26 April 2019
EMA/CHMP/323149/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Grasustek

International non-proprietary name: pegfilgrastim

Procedure No. EMEA/H/C/004556/0000

Note

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

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List of abbreviations

ADA	Anti-Drug Antibodies
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ANCOVA	Analysis of covariance
ARDS	Acute Respiratory Distress Syndrome
AS	Active Substance
AST	Aspartate Aminotransferase
AUC	Area Under Curve
AUC	Analytical Ultracentrifugation
AUEC	Area Under the Effect Curve
CI	Confidence Interval
CI	Critical Intermediate
CPP	Critical Process Parameters
CV%	Coefficient of variation
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
DSN	Duration of severe neutropenia
E. coli	Escherichia coli
ELISA	Enzyme Linked ImmunoSorbent Assay
EMA	European Medicines Agency
EOPCs	End of Production Cells
EPAR	European Public Assessment Report
EU	European Union
FAS	Full Analysis Set
FMEA	Failure Modes and Effect Analysis
FN	Febrile Neutropenia
FP	Finished Product
G-CSF	Granulocyte-Colony Stimulating Factor
GGT	Gamma glutamyl transferase
GMP	Good Manufacturing Practice
H	Hours
HCP	Host Cell Proteins
HV	Healthy volunteer
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Ion Exchange Chromatography

IMP	Investigational Medicinal Product
IRS	Internal Reference Standard
IWS	Internal Working Standard
kD	Kilo Daltons
KPP	Non-Critical Key Process Parameters
LLOQ	Lower Limit of Quantification
LS	Least square
MAA	Marketing Authorisation Application
MCB	Master Cell Bank
m-PEG	methoxy polyethylene glycopropionaldehyde, mPEG aldehyde, activated PEG)
MW	Molecular Weight
Nab	Neutralizing antibody
PD	Pharmacodynamics
PEG	Polyethylene Glycol
PEG-GCSF	Pegfilgrastim
PFS	Prefilled Syringe
Ph.Eur.	European Pharmacopoeia
PK	Pharmacokinetics
PP	Per-Protocol
PPQ	Process Performance Qualification
QC	Quality Control
QP	Qualified Person
rhG-CSF	Recombinant Human Granulocyte Colony-Stimulating Factor/Filgrastim
RP-HPLC	Reverse-Phase High-Performance Liquid Chromatography
SA	Scientific Advice
SC	Subcutaneous
SD	Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEC-HPLC	Size Exclusion Chromatography
SmPC	Summary of Product Characteristics
SPR	Surface Plasmon Resonance
TEAE	Treatment-Emergent Adverse Event
TSE	Transmissible Spongiform Encephalopathies
USP	United States Pharmacopeia
vs.	Versus
WBC	White Blood Cells
WCB	Working Cell Bank

1. Background information on the procedure

1.1. Submission of the dossier

The applicant USV Europe Limited submitted on 6 November 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Grasustek, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The applicant has changed to Juta Pharma GmbH during the procedure at Day 181.

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 leukaemia and myelodysplastic syndromes)."

The legal basis for this application refers to:

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

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

Information on Paediatric requirements

Not applicable

Information relating to orphan market exclusivity

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.

Scientific advice

Scientific Advice summary for AR – Grasustek

The applicant received Scientific Advice on the development relevant for the approved indication from the CHMP on 25 June 2009 (EMEA/H/SA/1307/1/2009/III), 20 September 2012 (EMEA/H/SA/1307/1/FU/1/2012/III), 26 June 2014 (EMEA/H/SA/1307/1/FU/2/2014/III) and 21 July 2016 (EMEA/H/SA/1307/1/FU/3/2016/III) . The Scientific Advice pertained to the following quality, non-clinical and clinical aspects of the dossier:

- Manufacturing process: master cell bank characterisation, reagents, key intermediate products, manufacturing scale-up, specifications and characterisation, choice of analytical methods, internal reference standards, impurity profile and limits, release and stability;
- Number of batches and statistical approach to analytical comparability to support MAA;
- Container closure system suitability and testing
- Definition of starting materials.
- Adequacy of the analytical testing programme to demonstrate physicochemical and biological comparability with originator and support MAA.
- Adequacy of the toxico-pharmacological development plans: in vitro potency comparability; design of animal studies (species, PK/PD, duration, route of administration, impurity spiking
- Bioequivalence trial design: population, parallel-group vs crossover design, low-dose study, choice of margins and acceptance criteria
- Clinical trials design: PK sampling, dose, choice of comparator, sample size, patient population, regions, endpoints, equivalence margins, immunogenicity testing, follow-up duration.
- Statistical analyses plans
- Overall adequacy of the package for MAA submission.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Koenraad Norga Co-Rapporteur: Martina Weise

The application was received by the EMA on	6 November 2017
The procedure started on	23 November 2017
The Rapporteur's first Assessment Report was circulated to all CHMP members on	12 February 2018
The Co-Rapporteur's first Assessment Report was circulated to all	9 February 2018

CHMP members on	
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	14 February 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 March 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	12 October 2018
The following GMP and GCP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
• A GCP inspection at two Clinical Investigator sites in Serbia and Georgia between 29 January 2018 and 16 March 2018. The outcome of the inspection carried out was issued on	28 May 2018
• A GCP inspection at the bioanalytical laboratory in the United Kingdom from 28 January – 01 February 2019. The outcome of the inspection carried out was issued on	21 February 2019
• A GMP inspection at two sites responsible for manufacture of the active substance and finished product in India on 23-27/07/2018 and 17-21/09/2018. The outcome of the inspection carried out was issued on	3 December 2018 and 3 January 2019, respectively
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	19 November 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	29 November 2018
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	13 December 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	20 March 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	10 April 2019
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 Grasustek on	26 April 2019

2. Scientific discussion

2.1. Problem statement

About the product

Grasustek (Grasustek) belongs to the pharmacotherapeutic group of immunostimulants, colony stimulating factor (ATC Code: L03AA13). It is a covalent conjugate of recombinant human G-CSF and

polyethylene glycol (PEG), which results in sustained blood levels due to decreased renal clearance. Its mechanism of action is through regulation of the production and release of neutrophils from the bone marrow.

Grasustek is a biosimilar to Neulasta. The same indication as the reference product Neulasta is claimed by the applicant:

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

The recommended posology is one 6 mg dose of pegfilgrastim injected subcutaneously for each chemotherapy cycle, given at least 24 hours after cytotoxic chemotherapy.

Type of Application and aspects on development

The chosen reference product is:

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- Marketing authorisation number: EU/1/02/227/004

The relevant EMA guidelines and SA procedures referred for this clinical development programme are listed below.

- Guideline on similar Biological Medicinal Products (CHMP/437/04);
- Guideline on similar Biological Medicinal Products (CHMP/437/04 Rev 1);
- Guidance on Similar Medicinal Products Containing Recombinant Granulocyte-Colony Stimulating Factor (EMEA/CHMP/BMWP/31329/2005);
- Guideline on Similar Biological Medicinal Products containing Biotechnology-Derived Proteins as Active Substance: Non-Clinical and Clinical Issues (EMEA/CHMP/BMWP/42832/2005);
- 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);
- Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins (CHMP/EWP/89249/2004);
- Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **);
- Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins (EMEA/CHMP/BMWP/14327/2006);
- Initial Scientific Advice, EMEA/CHMP/SAWP/357301/2009, Procedure No.: EMEA/H/SA/1307/1/2009/III, 25 June, 2009;
- Follow-up Scientific Advice, EMA/CHMP/SAWP/561213/2012, Procedure No.: EMEA/H/SA/1307/1//FU/1/2012/III, 20 September 2012;
- Scientific advice EMA/695467/2012 clarification letter: EMEA/H/SA/1307/1/FU/1/2012/III, 31 October 2012;
- Follow-up Scientific Advice, EMA/CHMP/SAWP/343876/2014, Procedure No.: EMEA/H/SA/1307/1/FU/2/2014/III, 26 June 2014;

- Follow-up Scientific Advice, EMA/CHMP/SAWP/476338/2016, Procedure No.: EMEA/H/SA/1307/1/FU/3/2016/III, 21 July 2016;
- Corrigendum EMA/CHMP/SAWP/517803/2016, Procedure No.: EMEA/H/SA/1307/1/FU/3/2016/III, 21 July 2016.

The applicant's clinical development programme comprises 3 studies, 2 studies in healthy volunteers at 2 different dose levels for evaluation of comparative pharmacokinetics (PK) and pharmacodynamics (PD), and a comparative safety and efficacy study in patients with breast cancer undergoing adjuvant myelosuppressive chemotherapy.

The clinical trials have been conducted in line with CHMP guidance and recommendations from scientific advices. The CHMP acknowledged that the additional PK/PD study at the sub-therapeutic dose (2 mg) was not a regulatory requirement but would strengthen the totality of evidence to support biosimilarity. Furthermore, it is considered that PK/PD trials are more sensitive than the efficacy trial in patients with breast cancer for the purpose of demonstrating comparable efficacy; in addition, this immunosuppressed population is not sensitive for the comparison of immunogenicity. Therefore, the results of this trial are considered as mainly supportive.

2.2. Quality aspects

2.2.1. Introduction

The active substance (AS) of Grasustek is pegfilgrastim. Pegfilgrastim (PEG-GCSF) is a long-acting, pegylated form of recombinant human granulocyte colony-stimulating factor (rhG-CSF or filgrastim) which has a longer half-life compared to its parent molecule, filgrastim.

The finished product (FP) is presented as a sterile solution for injection in a 1 mL prefilled syringe (PFS) containing 0.6 mL of a 10 mg/mL solution (6 mg pegfilgrastim per syringe) as AS for subcutaneous injection.

Other ingredients are D-sorbitol, polysorbate 20, sodium acetate (formed by titrating glacial acetic acid with sodium hydroxide) and water for injections (WFI).

The product is available in a pre-filled syringe (USP Type I glass, compliant to Ph.Eur.3.2.1), with a (butyl) rubber stopper and a stainless steel needle with automatic needle guard. The needle has a flexible, rigid needle shield.

Biosimilarity is claimed to NEULASTA.

2.2.2. Active Substance

General information

Pegfilgrastim AS is the N-terminally pegylated form of recombinant human granulocyte colony stimulating factor (rh-G-CSF) or filgrastim. Filgrastim protein has two intramolecular disulfide bridges between Cys37 - Cys43 and Cys65 - Cys75, which forms 2 small loop structures that maintain the biologically active conformation of granulocyte colony stimulating factor.

Due to expression in *E.coli*, filgrastim is a non-glycosylated (in contrast to the native hGCSF) protein of 175 amino acid residues with an additional methionine group attached to the human GCSF amino acid sequence. Pegfilgrastim is produced by covalent attachment of one ~21 kDa PEG (polyethylene glycol) molecule to the amino terminal methionine residue of the filgrastim protein. The relative molecular

mass of Pegfilgrastim is ~ 40 kDa (18.8 kDa for the protein component and ~ 21 kDa for the PEG component).

Manufacture, characterisation and process controls

The active substance is manufactured at USV Private Limited, D-115, TTC Industrial Area, Shirvane, Navi Mumbai - 400706 (Maharashtra, India). An appropriate GMP certificate has been provided further to a MO raised at D120.

The manufacturing process of pegfilgrastim AS can be described in two stages:

i) Manufacturing of filgrastim (stage I): a. Upstream manufacturing process; b. Downstream manufacturing process

ii) Manufacturing of pegfilgrastim AS (stage II): pegylation reaction of filgrastim and downstream manufacturing process.

Stage I: Filgrastim manufacturing process

The protein moiety of pegfilgrastim is expressed in *E.coli* starting with thawing of a cell bank vial followed by culture expansion and production fermentation.

The fermentation broth is then harvested and subjected to concentration and clarification, followed by homogenisation. The clarified, homogenised broth marks the end of upstream manufacture.

The downstream purification process of filgrastim involves solubilisation of the concentrated homogenate, followed by a refolding step, additional chromatographic and filtration steps. A final concentration and diafiltration step is followed by filtration of bulk filgrastim solution into specified storage bags that may be stored as an intermediate. Filgrastim lots are tested and released prior to use. Multiple batches of filgrastim within the stability period are pooled for the manufacturing of pegfilgrastim AS.

Stage II: Pegfilgrastim AS manufacturing process

Filgrastim batches are pooled to obtain the required quantity of filgrastim to manufacture a batch of pegfilgrastim. The pegylation reaction is performed and the PEGylated GCSF is purified by a series of chromatography and filtration steps prior to diafiltration, concentration and final 0.2 µm filtration. Pegfilgrastim is collected in specified storage bags and stored until further processing to finished product.

The batch numbering system and batch scale are in place and appropriately defined.

There is no reprocessing throughout the entire manufacturing process. Information on in-process controls is provided (see control of critical steps and intermediates section).

Control of materials

All raw materials used in the AS manufacturing process are described and are either compendial grade or are tested according to in-house standards. In house specifications for non-compendial raw materials are provided. AS is a pegylated form of filgrastim. There was a major objection at D120 and mPEG was subsequently reclassified as an intermediate. No human or animal derived materials are used in the active substance manufacturing process, nor used in the manufacture of the master cell bank (MCB) or working cell bank (WCB).

Filgrastim is expressed in *E. coli* during the manufacturing process (fermentation). The construction of the final expression vector involved multiple modifications and manipulations. *E. coli* cells were then transformed with the final construct.

A two tiered cell banking system is used. Both the master and working cell bank were manufactured under GMP conditions. The established cell banks are tested and characterized in accordance with ICH Q5D. Also end of production cells (EOPCs) have been tested. Cell banks are stored at -80 °C. The cell bank system has been adequately described, including information on genetic stability and protocols for future WCB preparation.

