was higher in subjects exposed to Neulasta in period 2 when compared to subjects exposed to BP14. These data do not raise concerns.

BP14-102 - There were 1075 immunogenicity samples analysed for ADA in the BP14-102 study, out of them 18 were screened positive and 8 confirmed positive. The immunogenic response observed for both BP14 and Neulasta was comparable.

15 subjects (3 subjects to Peg-GCSF, 5 subjects to GCSF and 7 subjects to Peg) had samples that were confirmed positive for anti-drug antibody in sequence treatment AB. A total of 9 subjects (2 subjects to GCSF

and 7 subjects to Peg) had samples that were confirmed positive for anti-drug antibody in sequence treatment BA category.

For Anti-PEG-ADA evaluation, slightly higher number of positive patients in test treatment compared to the reference treatment was observed during the first visit 10 (5.59%) vs 7 (3.89 %). For the visit 2 (24 hours) the numbers were identical for both treatment and the last visit (480 hours) showed higher percentage of positive results in the reference treatment (3.33% vs 2.23%)

For Peg-GCSF-ADA assessment by number/percentage of subjects, 0.56% (1 subject) were found positive at visit 1 and none of the subjects were found positive at visit 2 and visit 3 in the reference treatment. 1.68% (3 subjects) were found positive at visit 3 and none of the subjects were found positive at visit 1 and visit 2 in test (BP14) treatment. In the period II of the test treatment no positive subject was observed. The number of ADA to PEG-GCSF reactive post-dose samples was 28 (5.2%) in sequence AB and 25 (4.6%) in sequence BA. These numbers are balanced but differ from the incidences reported in study BP14-101 (12,7% and 9,8%, respectively). This difference in immunogenicity rates was explained by the applicant by the fact that the studies were conducted in two different geographies, the social, cultural environment differed and could have potentially impacted on the immune response observed in subjects of the two studies. The applicant also stresses that although the post-dose immune response differed between the two studies, the immune response within the study for the two arms (which was in fact the issue under study), was comparable. The applicant's justification is acknowledged and acceptable as the intra-study comparison did not demonstrate significant differences regarding the number of post-dose samples reactive for ADA to PEG-GCSF.

For GCSF-ADA evaluation, slightly higher number of positive patients in test treatment compared to the reference treatment was observed during the visit 1 and 2 (1 patient for each visit compared to the none in the test treatment). For the visit 3 (480 hours) the numbers of positive patients were identical for both treatment (3 each).

None of the subjects receiving Test (BP14) or Reference (Neulasta) had sample positive for NAb.

There were no injection site reactions reported for both the treatment groups. According with the applicant, no AE from SOC Immune system disorders was observed during the study. Based on the tabulated summary of Adverse event profiles of treatment-induced ADA/NAb positive subjects to those of ADA/NAb-negative subjects period-wise, only 1 subject with positive anti-Peg ADA experienced AE in the BP14 group 1 period only. The reported AE in the positive anti-peg ADA patient was body-ache. The AE resolved the same day as was observed. The patient completed both periods of the study without any clinically significant findings. The applicant concluded that the ADA response was a transient antibody response without significant clinical impact. This was acknowledged and no further action considered needed.

Provided results seems to be generally low and comparable between both treatments and no clinically significant observed immune response was noted.

Overdose

Overdosage information with BP14 is not available. However, single doses of 300 µg/kg have been administered subcutaneously to a limited number of healthy volunteers and patients with non-small cell lung cancer without serious adverse reactions.

Laboratory and other findings

BP14-101 Study

The relevant listings of individual laboratory parameters and other values concerning vital signs, physical examination and other observations related to safety were provided.

1 subject in study BP14-101 had abnormal physical examination results (muscular neck pain on the left side on full rotation) on Day 21 of Treatment Period 1 which was considered to be clinically significant and reported as a mild and Grade 1 TEAE.