Control of critical steps and intermediates

Process characterisation studies and prior knowledge were used to develop operational ranges for process parameters and in-process tests. Based on prior risk assessment and the outcome of process characterisation studies, the critical process parameters (CPP) were identified besides non-critical key process parameters (KPP) and general process parameters (GPP). Appropriate justifications were provided for the assigned operational ranges for each parameter. The control strategy developed is considered suitable to ensure that a product of acceptable quality will be manufactured and to ensure consistency of the downstream process with respect to product- and process-related variants. Filgrastim is the critical intermediate (CI) used in the manufacturing process of pegfilgrastim and the only stored intermediate. Manufacture is described in the manufacture of AS section. Filgrastim quality is appropriately controlled via the specifications, as supported by batch data.

With regards to the specification limit for host cell proteins (HCP), the applicant has committed to re-evaluate the HCP specification limit once a specified number of batches of filgrastim CI have been analysed using the in-house method (recommendation). Respective stability studies support the storage period in specified bags. Information is provided regarding the analytical procedures, method validation, specifications and stability of filgrastim.

Further to the MO raised at D120, mPEG was redefined as an intermediate and consequently more detailed information on its specification, control and stability was provided. The manufacturing process flow, process description, process controls and the in-process testing of mPEG is described and the process has been validated. Batch analysis data support the in-house specifications are provided. Information about m-PEG used in pegylation, on its specification, control and stability was provided. Sufficient details pertaining to the manufacturing process and its controls are described. Batch analysis data support the in-house specifications provided.

Based on the stability results, an appropriate shelf life in specified storage containers is accepted. Finally, an appropriate QP declaration to confirm that the intermediate is manufactured in accordance with GMP has been provided.

Process validation

The manufacturing processes for filgrastim and pegfilgrastim AS have been validated with the objective of producing product with predefined quality attributes while manufacturing within the specified operating ranges of process parameters of individual unit operations. A prospective approach was used for validation of filgrastim and pegfilgrastim manufacturing processes.

Process validation ranges were defined based on process characterization studies. The results of FMEA were analysed to determine the process parameters that should be studied in process characterization experiments. Process characterization studies further contributed to demonstration of process robustness and the justification of process control ranges used in the process validation activity. Process characterization experiments were performed using scale down models of the manufacturing process.

Several batches of filgrastim and pegfilgrastim AS were manufactured under the process validation campaign, according to the proposed commercial process and at commercial scale. All raw materials used were procured from qualified vendors and were tested and released upon receipt.

Based on the summary and evaluation of process validation data, it was demonstrated that the controls established at each unit operation were sufficient to deliver consistent output within the inherent variation of the respective unit operation. Each unit operation delivered consistent performance which was within the pre-defined acceptance criteria and yielded product with consistent quality attributes.

Hold time studies were performed for all intermediates intended to be held for a certain processing time and the hold times are considered supported.

Tabulated results of lifetime studies for chromatography resins have been provided on a concurrent basis at commercial scale. Membrane reuse cycles were established as well and the claimed reuse cycles are adequately supported by data.

Furthermore, the manufacturing process has also been validated with regards to hold times of media, buffers and accessories, membrane reuse, cleaning validation and shipping validation.

Manufacturing process development

Since development, the filgrastim and pegfilgrastim manufacturing process has evolved. Designation of a process variation or change was defined as a change in scale, or the addition/deletion of, or significant change to, a unit operation. The production scales for filgrastim (upstream and downstream) and pegfilgrastim AS for intended commercial manufacturing, have been validated.

Process comparability exercises have been conducted at different process development stages. The details of process changes in unit operation are described and considered minor. Comparison of relevant quality attributes at applicable unit operations between the scales were conducted and demonstrated that the pre- and post-change products are comparable. Accordingly, non-clinical material and clinical trial material are comparable to (and thus representative for) the process performance qualification (PPQ) and commercial batches.

Characterisation

Pegfilgrastim AS was analysed for physicochemical and biological characteristics.

Primary structure was verified using peptide mapping followed with electrospray ionisation tandem mass spectrometry (ESI MS/MS), peptide mapping followed by reverse-phase high-performance liquid chromatography (RP-HPLC) and western blot. Secondary structure was analysed by circular dichroism and peptide mapping to confirm disulphide linkage analysis, and free cysteine confirmation. Tertiary structure was verified using fluorescence spectroscopy. Furthermore, pegfilgrastim AS was also analysed using differential scanning calorimetry and analytical centrifugation. The characterisation studies confirmed that pegfilgrastim AS showed the expected structure. Purity was evaluated by a combination of different methods: size exclusion chromatography (SEC-HPLC), RP-HPLC, IEX-HPLC and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Overall purity level was very high.

The potency of pegfilgrastim was investigated by the compendial cell proliferation assay for filgrastim (in vitro cell line based proliferation assay). In this assay, pegfilgrastim binds to the G-CSF receptor on the cell surface and triggers intracellular signalling pathways resulting in dose-dependent cell proliferation. Cell proliferation is detected by a tetrazolium-based substrate that is reduced by mitochondrial enzymes of viable cells producing a coloured product which is detected photometrically. Relative potency is compared to a reference standard. A receptor binding assay by surface plasmon

resonance (SPR-BIAcore) has been employed and demonstrated that the association and dissociation of FP with the filgrastim receptor is highly comparable to that of EU-authorized Neulasta.

For process-related impurities a risk assessment was performed. Small scale spiking studies for specified process-related impurities (including host cell proteins (HCP) and residual DNA) were performed and demonstrated adequate clearance capability of the manufacturing process.

Data were provided to demonstrate that process-related impurities are cleared by the manufacturing process.

Analysis of product-related impurities/substances has been performed employing orthogonal chromatographic methods (RP-/SEC-/IEC-HPLC). Size-related variants were identified as dimer/di-pegylated and unpegylated filgrastim ll at low levels. Identification of related substances revealed oxidised and deamidated variants at low levels. Deamidated variants were found in pegfilgrastim at low levels and multiple potential deamidation sites were identified. Truncated forms, reduced forms and positional isomers were not observed. Overall, the characterisation of product-related impurities is considered comprehensive and the results are consistent across the orthogonal methods.

Specification

Specification

An active substance specification is provided including test parameters on identity, potency, content, purity, impurities, and microbiological safety. The list of parameters is considered comprehensive. The specification for active substance stability testing is nearly identical to release testing. During the procedure, a number of specifications for product related substances/ impurities were revised upon request.

Analytical methods

All methods used for release testing were developed in-house except for the compendial methods (description, pH, endotoxin and bioburden). Development summaries along with method comparability details, as applicable, are explained in the respective section for each test parameter.

Method validation reports have been submitted for the non-compendial analytical methods. Validations were performed in accordance with ICH Q2(R1). The presented data support the suitability of the methods for their intended use.

Batch analysis

Batch data were provided (non-clinical batches, clinical batches and an appropriate number of process validation batches -all commercial scale) showing that all batches were consistent and complied with the specifications.

Reference materials

The applicant follows a two-tier system for the reference standard in accordance with ICH Q6B. An internal reference standard (IRS) has ben developed, which is the primary standard, and internal working standard (IWS), which is the secondary reference standard. The IRS and IWS are characterized using orthogonal methods. Qualification of future reference standards will be based on a battery of test parameters and their respective acceptance criteria.

Container closure

Specified storage bags are used for storage of the AS. A study regarding potential extractables/leachables has been performed using AS and demonstrated that no relevant amounts of extractable/leachables are found. The container closure system has been adequately described.

Stability

An appropriate shelf-life and storage conditions for pegfilgrastim AS is proposed.

Stability studies of AS have been performed according to ICH under real time and accelerated conditions. The available stability data support the proposed AS shelf life.

Methods used were stability-indicating. Real time stability data from commercial scale batches filled in specified bags (representative of commercial product) are available for clinical batches and an appropriate number of process validation batches.

Photo stability studies have been performed. The studies demonstrated that pegfilgrastim is photosensitive.

Stability studies demonstrate that pegfilgrastim AS remains stable when stored in specified bags during the proposed shelf-life and storage conditions and these are therefore approved.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Grasustek pegfilgrastim injection (6 mg/0.6 mL) is supplied as a sterile, clear and colourless preservative-free solution for injection (pH 3.8 to 4.3) in a 1 mL USP Type 1 glass (compliant to Ph.Eur.3.2.1) pre-filled syringe (PFS). Grasustek final product will be supplied as a single pack containing a single pre-filled syringe with needle guard. The qualitative and quantitative composition of Grasustek injection (6 mg/0.6 mL) in pre-filled syringe is given in Table 4 below. Grasustek 6 mg/0.6 mL injection is not formulated with protein overages. It has a defined overfill (0.65 mL target fill volume) to ensure an extractable volume of 0.6 mL at the time of administration.

The quantitative and qualitative composition of Grasustek is the same as the formulation of the reference product Neulasta.

Specifications for the excipients used in the formulation of Grasustek final product conform to the current revision of each excipient's Ph. Eur. monograph. There are no excipients of human or animal origin used in formulation of Grasustek final product. Grasustek final product does not contain novel excipients.

The excipients include polysorbate-20 (stabiliser), D- Sorbitol (E420- isotonicity adjuster), sodium acetate (formed by titration of glacial acetic acid and sodium hydroxide- buffering agent) and water for injection (solvent vehicle).

Pharmaceutical development

Based on the formulation of the reference product Neulasta, formulation robustness studies were conducted. Optimal stability was found at pH of 4.0 with pegfilgrastim buffered in 10 mM sodium acetate and stabilised with sorbitol.

The Grasustek manufacturing process history is appropriately described. A comparison of the FP manufacturing processes conducted at development stages and the commercial process demonstrates that there are no significant differences. Minor changes were needed to account for a higher batch scale, different equipment and to adapt the process to the prefilled syringe container closure system. Thus, the conclusion that the changes did not impact Grasustek FP quality based on batch release data only, is considered supported. The pivotal clinical studies PEGF/USV/PI/001 and PEGF/USV/P3/003 were conducted with the final formulation.

Extractables and leachables studies were executed in order to evaluate the compatibility between primary packaging and Grasustek FP. Based on the extractables determined within the extraction studies, a suitable leachables study was initiated. After 36 months of storage at the recommended storage temperature or 6 months under accelerated conditions, no leachable was detected.

Manufacture of the product and process controls

Final product manufacture and quality control (QC) testing sites are specified. Final product manufacture and quality control (QC) testing sites are specified.

Juta Pharma GmbH, Germany is responsible for EU batch release of the final product. A major objection was raised at D120 requesting a valid GMP certificate for the FP manufacturing site. A valid certificate was supplied during the procedure. Therefore, it has been confirmed that all sites hold appropriate GMP authorisations.

The batch size is specified.

For pegfilgrastim final product manufacturing, formulation buffer is prepared, aseptically filtered and mixed with pegfilgrastim active substance to produce the formulated bulk solution (final product). The process description is found to be satisfactory to ensure homogeneity. Using an automatic syringe filling machine, the filtered formulated bulk solution is filled into syringes and the syringes are plunger-stoppered. Filled syringes are subsequently labelled and packaged. The process is adequately described. The process parameters and IPCs are indicated. Process hold times are indicated. The primary container components are purchased pre-sterilised.

Process controls

The pegfilgrastim final product QC strategy includes, but is not limited to, the following:

• Controls on material attributes, including critical raw materials, starting materials, reagents and primary packaging material

• Controls on design of the manufacturing process

• In-process manufacturing process controls

• Controls on the final product

The process control strategy for FP manufacturing is a risk based approach including FMEA. Upon request, the company revised the control strategy and criticality assignments for process parameters and in-process tests was based on process characterisation studies, FMEA risk assessment and process development knowledge. In addition, appropriate justifications were provided for the assigned operational ranges for each parameter. Based on the updated documentation, the control strategy developed is considered suitable to ensure that a product of acceptable quality will be manufactured.

Process validation/verification

The Grasustek proposed commercial manufacturing process was qualified according to a protocol that defined the sampling, analytical testing plan and acceptance criteria for each process step. The proposed commercial pegfilgrastim final product manufacturing process was validated during an appropriate number of consecutive Grasustek production batches.

A process performance qualification study was conducted for Grasustek 6 mg/0.6 mL pre-filled syringes. Critical process parameters and quality parameters across the manufacturing process were evaluated and found within limits. Sampling at different stages of manufacturing was performed as per the approved process performance qualification protocol. Equipment qualification and cleaning was verified. Adequate filter validation and compatibility studies have been conducted.

Finished product analytical data complied with the specification limit. In general, it can be concluded that the manufacturing process consistently produces product that meets its predetermined specification and quality characteristics. Shipping conditions for the transport to the EU importation site are provided and supported by data.

A program will be followed which collects on-going process monitoring and validation data for all subsequent batches to assure that the proposed manufacturing process continues to consistently produce the Grasustek final product. Any significant process changes will be covered via a new process performance qualification, with similar approach.

Product specification

Apart from some additional general tests (for which Ph. Eur. analytical methods are used), the final product specifications are very similar to those of the active substance. Method descriptions and validations are described in the AS section.

The FP specification includes tests on identity, impurities, biological activity, protein concentration, excipient and pharmaceutical particulars. An end of shelf-life specification is also provided. Upon request, a test for content of a specified excipient has been added to the FP specifications. The specification proposed for this is agreed based on the submitted data although the applicant is recommended to review this specification in the light of further manufacturing experience (see recommendations).

Currently, three different analytical methods have been established for FP impurity control- RP-HPLC, SEC and cation IEC. No new impurities are introduced during the FP manufacturing process.

A rationale for the specification acceptance criteria is presented. The justification provided can be accepted, including acceptance limits proposed for the impurities and product-related substances of Grasustek FP determined by RP-HPLC, SEC and IEC.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch data were provided (non-clinical batches, clinical batches and an appropriate number of process validation batches -all commercial scale) showing that all batches were consistent and complied with the specifications.

Reference materials

The reference standards used are the same as for the AS (see reference standards section).

Container closure

A 1 mL pre-filled syringe is used as the container closure system for Grasustek FP which is comprised of the following components:

- A sterile, clean glass syringe barrel assembled with a stainless steel needle. The glass barrel is of Type I quality according to Ph. Eur. 3.2.1.

- Rigid Needle-Shield.

• A grey bromobutyl plunger stopper

• A needle guard system.

Relevant information on the packaging components has been presented. The components comply with relevant European standards.

Stability of the product

A shelf-life of 36 months for Grasustek finished product batches when stored at 5 ± 3 °C is proposed.

Stability studies of final product have been performed under real time (5 ± 3 °C) and accelerated (25 ± 2 °C) conditions. Test methods include stability-indicating parameters.

The proposed shelf-life is supported by the stability studies. Stability studies demonstrate that Grasustek final product remains stable up to 36 months when stored at 2-8 °C.

Real time stability data (according to ICH) are available for non-clinical, clinical batches and a suitable number of process validation batches. . All stability results are compliant with the specifications. Accelerated stability studies (25 ± 2 °C) were performed up to 6 months on commercial scale batches.

Results of the photo stability study show that the finished product is photosensitive. Therefore, the product information includes a warning to keep the container in the outer carton in order to protect from light.

Thermal excursion was additionally studied for the FP at different conditions. As described in the SmPC, Grasustek may be exposed to room temperature (not above 30°C) for a maximum single period of up to 72 hours. Grasustek left at room temperature for more than 72 hours should be discarded. The thermal excursion data also support the SmPC statement that accidental exposure to freezing temperatures for a single period of less than 24 hours does not adversely affect the stability of Grasustek.

A shelf-life of 36 months for Grasustek when stored at 5 ± 3 °C is approved.

Adventitious agents

The manufacture of pegfilgrastim AS does not utilize any raw materials of human or animal origin therefore viral and TSE safety is assured. Where applicable, bioburden and/or endotoxin specifications for incoming raw materials used in manufacturing have been established.

In addition to raw material contamination controls, the pegfilgrastim AS manufacturing process contains multiple preventive measures against adventitious agent contamination. All equipment is cleaned and either sanitized or sterilized, using in-house procedures. Open operations are minimized; those that are required are conducted in controlled environments and by personnel wearing protective gowns according to environmental requirements. In-process sampling to monitor bioburden and endotoxin is conducted throughout the process. Resins used in manufacturing are cleaned and sanitized as appropriate.

Sufficient information has been presented to give reassurance on viral/TSE safety.

Biosimilarity

The development of the biosimilar product Grasustek is based on comparability to the reference product Neulasta derived from the EU market. The quality target product profile (QTPP) of Grasustek is based on EU Neulasta. Preclinical and clinical studies were performed in comparison to EU Neulasta. To establish biosimilarity of Grasustek to EU Neulasta, a head-to-head analytical similarity study was performed. An overview of the biosimilarity analyses is provided in Table 1 below.

Table 1: Overview of the biosimilarity analyses

Molecular parameter	Attribute	Methods for control and characterization	Key findings
Primary structure	Amino acid sequence Pegylation site Disulfide bridges	Non-reducing/ Reducing peptide mapping (MS)	Identical primary sequence with confirmed pegylation site and disulphide bridges
Higher order structure	Secondary and tertiary structure	CD spectroscopy, FTIR, DSC, fluorescence spectroscopy	Comparable higher order structure
Intact mass	Molecular weight	ESI MS	Slight difference in MW, no impact
Mass of PEG-aldehyde	Molecular weight	ESI MS	Slight difference in MW, comparable polydispersity, no impact
High molecular weight proteins	Aggregates	SEC, AUC, DLS	Lower amount of HMW impurities than in Neulasta
Hydrophobic impurities	Oxidised forms, deamidated forms	RP-HPLC	Lower amount of hydrophobic impurities than in Neulasta, except for certain impurity
Charged impurities	Charged impurities Free filgrastim	IEC	Comparable amount of free filgrastim
Impurity	Free PEG-aldehyde	RP-HPLC-ELSD	Comparable amount of free PEG-aldehyde
Activity	Biological activity	Cell proliferation	Comparable
Activity	Biological activity	Receptor binding	Comparable
Strength	Protein content	UV280	Comparable

Depending on the method, number of batches of Grasustek and EU Neulasta were included in the analytical similarity study. Overall, the number of reference and test batches included in the study is considered acceptable. Head-to-head analytical similarity studies were performed with test batches reflecting all development phases of Grasustek. Analytical methods used for analytical similarity studies were those developed at the time of analysis.

Primary structure analysis confirmed identical amino acid sequence and pegylation site in the test and the reference product. Slight differences in molecular mass of pegfilgrastim and reference Neulasta was observed. This is attributed to the PEG-aldehyde used for Grasustek. Secondary and tertiary structures including the correct presence of disulphide bridges in the test and reference products is appropriately analysed using diverse state-of the art analytical methods. Based on the results, biosimilarity in terms of higher order structures is demonstrated.

Aggregates in the test and reference products are investigated using SEC, analytical ultracentrifugation (AUC) and dynamic light scattering (DLS). Within the limitations of the methods used it is confirmed that the amount of high molecular weight impurities including dimer co-eluting with dipegylated filgrastim impurities, trimer and aggregates is within or even lower than the range of the reference

product. In addition the amount of unpegylated filgrastim seems to be lower in pegfilgrastim than in Neulasta.

RP-HPLC is used to compare the amount of oxidised and deamidated forms, i.e. oxidation at M127 ("ox-1") and M138 and deamidation at multiple sites. Overall, RP-HPLC impurities of the test product are found within the range of Neulasta or even lower. Although certain specified attributes are higher in Grasustek, the total amount of these impurities in Grasustek is still very low and not considered clinically relevant.

Comparative IEC analysis demonstrates the total of IEC impurities in Grasustek to be below the reference range of Neulasta. The amount of free PEG aldehyde in Grasustek and EU Neulasta was studied and found to be lower in Grasustek than in Neulasta. The impression of an overall higher purity in Grasustek in comparison to the reference product Neulasta is also supported by the comparative stability data provided.

Biological activity in terms of cell proliferation and receptor binding was investigated. Comparable biological activity has been demonstrated for the test product and EU Neulasta. Upon request, the applicant provided the qualification report of the method that was used to analyse receptor binding (SPR) in the biosimilarity analysis. The method has been properly qualified.

Protein concentration results demonstrate high similarity between the candidate biosimilar and reference product. Summarising, biosimilarity of Grasustek with the reference product EU Neulasta with respect to primary, secondary and higher order structures has been confirmed. Grasustek has been demonstrated to have higher purity than Neulasta with respect to oxidized and reduced, deamidated and charged variants, dimers, trimers and aggregates as well as free filgrastim and free PEG-aldehyde. This is also supported by the comparative stability data provided so far. In addition, slight differences between Grasustek and the reference product Neulasta exist with respect to certain attributes. Taking these differences into consideration and based on the totality of data, Grasustek could be considered as biosimilar to EU Neulasta from a quality point of view.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Development, characterisation, manufacture and control of pegfilgrastim AS and FP are described with a high level of detail.

During the procedure two major objections (MOs) were raised. One of them concerned the request for GMP certificates for the AS and FP manufacturing sites. The other major objection related to a request for reclassification of the mPEG, which resulted in the need for: a) confirmation that the intermediate is manufactured to appropriate GMP standards and b) more detailed information requirements in the dossier to support use of this intermediate in further downstream processing of the AS to produce pegfilgrastim. Both MOs were satisfactorily resolved as detailed in the report.

Biosimilarity of Grasustek with the reference product EU Neulasta has been confirmed with respect to primary, secondary and higher order structures. A higher purity than determined for Neulasta has been demonstrated for Grasustek. Slight differences between Grasustek and Neulasta exist with respect to M127 oxidation and molecular weight. Comparability with respect to biological activity and protein content is confirmed. Based on the totality of data, Grasustek can be considered as biosimilar to EU Neulasta from a quality point of view.

The applicant is recommended to re-evaluate and revise the specification limits for a specified excipient when data from more batches are available (see recommendations in Annex II).

2.2.5. 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. Information has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendation(s) 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:

Area	Number	Description	Classification *
Quality	001	The applicant is asked to re-evaluate the specification limit for HCP once a specified number of batches of Filgrastim CI have been analysed and to provide this revised specification limit upon availability.	REC
	002	The applicant is asked to re-evaluate the specification limits for a specified excipient once a certain number of FP batches have been analysed and to provide these revised specification limits upon availability.	REC

*REC - recommendation

2.3. Non-clinical aspects

2.3.1. Introduction

The nonclinical programme of pegfilgrastim included a series of head-to-head *in vitro* comparative studies including binding studies and cell based assays. A non-clinical comparative study in healthy and neutropenic rats, which evaluated pharmacokinetics (PK) and pharmacodynamics (PD) after a single dose of 50, 150, or 450 µg/kg, was conducted to further support the similarity demonstration between Grasustek and Neulasta. Finally, a 4-week (total of 5 administrations of 100; 300 and 1000 µg/kg) repeat-dose toxicity study including toxicokinetic assessments on days 1 and 29 was performed in CD rats and a dedicated local tolerance study was performed in New Zealand White rabbits.

Safety pharmacology, genotoxicity, carcinogenicity, single and reproductive and developmental toxicity studies were not submitted and are not required in this case.

2.3.2. Pharmacology

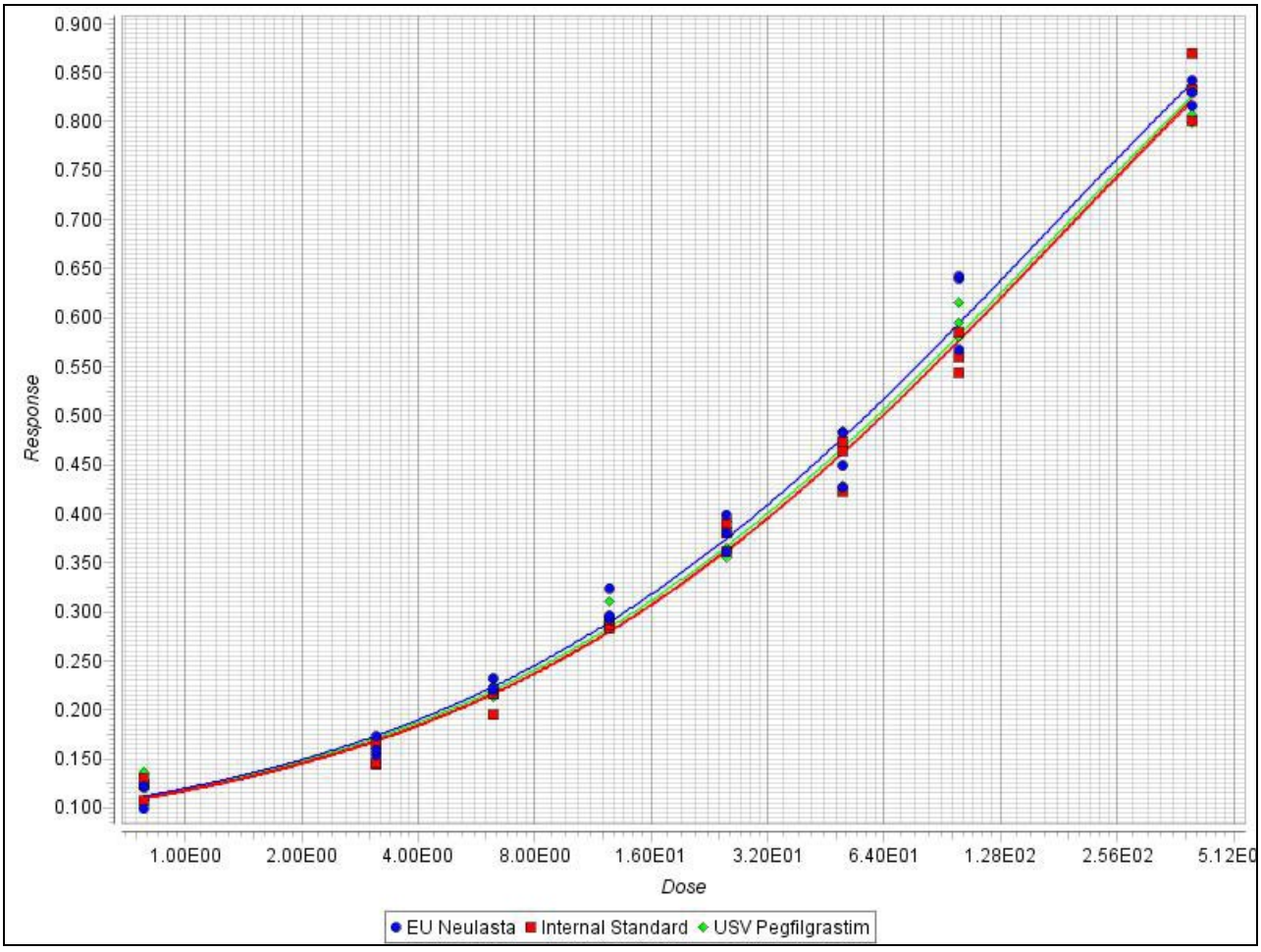
Primary pharmacodynamic studies

The nonclinical pharmacology *in vitro* and *in vivo* studies were conducted to assess the biosimilarity of Pegfilgrastim (Grasustek) compared to Neulasta, using a variety of batches of both Grasustek and Neulasta. Biosimilarity studies included *in vitro* cell-based models (M/G-NFS-60 cells, a murine

myeloblastic cell line) and receptor binding assays by Surface Plasmon Resonance (SPR); and *in vivo* PK/PD studies in normal and neutropenic rats.

Cell proliferation assay

G-CSF stimulates the proliferation of a murine myeloblastic cell line, designated as NFS-60. This was used to determine the relative biological potency of pegfilgrastim and compare it between Grasustek and Neulasta in an appropriate number of batches. Figure 1 below shows two concentration-response curves taken from two experiments.