2 TEAEs were reported for the abnormal lab parameters. 1 subject in BP14 treatment group experienced elevated AST and 1 subject in the Neulasta treatment group experienced neutropenia. Both TEAEs were considered probably related to the treatment and resolved during the study. A transient elevation in AST is expected with pegfilgrastim, while neutropenia would be considered disease related.

No TEAEs were reported for Vital signs and ECG findings in the entire study.

BP14-102 Study

The relevant listings of individual laboratory parameters and other values concerning vital signs, physical examination and other observations related to safety were provided.

Comparison tables of abnormal vital signs, physical findings and ECG results did not show any clinically relevant differences between treatment groups. Only subjects with normal limit or clinically non-significant limit of these tests were dosed in each period.

There was 1 case associated with laboratory treatment-related AEs which led to treatment discontinuation. The subject had Blood Creatinine Increased which was documented as an adverse event and discontinued from the study.

The ECG was repeated for one subject in study BP14-102 at the time of checkout of Period 2 as per investigator/physician discretion and documented as an adverse event. The subject experienced Sinus Tachycardia which was mild in severity following administration of Treatment B. AE was evaluated as unlikely related by the applicant.

Total WBC count of 2 subjects in study BP14-102 were not measured in Period 1, for deviation. In the safety assessment test, total WBC count was found normal and clinically non-significant in the study.

2.5.8. Conclusions on the clinical safety

The collected safety data for the intended biosimilar product are acceptable considering the known safety profile of the active substance. The similarity of BP14 to the reference product Neulasta in terms of clinical safety and immunogenicity is considered sufficiently addressed.

2.6. Risk Management Plan

2.6.1. Safety concerns

Table SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	Capillary leak syndrome
	Acute respiratory distress syndrome
	Sickle cell crisis in patients with sickle cell disease
	Glomerulonephritis
Important potential risks	Cytokine release syndrome
Missing information	None

2.6.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.6.3. Risk minimisation measures

Table V.3: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	**Risk minimisation measures**	**Pharmacovigilance activities**
Important Identified Risks		
Capillary leak syndrome	Routine risk minimisation measures: Listings in SmPC section 4.4 Special warnings andprecautions for use and 4.8 Undesirable effects Listings in PIL section 2. What you need to know before youuse pegfilgrastim and 4. Possible side effects Other routine risk minimisation measures beyond the ProductInformation: Symptoms of capillary leak syndrome should be closely monitored and receive standard symptomatic treatment, which may include a need for intensive care o Prescription only medicine.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: AE follow-up form for adverse reaction
Sickle cells crisis in patients with sickle cell disease	Routine risk minimisation measures: Listings in SmPC section 4.4. Special warnings and precautions for use and 4.8 Undesirable effects Listings in PIL section 2. What you need to know before you use pegfilgrastim	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None

	Other routine risk minimisation measures beyond the Product Information: Physicians should use caution when prescribing pegfilgrastim in patients with sickle cell trait or sickle cell disease, should monitor appropriate clinical parameters and laboratory status and be attentive to the possible association of this medicine with splenic enlargement and vaso-occlusive crisis. o Prescription only medicine.	
Glomerulonephritis	Routine risk minimisation measures: Listings in SmPC section 4.4 Special warnings and precautions for use and 4.8 Undesirable effects Listings in PIL section 2. What you need to know before you use pegfilgrastim and 4. Possible side effects Other routine risk minimisation measures beyond the Product Information: Urinalysis monitoring is recommended. o Prescription only medicine.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None
Acute respiratory distress syndrome	Routine risk minimisation measures: Listings in SmPC section 4.4 Special warnings and precautions for use and 4.8 Undesirable effects Listings in PIL section 2. What you need to know before you use pegfilgrastim and 4. Possible side effects Other routine risk minimisation measures beyond the Product Information: o Prescription only medicine.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None
Important Potential Risks		
Cytokine release syndrome	Routine risk minimisation measures: None proposed Other routine risk minimisation measures beyond the Product Information: Prescription only medicine.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: AE follow-up form for adverse reaction
Missing information None		

2.6.4. Conclusion

The CHMP considers that the risk management plan version 0.3 is acceptable.