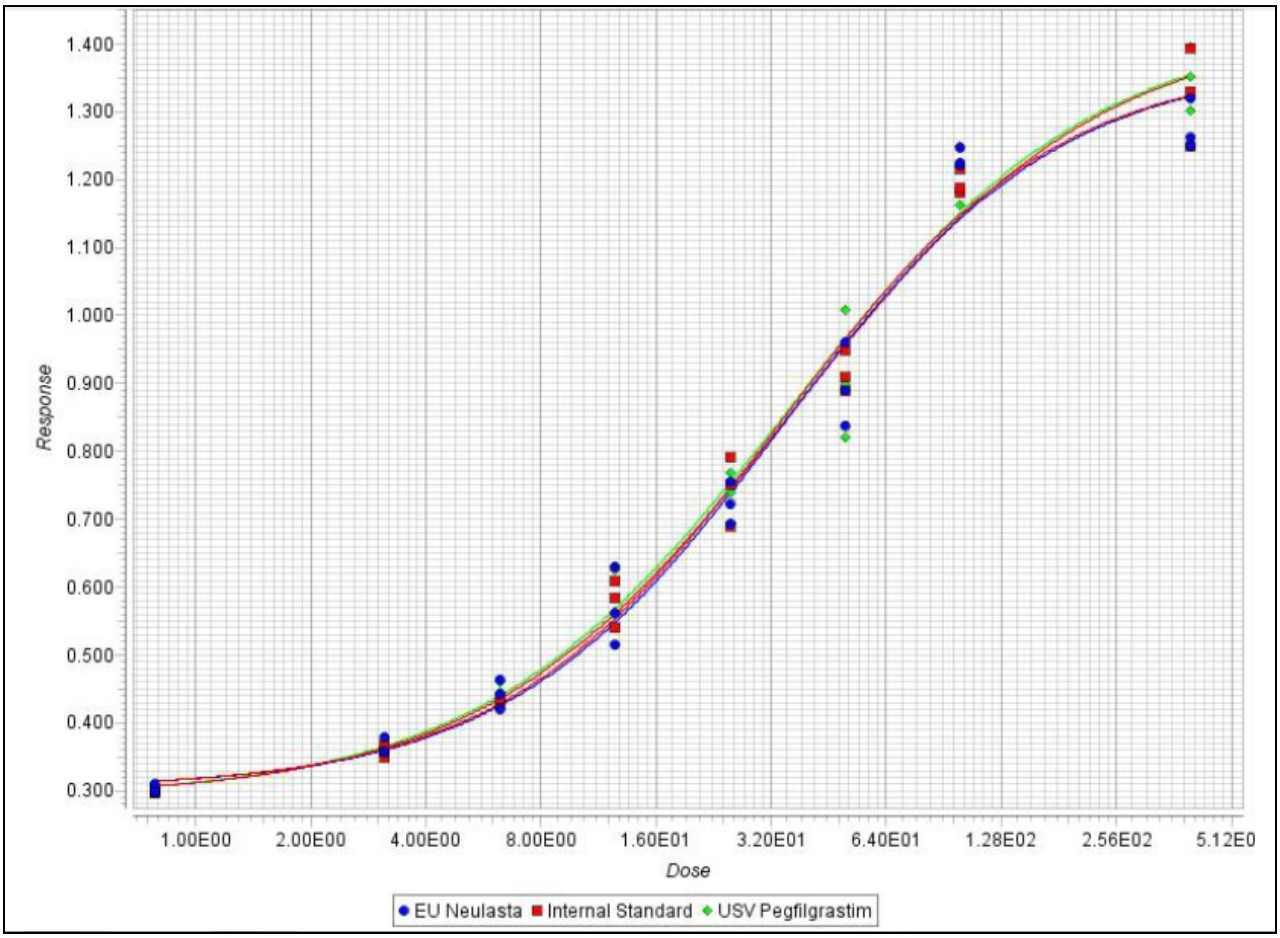


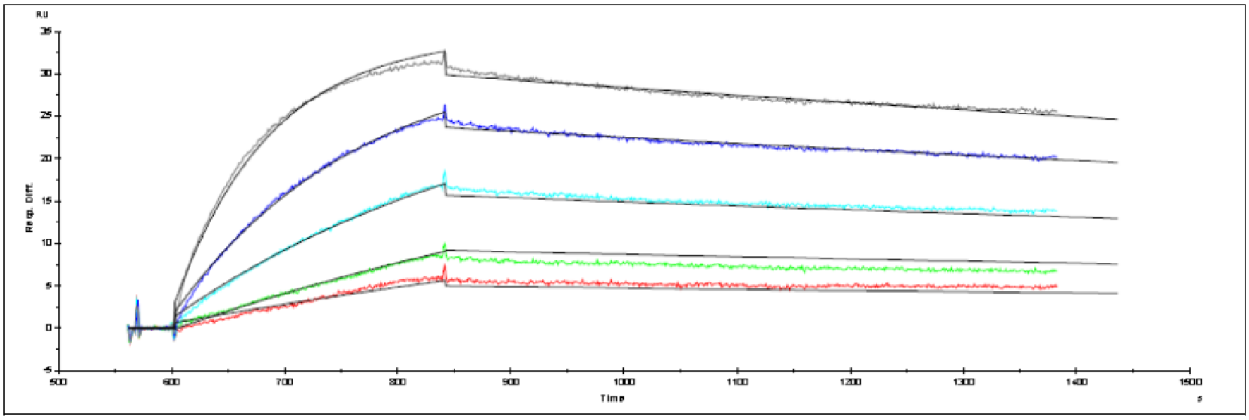
Figure 1. Representative dose response curves (PLA) shown for cell proliferation following treatment with two batches of Grasustek and EU-authorized Neulasta

For each batch tested, a relative potency value (relative to the internal standard) was calculated during the comparability studies.

In general, relative potency was around 1 in most cases.

Receptor binding assay (RBA) by SPR

The receptor binding affinity (RBA) of pegfilgrastim to the G-CSF receptor was investigated by SPR using Biacore 3000 and T200 instruments. RBA results from all studies show that the association and dissociation of Grasustek with the filgrastim receptor is comparable. The results of a representative Biacore experiment, measuring binding and dissociation with ascending doses of pegfilgrastim, are shown in Figure 2 below.



`Legend red: 12.5 ng/mL; green 25 ng/mL, cyan: 50 ng/mL, blue: 100 ng/mL, grey: 200 ng/mL; black: fitted curves

Figure 2. Sensorgrams of the Biacore receptor binding kinetics

The results of receptor binding assay such as K_D, K_{on} and K_{off} are found to be comparable between Grasustek and Neulasta®.

In vivo study in rats

The applicant additionally used a rat model with or without pre-treatment with 50 mg/kg of cyclophosphamide one day prior to administration of either of Grasustek/Neulasta to compare the efficacy of both treatments in terms of absolute neutrophil counts (ANC) in normal and neutropenic rats after a single s.c. injection of 0 (vehicle control), 50, 150 or 450 µg/kg of Grasustek or Neulasta.

Absolute neutrophil counts (ANC) were assessed in all treatment groups from two subsets of 6 animals of both neutropenic and non-neutropenic at 0, 4, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours post dose. In the non-neutropenic group, the ANC gradually increased with the highest levels observed at 72 hours post-dose at the dose of 450 µg/kg body weights for both Neulasta and Grasustek. In the case of neutropenic animals, peak ANC levels were observed 144 hours post dose for the test product and 216 hours post-dose for Neulasta.

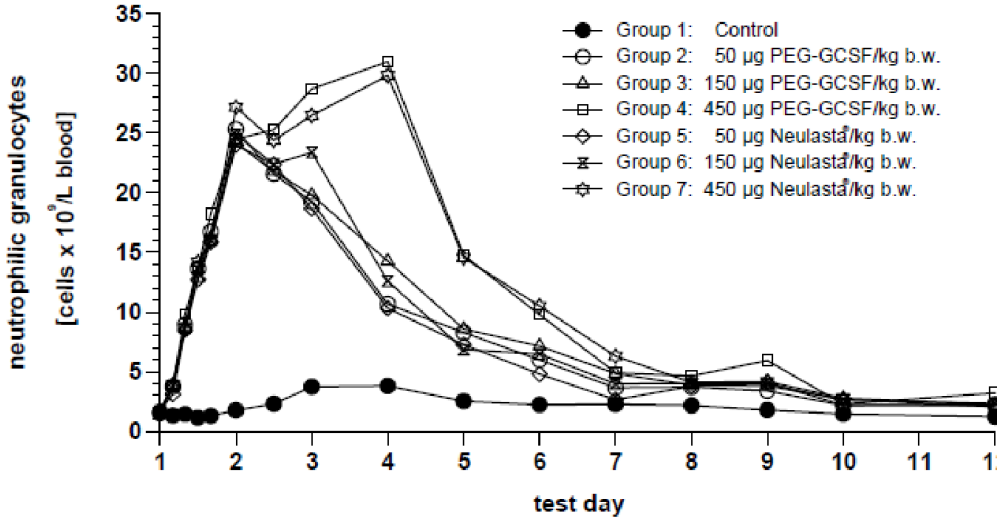


Figure 3. Absolute Neutrophil Counts in <u>non-neutropenic male rats</u> Following Single Dose Administration of Grasustek and Neulasta

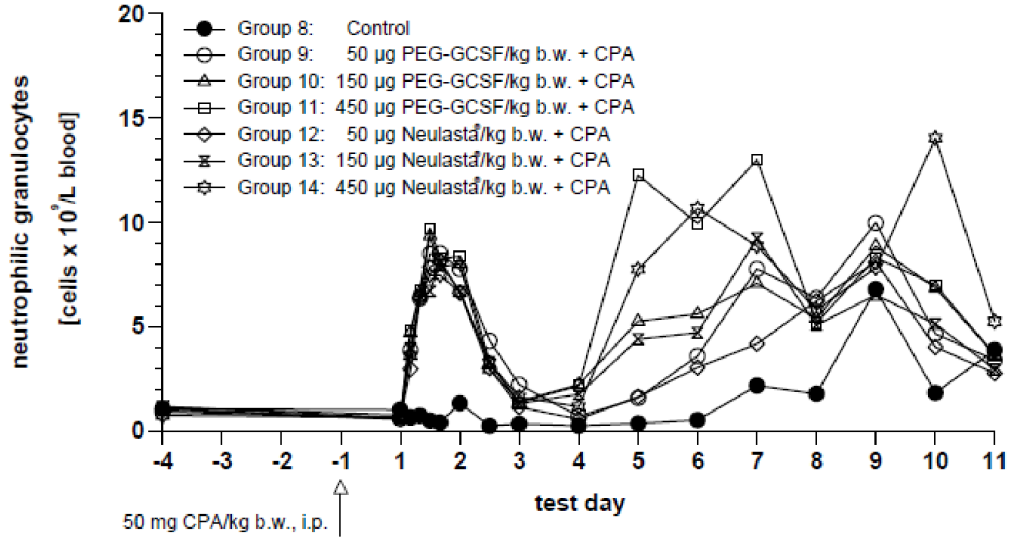


Figure 4. Absolute Neutrophil Counts in <u>neutropenic male rats</u> Following Single Dose Administration of Grasustek and Neulasta

The pharmacodynamics parameters (AUCeff and Emax) for the neutrophil response in non-neutropenic and neutropenic rats were obtained on one batch of the biosimilar and one batch of the reference product. The results were compared between Grasustek and Neulasta (Emax Ratio Test item / Reference) and are provided in Table 2 below.

Table 2 Pharmacodynamic parameters in non-neutropenic and neutropenic male rats

Dose µg/kg body weight	Area under ANC Time curve (Number of cells x 10^9/L blood-hour)			E_{max} # (Number of cells x 10^9/L blood)		
	PEG-GCSF injection (USV) (Test item)	Neulasta® (Reference)	AUC Ratio (Test item/ Reference)	PEG-GCSF injection (USV) (Test item)	Neulasta® (Reference)	E_{max} Ratio (Test item/ Reference)
Healthy male rats (non-neutropenic group)						
Control	545.89*	-	-	3.82*	-	-
50	2003.31	1908.54	1.05	25.32	23.99	1.06
150	2170.63	2143.07	1.01	24.16	24.86	0.97
450	3082.78	3007.63	1.02	30.98	29.81	1.04
Neutropenic male rats						
Control	407.96*	-	-	6.79*	-	-
50	1166.94	939.27	1.24	9.97	8.12	1.23
150	1304.91	1165.87	1.12	9.35	9.22	1.01
450	1713.81	1658.64	1.03	12.99	14.03	0.93

*Values for control group
Values obtained from analytical results, all other values calculated by pharmacodynamic analysis
E_{max} -highest measured neutrophilic granulocyte concentration

Secondary pharmacodynamic studies

The applicant did not submit secondary pharmacodynamic studies (see non-clinical discussion).

Safety pharmacology programme

The applicant did not submit safety pharmacology studies (see non-clinical discussion).

Pharmacodynamic drug interactions

The applicant did not submit pharmacodynamic drug interactions studies (see non-clinical discussion).

2.3.3. Pharmacokinetics

A comparative evaluation of the PK profiles of Grasustek and Neulasta was conducted in non-neutropenic and neutropenic rats administered 0 (vehicle control), 50, 150 or 450 µg/kg as described above. Blood sampling for pharmacokinetic evaluation was done for all treatment groups under isoflurane anaesthesia from two subsets of animals having 6 animals each at 0, 4, 8, 12, 16, 24, 36, 48, 72, 96,120, 144 and 168 hours post dosing (see figures 5 and 6).

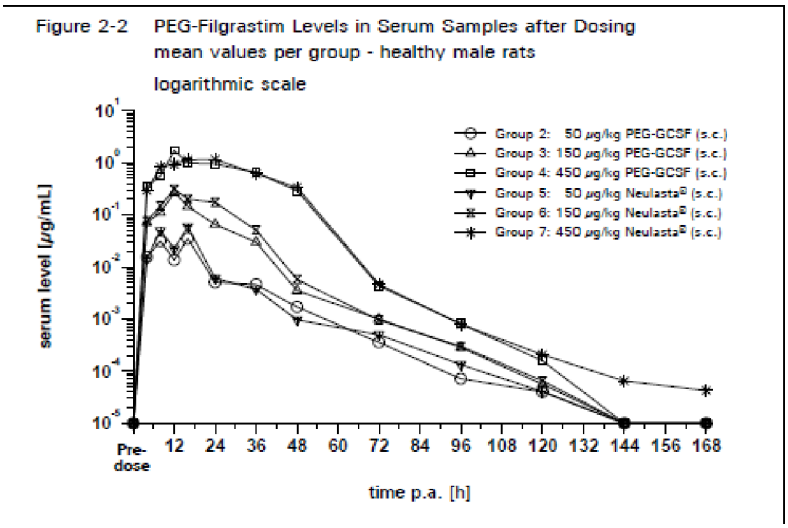


Figure 5 Pegfilgrastim levels after single dosing - mean values per group (N=6) - healthy male rats (logarithmic scale)

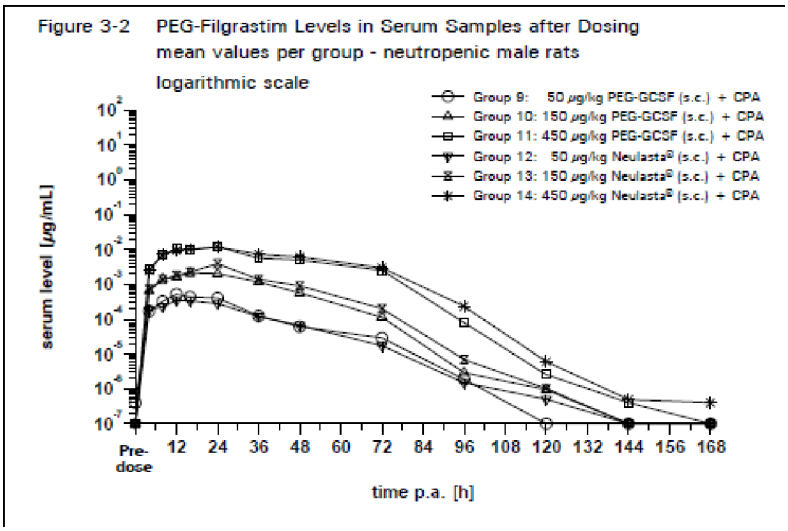


Figure 6 Pegfilgrastim levels after single dosing - mean values per group (N=6) - neutropenic male rats (logarithmic scale)

The rate and extent of exposure resulting from a single administration of Grasustek or Neulasta were compared during the PK/PD study conducted in healthy and neutropenic rats after a single s.c. injection of 0 (vehicle control), 50, 150 or 450 µg/kg of Grasustek or Neulasta. The results of the pharmacokinetic analysis after a single dose tend to show similar AUC and Cmax for Grasustek and Neulasta.

2.3.4. Toxicology

The applicant performed a GLP-compliant 4-week toxicity study (including a 4-week recovery period) comparing test or reference product administered once weekly on 5 occasions at 300 or 1000 µg/kg in CD strain rats of both sexes. Local tolerance was also assessed in NZW rabbits administered 6mg of Grasustek intra-arterial, intramuscular, intravenous, subcutaneous and 0.1 mL paravenous. There are no reproduction toxicology, mutagenicity and carcinogenicity studies reported.

Single dose toxicity

The applicant did not submit single dose toxicity studies (see non-clinical discussion).

Repeat dose toxicity

The applicant performed a GLP-compliant 4-week toxicity study (including a 4-week recovery period) comparing test or reference product administered once weekly on 5 occasions at 300 or 1000 µg/kg in CD strain rats of both sexes. In this study the animals received 5 weekly doses of 100, 300 or 1000 µg/kg body weight. That study included toxicokinetic investigations carried out on Day 1 and Day 29, including for the presence of ADA.

Table 3 Toxicity results

Parameters	**Results**
Mortality	None of the animals died prematurely
Clinical signs	No changes in behaviour or external appearance were observed
Local tolerance	No local reactions were observed
Body weight, food consumption	No test or reference item-related influence was noted.
Haematology	A marked increase in the absolute number of leucocytes predominantly caused by an increase in the number of neutrophils was noted at all dose levels except control. The effect was dose-dependent.
Clinical chemistry	Decreased plasma levels of cholesterol, glucose and triglycerides and as well as increased plasma levels of alkaline phosphatase were noted partly starting at 100 µg Grasustek or 300 µg Neulasta/kg. No differences were noted between the animals treated subcutaneously with 1000 µg Grasustek/kg compared to animals treated with 1000 µg Neulasta/kg
Ophthalmology	No test or reference item-related influence was noted.
Macroscopic pathology	An enlarged spleen was noted for 7 of 10 male and for 1 of 10 female animals treated with 100 µg Grasustek/kg, for 5 of 10 male and for 4 of 10 female rats treated with 300 µg Grasustek/kg and for 7 of 10 male and for all female rats treated with 1000 µg Grasustek/kg. The same finding was also noted for 5 of 10 male and for 5 of 10 female animals treated with 300 µg Neulasta/kg as well as for 4 of 10 male and for 9 of 10 female rats treated with 1000 µg Neulasta/kg. Furthermore, two of ten male animals treated with 1000 µg Grasustek/kg and 1 of 10 male animals treated with1000 µg Neulasta/kg revealed a red-stained discoloured thymus at terminal sacrifice. At 100, 300 or 1000 µg Grasustek or 300 or 1000 µg Neulasta/kg, the relative and absolute weights of the lungs and the spleen were increased. No differences were noted between the animals treated subcutaneously with 1000 µg Grasustek/ kg compared to animals treated with 1000 µg Neulasta/kg.
Histology	Test or reference item-related organ changes in form of increased granulocytopoiesis were noted in the bone marrow. Activation of haematopoiesis was noted in the spleen and in the liver. Further, granulocytosis was noted in the lungs. These changes are considered to be pharmacodynamic effects of the test or reference item. There was no difference in the effects between Grasustek and Neulasta. Test or reference item-related pathological changes in form of bone remodelling of the femur and tibia was observed. Both products showed similar effects on the bone structure.

Parameters	Results
Recovery period	The relative and absolute number of lymphocytes was still slightly increased by 14% (rel.) or by 42% (abs.) for the male animals previously treated with 1000 µg Grasustek/kg. The relative and absolute number of lymphocytes was still slightly increased by 8% (rel.) or by 15% (abs.) for the male animals previously treated with 1000 µg Neulasta/kg. Two of five male animals previously treated with 1000 µg Grasustek/kg and 1 of 5 male animals previously treated with1000 µg Neulasta/kg showed a red-stained discoloured thymus at recovery sacrifice.

The results presented showed no difference observed in the pharmacodynamics and toxicological effect of either the biosimilar or the reference.

Genotoxicity

The applicant did not submit genotoxicity studies (see non-clinical discussion).

Carcinogenicity

The applicant did not submit carcinogenicity studies (see non-clinical discussion).

Reproduction Toxicity

The applicant did not submit reproduction toxicity studies (see non-clinical discussion).

Toxicokinetic data

The 4-week repeat-dose toxicity study included toxicokinetic investigations carried out on Day 1 and Day 29. The exposures were higher in all the Neulasta groups compared to Grasustek. There was no sign of accumulation in either sex and the exposures appear lower after 5 weekly administrations in all the groups.

Table 4 Mean values of toxicokinetic parameters after one and repeated administrations

Dosage [µg/kg]	Sex	Non-compartment analysis of PEG-Filgrastim							
		C_{max}#[1] [µg/mL]	t_{max} [h]	$t_{1/2}$ [h]	K_{el} [1/h]	$AUC_{0\text{-}t\ last}$ [µg*h/mL]	$AUC_{0\text{-}inf}$ [µg*h/mL]	R	DPF
Test day 1									
100 µg PEG-GCSF/kg	m	0.21	12.00	6.70	0.10	4.48	4.48	1	-
	f	0.35	12.00	6.24	0.11	6.00	6.00	1	-
300 µg PEG-GCSF/kg	m	0.73	24.00	6.50	0.11	25.70	25.70	1	1.91
	f	0.87	24.00	6.58	0.11	27.93	27.93	1	1.55
1000 µg PEG-GCSF/kg	m	3.41	16.00	7.00	0.10	119.20	119.20	1	2.66
	f	4.14	24.00	6.94	0.10	158.76	158.76	1	2.65
300 µg Neulasta®/kg	m	0.82	12.00	6.57	0.11	29.10	29.10	1	-
	f	0.97	8.00	6.40	0.11	37.73	37.73	1	-
1000 µg Neulasta®/kg	m	3.17	16.00	8.02	0.09	129.56	129.56	1	1.34
	f	4.36	16.00	7.92	0.09	155.53	155.53	1	1.24
Test day 29									
100 µg PEG-GCSF/kg	m	0.07	16.00	10.63	0.07	1.18	1.18	0.26	-
	f	0.16	12.00	7.77	0.09	2.94	2.94	0.49	-
300 µg PEG-GCSF/kg	m	0.48	16.00	9.22	0.08	10.20	10.20	0.40	2.88
	f	1.10	12.00	8.16	0.08	15.97	15.97	0.57	1.81
1000 µg PEG-GCSF/kg	m	2.08	16.00	8.90	0.08	72.90	72.90	0.61	6.18
	f	3.19	16.00	8.37	0.08	108.45	108.45	0.68	3.69
300 µg Neulasta®/kg	m	0.50	12.00	9.21	0.08	12.97	12.97	0.45	-
	f	0.99	12.00	8.30	0.08	29.01	29.02	0.77	-
1000 µg Neulasta®/kg	m	3.03	24.00	8.83	0.08	113.38	113.38	0.88	2.62
	f	3.52	24.00	8.47	0.08	131.19	131.19	0.84	1.36

m: Male
f: Female
#[1]: Values obtained from serum analysis of PEG-Filgrastim, all other values calculated by pharmacokinetic analysis
R Accumulation factor ($AUC_{TD1\ 0\text{-}t\ last}$ or $AUC_{TD29\ 0\text{-}t\ last}/AUC_{TD1\ 0\text{-}t\ last}$)
DPF: Dose proportion factor
[$AUC_{0\text{-}t\ last}$ (intermediate or high dose)/$AUC_{0\text{-}t\ last}$ (low doe)]/
[(intermediate or high dose)/ (low dose)] for the same day

Local Tolerance

Local tolerance was also assessed in New Zealand White (NZW) rabbits administered 6mg of Grasustek intra-arterial, intramuscular, intravenous, subcutaneous and 0.1 mL paravenous and no particular findings were noted. Local adverse effects were not observed in rats after repeated administrations.

Other toxicity studies

Immunogenicity

The presence of ADAs was assessed by a validated semi-quantitative ELISA method as part of the four-week repeated toxicity study in animals given either the test product or Neulasta at all dose levels, at the end of the 4-week dosing period and at the end of 4-week recovery period. Higher incidence of ADAs was detected for Grasustek treated animals compared to those given Neulasta without any consistent pattern of ADA incidence across all doses / gender. There was no apparent impact on ANCs.

Studies on impurities

The levels of oxidized-I form found in Grasustek are twice as high as in the reference product Neulasta. This level is similar to that contained in the lot used in the clinical studies (Phase I- PEGF/USV/P1/001, Phase III- PEGF/USV/P3/003 and Phase I- PEGF/USV/P1/003). Those batches did not show any relevant immunogenicity.

2.3.5. Ecotoxicity/environmental risk assessment

Based on the CHMP Guideline on the environmental risk assessment of medicinal products for human use (CHMP/SWP/4447/00 corr. 2) which states that proteins are exempted from the need to submit studies because they are unlikely to result in a significant risk to the environment due to their nature, the applicant submitted a justification for not submitting an environmental risk assessment. Pegfilgrastim is already used in existing marketed products and no significant increase in environmental exposure is anticipated.

2.3.6. Discussion on non-clinical aspects

Comparability on the non-clinical level was adequately addressed according to the current regulatory requirements. Biosimilarity between Grasustek and the reference medicinal product Neulasta was demonstrated by similar potency to stimulate proliferation of G-NFS-60 cells (a murine myeloblastic cell line) and by similar receptor binding affinity to the G-CSF receptor. The applicant submitted the raw data (sensograms for the Biacore assay and concentration-response curves for the G-NFS-60 cell proliferation assay) and it was concluded that the binding affinity and potency values provided are reliable.

An in vivo PK/PD study was performed in normal and neutropenic rats with the aim to compare Grasustek versus Neulasta. This experiment has shown a comparable activity of either product in terms of absolute neutrophil counts. The rate and extent of exposure resulting from a single administration of Grasustek or Neulasta were compared during that same study. The results of the pharmacokinetic analysis after a single dose tend to show similar AUC and Cmax for Grasustek and Neulasta.

The applicant also conducted comparative toxicokinetic evaluations as part of a repeat-dose toxicity study in rats described below. The toxicokinetic investigations were conducted on Day 1 and Day 29. The exposures were higher in all the Neulasta groups compared to Grasustek. There was no sign of accumulation in either sex and the exposures appear lower after 5 weekly administrations in all the groups. Differences in time points of highest serum concentrations (tmax) between Grasustek and Neulasta can be attributed to normal variability observed between animals, small number of animals at each time point and non-sequential PK sampling, in line with the follow-up scientific advice EMA/CHMP/SAWP/343876/2014.

The Grasustek and Neulasta concentrations in the formulation vehicle and in the animal sera were determined by ELISA. The information provided on the validation of the commercial ELISA used to

dose the pegfilgrastim in the rat plasmas is somewhat limited to accuracy, precision and LLOQ. Further details would be needed, in order to be able to judge whether these data are adequate. The in vivo PK data are nonetheless considered as supportive only and it is considered that the in vitro assays described above are more informative together with the PK/PD data obtained in healthy volunteers and patients.