2.7. Pharmacovigilance

2.7.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.7.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.8. Product information

2.8.1. User consultation

A justification for not performing a full user consultation with target patient groups on the package leaflet has been submitted by the applicant and has been found acceptable for the following reasons:

Full user consultation is not a mandatory requirement for a scientific opinion on a medicinal product under Article 58 of Regulation (EC) No 726/2004. The applicant provided a comparative table between the information mentioned in the reference package leaflet and proposed biosimilar product package.

2.8.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Dyrupeg (Pegfilgrastim) is included in the additional monitoring list as it is a biological product.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Biosimilarity assessment

3.1. Comparability exercise and indications claimed

A comprehensive analytical similarity exercise has been performed via comparison of the proposed biosimilar to the EU-authorised Neulasta reference medicinal product. A wide selection of orthogonal methods was employed in analytical exercises. The side-by-side analytical testing included methods for identity (including confirmation of PEGylation site and primary protein sequence), purity, content, product related variants and impurities (including size and charged variants), post-translation modifications, protein higher order structures and biological activity (including potency by bioassay, binding assay and immunogenicity properties by *in vitro* analysis). The applicant also performed a comparative evaluation of degradation profiles

of the proposed biosimilar and reference medicinal product. The batches used for both the analytical exercise and the comparative stability evaluation are considered representative of the proposed Dyrupeg commercial process.

3.2. Results supporting biosimilarity

Overall, the results of the performed analytical similarity exercise support the biosimilarity claim. Both structural, functional, and immunogenic similarity was demonstrated for the proposed biosimilar and the EU-authorised reference medicinal product. The identified minor excursions from the pre-defined similarity ranges were appropriately discussed and the similarity claim is also supported by the performed comprehensive comparison of degradation profiles.

The applicant conducted three *in vitro* immunogenicity studies. These qualitative tests comparatively assessed the immunogenicity of BP14 against reference medicinal product Neulasta.

Two clinical PK/PD studies BP14-101 and BP14-102 were performed. Study BP14-102 demonstrated bioequivalence between BP14 and EU-approved Neulasta. Confidence intervals for both primary PK parameters C_{max} (98.92% to 114.78%) and AUC_t (100.92% to 118.31%) were within the predefined acceptance range 80-125% and bioequivalence was concluded.

The primary analysis in the study BP14-102 was based on PK data without potency correction which is considered appropriate. The applicant also performed additional analysis with potency corrected PK data for difference in protein content between test and reference batch. This analysis also concluded bioequivalence with GMRs closer to 100%.

To investigate the effect of ADA on PK, the applicant performed analysis excluding ADA positive subjects. This analysis confirms bioequivalence of the products tested. The impact of ADA on PK was minimal.

The applicant also performed a meta-analysis of both submitted PK/PD studies BP14-101 and BP14-102 showing that the 90% CI of pooled results for the primary PK parameters were within the standard ER (80%, 125%). Pharmacokinetic equivalence between BP14 and Neulasta can be therefore concluded.

From a PD perspective BP14 and Neulasta are equivalent with respect to primary parameters ANC E_{max} and $AUC_{(0-t)}$ as shown in both submitted PK/PD studies. Corresponding 95% CI were (99.70%, 107.00%) and (98.53%, 102.15%) for ANC E_{max} and corresponding 95% CI for ANC $AUC_{(0-t)}$ were (98.40%, 106.50%) and (99.62%, 102.51%) in the studies BP14-101 and BP14-102, respectively.