The applicant did not provide results of distribution, metabolisation, excretion or additional studies; those studies are not needed.

The applicant performed a GLP-compliant 4-week repeat-dose toxicity study (including a 4-week recovery period) comparing test or reference product administered once weekly on 5 occasions at 300 or 1000 µg/kg in CD strain rats of both sexes. The results presented showed no difference observed in the toxicological effect of either the biosimilar or the reference.

No particular findings were noted in a local tolerance study. The presence of ADAs was assessed as part of the four-week repeated toxicity study in animals. However, immunogenicity assessment in animals is generally not predictive for immunogenicity in humans and is thus not requested for biosimilars. The studies performed in animals are in this particular case not needed and the resulting information is somewhat limited.

The levels of oxidized-I form found in Grasustek are twice as high as in the reference product Neulasta (0.23 % to 0.50 % in Grasustek lots versus 0.13% to 0.21 % in EU-authorized Neulasta lots). This level is similar to that contained in the lot used in the clinical studies (Phase I- PEGF/USV/P1/001, Phase III- PEGF/USV/P3/003 and Phase I- PEGF/USV/P1/003), where the Grasustek batches had a level of Oxidized-1 form ranging from 0.35-0.41% (against EU-authorized Neulasta range of 0.1-0.2%). Those batches did not show any relevant immunogenicity.

No data from secondary PD, safety pharmacology, pharmacodynamic drug interaction studies, single dose studies, genotoxicity, carcinogenicity, reproduction, fertility and developmental toxicity studies was submitted. These studies are not required for a biosimilar development program in accordance with EMEA/CHMP/BMWP/42832/2005 Rev. 1 and EMEA/CHMP/BMWP/31329/2005.

The justification for not submitting environmental risk assessment studies is acceptable. It is unlikely that residues of pegfilgrastim would persist in the environment or cause inadvertent environmental effects. The approval of Grasustek is not expected to cause increases in environmental exposure above existing levels for this active substance or result in any additional hazards to the environment during storage, distribution, use and disposal. Considering the expected exposure and the nature of the product, the absence of formal environmental risk assessment studies for Grasustek is considered justified. This is in accordance with the CHMP Guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 corr 2).

2.3.7. Conclusion on the non-clinical aspects

The non-clinical aspects of pharmacology, pharmacokinetic and toxicology for Grasustek have been well characterised and are considered acceptable. There were no further changes to the SmPC and the product information is aligned with the reference product Neulasta.

2.4. Clinical aspects

2.4.1. Introduction

GCP

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

Table 5 Tabular overview of clinical studies

Study type	Study identifier	Study objectives	Study design	Test products	Number and type of subjects	Duration of treatment
PK/PD	PEGF/USV/P1/001	Primary: PD & PK comparison of USV Pegfilgrastim and Neulasta	Randomised, double-blind, 2-treatment, 2-period, 2-sequence cross-over study	SC injection of pegfilgrastim 6 mg	156 healthy male & female	single dose
		Secondary: safety and local tolerance				
PK/PD	PEGF/USV/P1/003	Primary: PD comparison of USV Pegfilgrastim and Neulasta	Randomised, double-blind, 2-treatment, 2-period, 2-sequence cross-over study	SC injection of pegfilgrastim 2 mg	54 healthy male	single dose
		Secondary: PK comparison, safety and local tolerance				
Efficacy/safety	PEGF/USV/P3/003	Primary: efficacy comparison of USV Pegfilgrastim and Neulasta with respect to DSN during cycle 1 of chemotherapy	Randomised, multicentre, double-blind, parallel group	SC injection of pegfilgrastim 6 mg	254 (172 USV-PEGF/82 Neulasta) female patients with breast cancer undergoing myelosuppressive chemotherapy	one dose per cycle for 6 cycles (18 weeks)
		Secondary: efficacy, safety, immunogenicity comparison				

2.4.2. Pharmacokinetics

Absorption

The PK comparability of Grasustek and Neulasta was evaluated in a pivotal PK/PD trial (PEGF/USV/P1/001) and a supportive PK/PD trial (PEGF/USV/P1/003).

Analytical methods

Pegfilgrastim assay

A solid phase sandwich ELISA assay was developed to measure pegfilgrastim in serum and in general validated appropriately including evidence of similar precision and sensitivity for Neulasta and Grasustek from analysis of samples in the same analytical runs using Neulasta standard curves. Incurred sample reanalysis was used to indicate no significant matrix interference with haemolysed and lipaemic samples. Anti-pegfilgrastim antibodies, but not anti-PEG antibodies, may interfere in this assay.

The assay measures pegfilgrastim as well as endogenous G-CSF.

Anti-pegfilgrastim antibody (ADA) assay

Methods have been developed to measure binding ADAs. A neutralising bioassay has been developed using NFS-60 cells, which proliferate in the presence of G-CSF. The assay has been validated.

Study PEGF/USV/P1/001

Design

This was a randomised, double-blind comparative study in healthy male and female subjects with a 2-treatment, 2-period, 2-way crossover design. The primary objective of this pivotal PK/PD trial was to compare the PK and PD of Grasustek and EU-sourced Neulasta following a single SC dose of 6 mg.

Methods

Pharmacokinetic parameters were estimated with non-compartmental analysis and compared using an analysis of variance (ANOVA) including terms for treatment, period, sequence and subject within sequence.

Results

A total of 156 subjects were randomised and administered at least one treatment dose (PK population); 142 subjects (91%) completed the study, 71 in each treatment sequence, and constitute the PK dataset. A total of 14 subjects withdrew from the study for various reasons (consent withdrawal, clinically significant AE, poor venous access, failure to attend period 2 on time, concurrent illness).

The pegfilgrastim concentration curves over time and PK parameter estimates for the test and reference products are shown in Figure 7 and Table 6, respectively. The comparison of the key PK parameters is presented in Table 7. The 90% CI of the geometric mean ratio for the three key parameters (AUC_{last}, C_{max}, AUC_{0-inf}) is contained within the standard bioequivalence range of 80.00-125.00%.

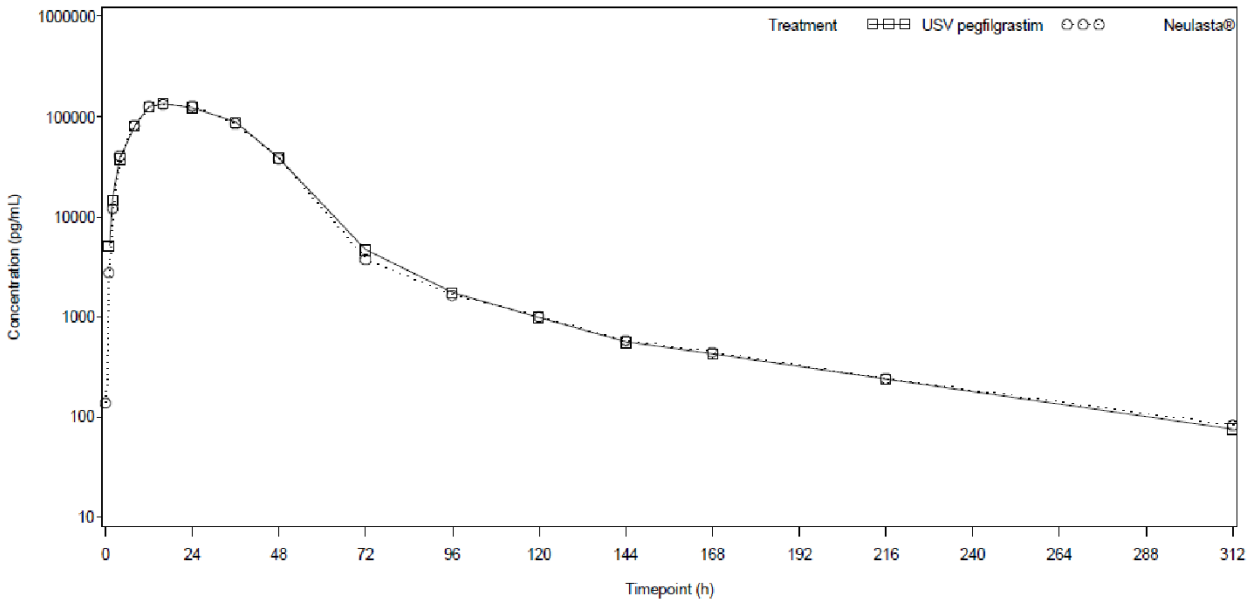


Figure 7: Geometric mean serum pegfilgrastim concentration over time (Log10/Linear) – PK population

Table 6: Geometric mean estimates (CV %) of PK parameters (PK dataset)

Parameter (Unit)	Test (Treatment A) USV pegfilgrastim 6 mg SC n = 142	Reference (Treatment B) Neulasta® 6 mg SC n = 142
Tlag[a] (h)	0.000 (0.00–1.00)	0.000 (0.00–0.00)
Tmax[a] (h)	16.000 (8.00–36.03)	16.000 (4.00–36.17)
Cmax (pg/mL)	107000 (114%)	109000 (112%)
AUC(last) (pg·h/mL)	3540000 (109%)	3480000 (115%)
AUC(0–inf) (pg·h/mL)	3570000 (108%)	3510000 (114%)
%AUC	0.485 (126.0%)	0.459 (148.5%)
T½el (h)	41.784 (29.0%) [n = 68]	45.341 (31.5%) [n = 71]

SC; subcutaneous
[a] median (range)

Table 7: Assessment of comparability for PK parameters (PK dataset)

Parameter (Unit)	Adjusted Geometric Mean[a]		Ratio[b]	90% Confidence Interval[c]
	USV Pegfilgrastim n = 142	Neulasta® n = 142		
AUC(last) (pg·h/mL)	3540000	3480000	101.70	(92.86, 111.38)
AUC(0-inf) (pg·h/mL)	3570000	3510000	101.70	(92.95, 111.27)
Cmax (pg/mL)	107000	109000	98.32	(89.38, 108.15)

[a] Adjusted geometric mean from analysis of variance model
[b] Ratio of adjusted geometric means defined as USV pegfilgrastim/Neulasta®
[c] Confidence Interval for the ratio of adjusted geometric means

Following CHMP request, the PK results have been presented by period (Figure 8 and Table 8). There is a significant period effect with geometric mean values being higher in Period 1 when compared to Period 2.

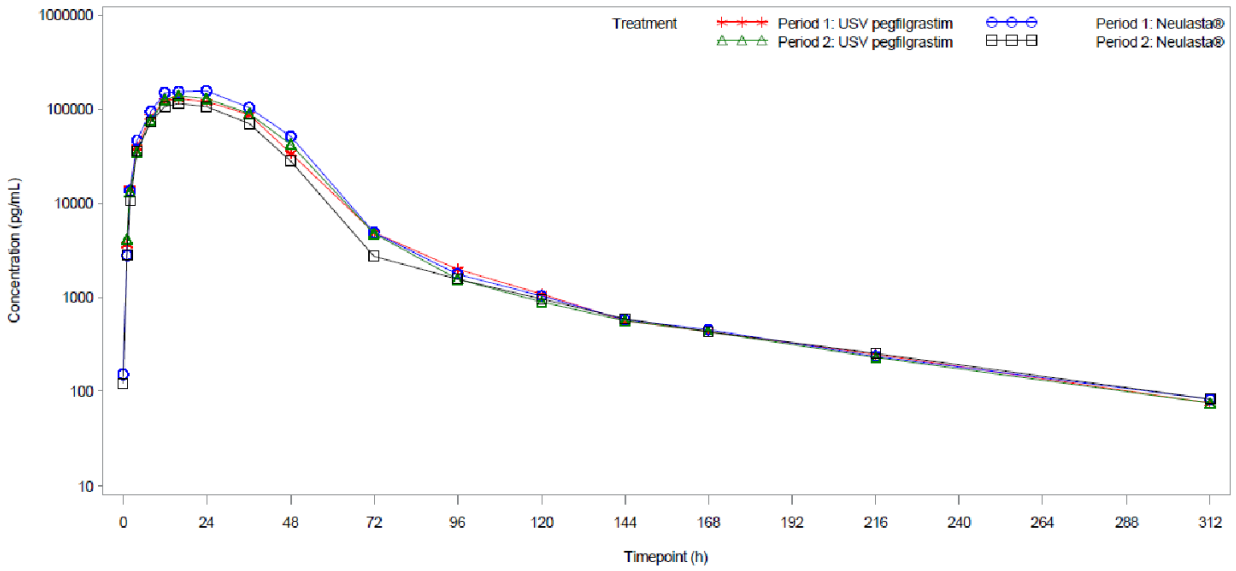


Figure 8: Mean Serum Concentrations of pegfilgrastim (pg/mL) - Log10/Linear Scale - PK Dataset

Table 8: Geometric means of PK parameters by period and treatment - PK data set

PK Dataset	AUC(last) (pg.h/mL)		AUC(0-inf) (pg.h/mL)		Cmax (pg/mL)	
Treatment	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
USV pegfilgrastim	3550000	3540000	3580000	3560000	107000	107000
Neulasta	4190000	2900000	4220000	2930000	127000	93400

Study PEGF/USV/P1/003

Design

This was a randomised, double-blind comparative study in healthy male subjects with a 2-treatment, 2-period, 2-way crossover design. The primary objective of this supportive PK/PD trial was to compare the PD of Grasustek and EU-sourced Neulasta following a single SC dose of 2 mg. PK comparison was a secondary objective and was only descriptive.

Methods

The study was conducted according to the protocol and in the same centre as the previous trial.

Results

A total of 64 subjects were randomised and administered at least one treatment dose (PK population); 60 subjects (94%) completed the study, 30 in each treatment sequence, and constitute the PK dataset. A total of 4 subjects withdrew from the study for various reasons (AE, consent withdrawal, alcohol consumption).

The pegfilgrastim concentration curves over time and PK parameter estimates for the test and reference products are shown in Figure 9 and Table 9, respectively.

Following CHMP request, formal equivalence testing has been performed, which fails to show PK equivalence. Although a period effect is also apparent for both products, it is only significant for one of the three PK parameters (AUC(0-last)).

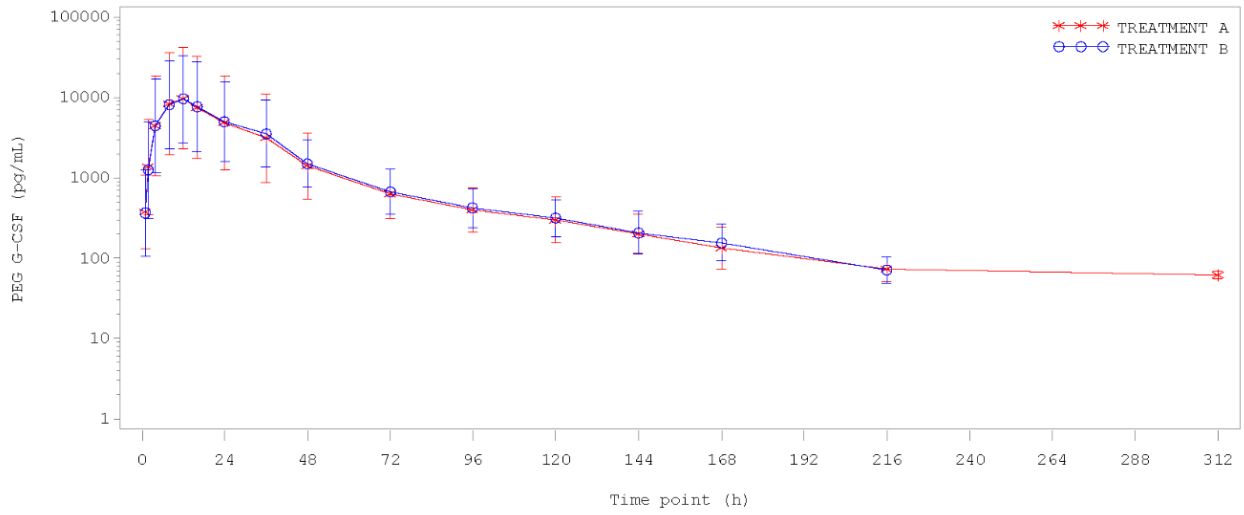


Figure 9: Geometric mean (± SD) serum pegfilgrastim concentration over time (Log10/Linear) – PK population (Treatment A = Grasustek; Treatment B = Neulasta)

Table 9: Geometric mean estimates (CV %) of PK parameters (PK dataset)

Treatment No. of Subjects Parameter	2 mg USV Pegfilgrastim (Treatment A) Subcutaneous Injection N=60	2 mg Neulasta® (Treatment B) Subcutaneous Injection N=60
Tlag (h) [a]	0.000 (0.00 – 4.00)	0.000 (0.00 – 2.02)
Tmax (h)[a]	12.000 (4.03 – 36.10)	12.000 (4.00 – 48.02)
Cmax (pg/mL)	10600 (231.2%)	10700 (164.1%)
AUC(0-last) (pg.h/mL)	299000 (281.0%)	333000 (121.8%)
AUC(0-inf) (pg.h/mL)	410000 (90.9%) (n=49)	417000 (99.1%) (n=40)
AUC%extrap (%)	2.506 (101.8%) (n=49)	2.451 (124.8%) (n=40)
T1/2 (h)	43.021 (36.0%) (n=49)	41.214 (25.7%) (n=40)
Frel AUC(0-inf) (%) [b]	107.068 (97.4%) (n=32)	NC
Frel AUC(0-last) (%) [b]	89.843 (245.0%)	NC
Frel Cmax (%) [b]	99.306 (181.2%)	NC

[a] Median (range); [b] i.e. within subject ratio of AUC(0-inf), AUC(0-last) and Cmax, NC = Not calculated

Distribution

The applicant did not submit distribution studies (see pharmacology discussion).

Elimination

The applicant did not submit elimination studies (see pharmacology discussion).

Dose proportionality and time dependencies

The applicant did not submit dose proportionality and time dependency studies (see pharmacology discussion).

Special populations

The applicant did not submit special population studies (see pharmacology discussion).

Pharmacokinetic interaction studies

The applicant did not submit pharmacokinetic interaction studies (see pharmacology discussion).

Pharmacokinetics using human biomaterials

The applicant did not submit pharmacokinetic studies using human biomaterials (see pharmacology discussion).

2.4.3. Pharmacodynamics

Mechanism of action

The applicant did not submit mechanism of action studies (see pharmacology discussion).

Primary and Secondary pharmacology

The PD comparability of Grasustek and Neulasta was evaluated in the two trials previously described; it is based on two blood markers: the ANC and CD34+ cell count.

Analytical methods

The two PD markers were measured by flow cytometry in the same central laboratory for the two trials.

Study PEGF/USV/P1/001

PD parameters were estimated with non-compartmental analysis: AUEC (the area under the effect curve of the ANC and CD34+ response), Emax (the maximum effect observed of the ANC and CD34+ response), and Tmax (the time to reach the maximum observed effect of the ANC and CD34+ response). AUEC and Emax were compared using an analysis of covariance (ANCOVA) including terms for treatment, period, sequence and subject within sequence. The baseline concentration value (pre-dose value) was used as the covariate. A 95% CI for the ratio of the adjusted geometric means for Grasustek/Neulasta was calculated. In order to demonstrate comparability, the 95% CI for AUEC (ANC), the primary PD parameter, was to be contained within the acceptance limit of 90.00% to 111.11%. The PD dataset was the same as the PK dataset.

The ANC and CD34+ cell count curves over time and PD parameter estimates for the test and reference products are shown in Figures 10 and 11 and Tables 10 and 11, respectively. The comparison of the PD parameters is presented in Table 12. The 95% CI of the geometric mean ratio for the key parameters (AUEC and Emax) for both cell counts is contained within the pre-defined equivalence range of 90.00-111.11%.

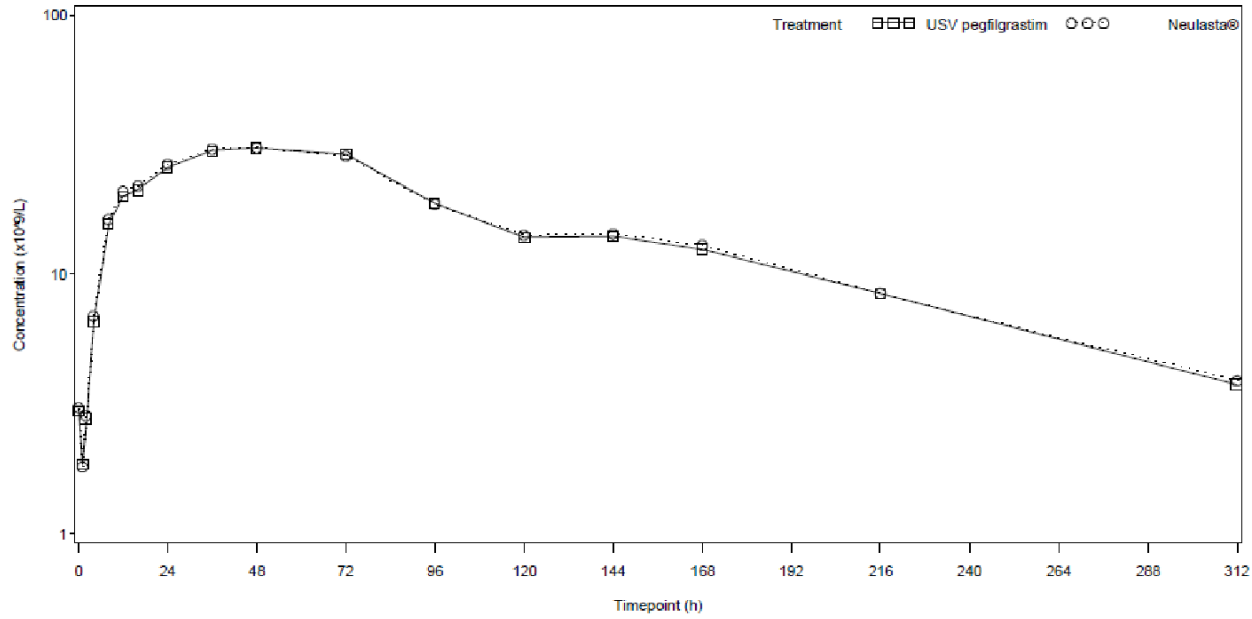


Figure 10: Geometric mean blood ANC values over time (Log10/Linear) – PD population

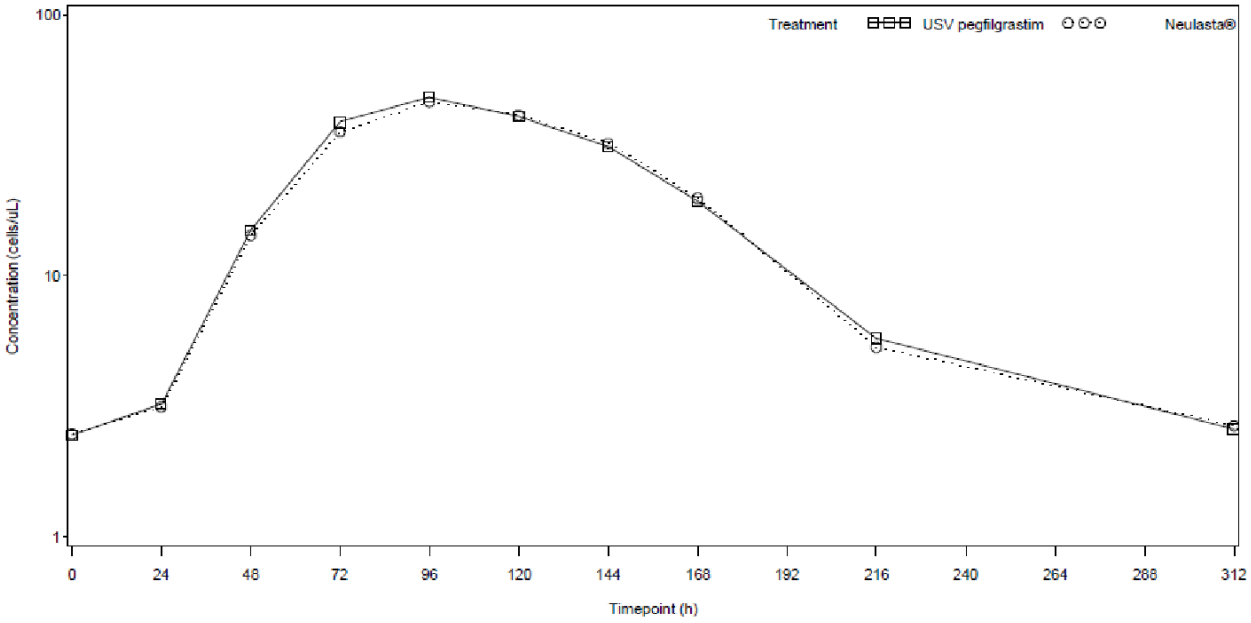


Figure 11: Geometric mean blood CD34+ cell count values over time (Log10/Linear) – PD population

Table 10: Geometric mean estimates (CV %) of ANC parameters (PD dataset)

Parameter (Unit)	Test (Treatment A) USV pegfilgrastim 6 mg SC n = 142	Reference (Treatment B) Neulasta® 6 mg SC n = 142
Tmax[a] (h)	48.0 (16–96)	48.0 (24–72)
Emax (× 10^9/L)	31.83 (24.1%)	31.74 (25.3%)
AUEC (× 10^9/L·h)	4420 (23%)	4440 (23.7%)

Table 11: Geometric mean estimates (CV %) of CD34+ parameters (PD dataset)

Parameter (Unit)	Test (Treatment A) USV pegfilgrastim 6 mg SC n = 142	Reference (Treatment B) Neulasta® 6 mg SC n = 142
Tmax[a] (h)	96.0 (72–144)	96.0 (72–144)
Emax (cells/µL)	43.4 (71.5%)	42.6 (64.6%)
AUEC (cells/µL·h)	4590 (68.6%)	4510 (65.8%)

SC; subcutaneous
[a] median (range)

Table 12: Assessment of comparability for PD parameters (PD dataset)

Parameter (Unit)	Analyte	Adjusted Geometric Mean[a]		Ratio[b]	95% Confidence Interval[c]
		USV pegfilgrastim n = 142	Neulasta® n = 142		
AUEC (× 10^9/L/h)	ANC	4420	4440	99.66	(97.46, 101.91)
Emax (× 10^9/L/h)	ANC	31.84	31.73	100.35	(97.47, 103.31)
AUEC (cells/µL·h)	$CD34^+$	4590	4510	101.60	(96.68, 106.76)
Emax (cells/µL)	$CD34^+$	43	43	102.00	(95.43, 109.02)

ANC; absolute neutrophil count
[a] Adjusted geometric mean from analysis of covariance model
[b] Ratio of adjusted geometric means defined as USV pegfilgrastim/Neulasta®
[c] Confidence Interval for the ratio of adjusted geometric means

Following CHMP request, the PD results have been presented by period (Figure 12 and Table 13). There is a significant period effect with geometric mean values being higher in Period 2 when compared to Period 1.