The safety assessment of BP14 is based on two phase I randomized, single-dose, double-blind, two-sequence, two-period crossover studies to compare immunogenicity and safety (secondary objectives) of BP14 (pegfilgrastim) with EU-approved Neulasta in healthy male adult subjects (BP14-101 and BP14-102). In these studies, subjects received a single dose of 6 mg SC injection of either BP14 or Neulasta on Day 1 of each treatment period. According to the low incidence (discrepancy based mainly on individual basis), majority of observed cases with recovered outcomes, no safety concerns were raised, and the groups (BP14 vs reference medicinal product) were considered comparable from the safety point of view. The similarity of BP14 to the reference product Neulasta in terms of clinical safety and immunogenicity is considered sufficiently addressed.

3.3. Uncertainties and limitations about biosimilarity

The pharmacokinetic equivalence was not shown in the study BP14-101 as the confidence intervals for both primary PK parameters were outside predefined limits 80-125%. 90% CI for C_{max} was (103.70%, 129.30%) and 90% CI for $AUC_{(0-t)}$ was (108.10%, 136.60%). The GMR estimates for the both primary parameters were also higher for BP14 (1.158 for C_{max} and 1.215 for $AUC_{(0-t)}$). Nevertheless, pharmacokinetic equivalence was confirmed in the second study BP14-102 and metanalysis of both the studies.

With respect to the secondary parameters CD34+ Emax and AUC(0-t), bioequivalence was not concluded between BP14 and Neulasta in the study BP14-101, as these parameters were not in the specified equivalence range (corresponding 95% CI was (100.90%, 121.10%) for CD34+ Emax and 95% CI was (99.60%, 111.30%) for CD34+ AUC(0-t)). Higher levels of CD34+ for BP14 might corelate with higher exposure observed in the study BP14-101. Nevertheless, as these are secondary parameters in this Phase I study, impact on bioequivalence of these parameters is not considered critical.

3.4. Discussion on biosimilarity

Both the presented data for the analytical similarity and the overall strategy (QTPP, selection of methods and batches, statistical analysis and comparative stability) are found comprehensive and support the biosimilarity conclusion.

The first submitted PK/PD study BP14-101 failed to show similarity in pharmacokinetics between BP14 and EU-Neulasta. The second submitted PK/PD study BP14-102 demonstrated pharmacokinetic equivalence between BP14 and EU-Neulasta. The meta-analysis of both submitted Phase 1 PK/PD studies confirmed the pharmacokinetic equivalence.

Equivalence between BP14 and EU-Neulasta was concluded from the PD perspective with respect to primary parameters in both submitted PK/PD trials. Equivalence was not concluded between BP14 and Neulasta with respect to secondary parameters for CD34+ in the study BP14-101, as these parameters were not in the prespecified equivalence range. Nevertheless, impact on bioequivalence of these secondary parameters is not considered critical.

Based on the submitted results, biosimilarity between BP14 and the reference product Neulasta can be concluded from the PK and PD perspective.

The safety data collection is considered reliable and sufficient for establishment of safety for this product taking into account a well-known safety profile of this active substance and its nature, i.e. biosimilar.

The incidence of recorded AEs in adults was not unexpected as these events are mostly known and well reported for pegfilgrastim treatment. No new AEs with clinically significant difference between both treatment groups were identified and no known important risks for pegfilgrastim treatment were observed. As for the provided data, no relevant trends or clinically significant findings opposing the comparability of BP14 with the reference medicinal product were observed. The similarity of BP14 to the reference product Neulasta in terms of clinical safety and immunogenicity is considered sufficiently addressed.

3.5. Extrapolation of safety and efficacy

The claimed indication is the only indication currently approved for Neulasta ("Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for

malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes"). Therefore, no extrapolation to other indications is needed for this biosimilar application.

3.6. Additional considerations

Not applicable.

3.7. Conclusions on biosimilarity and benefit risk balance

Based on the review of the submitted data, Dyrupeg is considered biosimilar to Neulasta. Therefore, a benefit/risk balance comparable to the reference product can be concluded.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Dyrupeg is favourable in the following indication(s):

Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes).

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information

being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.