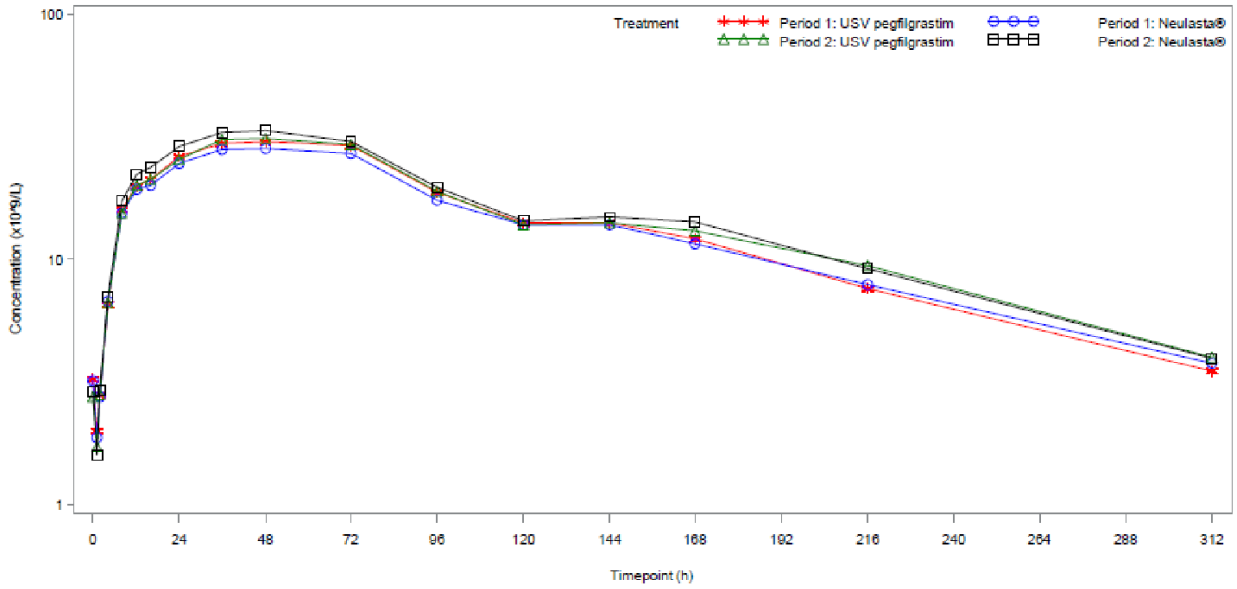


Figure 12: Geometric blood ANC values over time (Log10/Linear) – PD analysis data set

Table 13: Geometric means of ANC PD parameters by period and treatment - PD data set

ANC	AUEC (x10^9/L.h))		Emax (x10^9/L)	
Treatment	Period 1	Period 2	Period 1	Period 2
USV pegfilgrastim	4350	4490	31.46	32.19
Neulasta	4140	4760	29.33	34.34

Immunogenicity

The 156 pre-dose samples from all study subjects were tested. 2 were screened positive with the anti-Grasustek (1.3%) and 13 with the anti-Neulasta assay (8.3%), one sample being screened positive with both assays. One follow-up sample (Neulasta/Grasustek) was confirmed weakly positive for anti-Grasustek antibodies and screened positive for anti-Neulasta antibodies; it is possible that serum from this subject exhibited high non-specific binding.

Study PEGF/USV/P1/003

The same analysis was performed as in the previous trial, except that the 95% CI for AUEC and Emax for ANC (the primary PD parameters) was to be contained within wider acceptance limits (80.00 - 125.00%). The PD dataset was the same as the PK dataset.

The ANC and CD34+ cell count curves over time and PD parameter estimates for the test and reference products are shown in Figures 10 and 11 and Tables 14 and 15, respectively. The comparison of the PD parameters is presented in Tables 16 and 17. The 95% CI of the geometric mean ratio for the key parameters (AUEC and Emax) for both cell counts is contained within the pre-defined equivalence range of 80.00-125.00%.

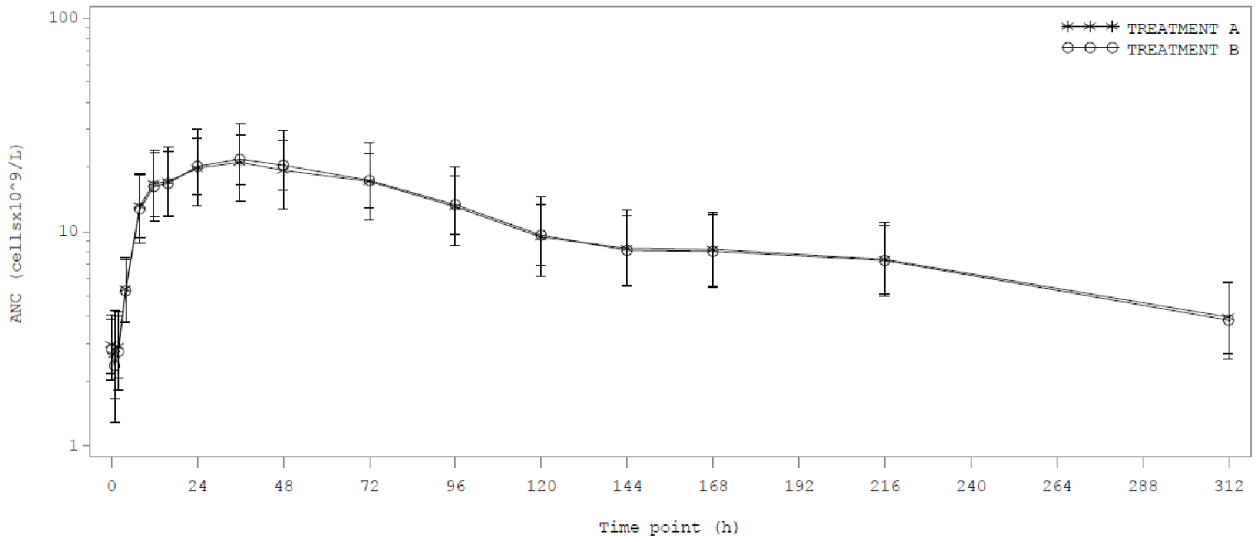


Figure 13: Geometric mean (±SD) blood ANC values over time (Log10/Linear) – PD analysis data set (Treatment A=Grasustek; Treatment B=Neulasta)

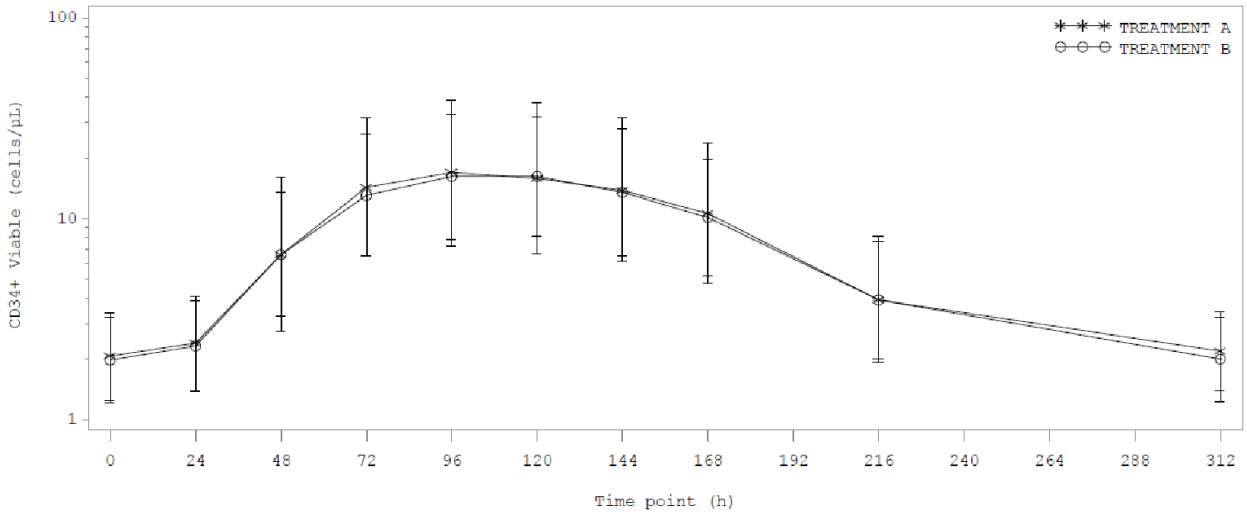


Figure 14: Geometric mean (±SD) blood CD34+ cell count values over time (Log10/Linear) – PD analysis data set

Table 14: Geometric mean estimates (CV %) of ANC parameters (PD dataset)

Treatment	2 mg USV Pegfilgrastim (Treatment A) Subcutaneous Injection	2 mg Neulasta® (Treatment B) Subcutaneous Injection
No. of Subjects	N=60	N=60
Parameter		
Tmax (h) [a]	36.000 (8.00-168.00)	36.000 (16.00-72.00)
Emax (cells×10^9/L)	21.9 (39.6%)	22.5 (27.1%)
AUEC (cells×10^9/L.h)	3320 (33.0%)	3330 (24.0%)
Frel Emax (%)[b]	97.440 (34.2%)	NA
Frel AUEC (%)[b]	99.837 (22.6%)	NA

Table 15: Geometric mean estimates (CV %) of CD34+ parameters (PD dataset)

Treatment	2 mg USV Pegfilgrastim (Treatment A) Subcutaneous Injection	2 mg Neulasta® (Treatment B) Subcutaneous Injection
No. of Subjects	N=60	N=60
Parameter		
Tmax (h) [a]	96.00 (48.00-216.00)	96.00 (48.00-144.00)
Emax (cells/µL)	19.3 (88.1%)	18.6 (79.8%)
AUEC (cells/µL.h)	2640 (75.8%)	2510 (66.9%)
Frel Emax (%)[b]	103.631 (51.2%)	NA
Frel AUEC (%)[b]	104.975 (34.6%)	NA

[a] Median (range); [b] i.e. within subject ratio of Emax or AUEC; NA = Not applicable

Table 16: Assessment of comparability of ANC parameters (PD dataset)

Parameter	Adjusted Geometric Mean		Ratio[a]	95% CI[b]	p-value[c]	CVw (%)[d]
	2 mg USV Pegfilgrastim (Treatment A) SC Injection (N = 60)	2 mg Neulasta® (Treatment B) SC Injection (N = 60)				
Emax (cells×10^9/L)	21.8	22.5	97.01	(88.90, 105.85)	<0.001	23.89
AUEC (cells×10^9/L.h)	3320	3330	99.72	(93.96, 105.83)	<0.001	16.15

Table 17: Assessment of comparability of CD34+ count (PD dataset)

Parameter	Adjusted Geometric Mean 2 mg USV Pegfilgrastim (Treatment A) SC Injection (N = 60)	2 mg Neulasta® (Treatment B) SC Injection (N = 60)	Ratio[a]	95% CI[b]	CVw (%)[c]
Emax (cells/µL)	19.2	18.6	103.06	(90.72, 117.08)	35.64
AUEC (cells/µL.h)	2630	2520	104.21	(95.48, 113.74)	24.07

Results obtained from ANCOVA model of natural log transformed PD parameters including terms for treatment, sequence, period and subject within sequence fitted as fixed effects and baseline fitted as a covariate.
[a] Ratio of adjusted geometric means for USV Pegfilgrastim/Neulasta®; [b] CI = confidence interval for ratio of adjusted geometric means; [c] CVw = Intra-subject variability.

No period effect was shown in this trial.

Immunogenicity

In Study PEGF/USV/P1/001, out of 454 samples, none was confirmed ADA positive.

In Study PEGF/USV/P1/003, out of 188 samples, five samples (2.7%) from 3 subjects were confirmed positive; these subjects were ADA negative pre-dosing and first received Grasustek followed by Neulasta. Two subjects were confirmed positive after both Grasustek and Neulasta, while the last was negative after Neulasta. One sample had a titre of 16, whereas the other confirmed ADA positive samples had titres ≤ 2. All five samples were negative in the neutralising assay.

In all three subjects, the ANC and CD34+ cell count profiles are similar after Grasustek and Neulasta. However, the PK profile in these three subjects shows lower pegfilgrastim concentration after Neulasta administered in Period 2 than after Grasustek administered in Period 1.

Following CHMP request, an analysis of the subjects with screened positive samples was conducted.

Table 18 Subjects with ADA+ samples (N %)

When ADA positive?	Study 001 N=156			Study 003 N=64	
+ BL	3	1.9%		5	7.8%
+ BL & any period	3	1.9%		3	4.7%
+ BL total	6	3.8%		8	12.5%
+ after Grasustek*	4	2.6%		5	7.8%
+ after Neulasta*	8	5.1%		2	3.1%

* with negative sample before administration – BL= baseline

Individual PK and PD curves in these subjects have been examined and no consistent pattern has been identified.

2.4.4. Discussion on clinical pharmacology

Filgrastim (recombinant human G-CSF) and pegfilgrastim have the same mechanism of action, pegfilgrastim being a sustained duration form of filgrastim due to decreased renal clearance. They bind to specific cell surface receptors, thereby stimulating cell proliferation and differentiation. Their PD effects are characterised by the elevation of ANC by release of mature neutrophils from the marrow into the peripheral blood and by stimulation of proliferation and differentiation of neutrophil precursors in the bone marrow, and mobilisation of progenitor (CD34+) cells to the peripheral blood.

Pegfilgrastim is used to reduce the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with myelosuppressive chemotherapy for malignancy. Therefore, ANC is a valid surrogate marker of efficacy while CD34+ cell count may further support the evaluation of the PD profile.

The pharmacokinetics of pegfilgrastim are complex with both dose and time-dependency, due to target-mediated drug disposition; neutrophil-mediated clearance is the predominant elimination pathway for pegfilgrastim.

In healthy subjects, after subcutaneous administration in a dose range of 30 to 300 µg/kg, pegfilgrastim exhibits nonlinear pharmacokinetics and exposure to pegfilgrastim increases in more than a dose-proportional manner, suggesting that the clearance of pegfilgrastim decreases with increased dosing. A 10-fold increase in the dose results in an approximately 25-fold increase in the maximum observed concentration (Cmax) and an approximately 75-fold increase in the AUC. In contrast, the magnitude and duration of ANC elevation is also dose dependent, but less than dose-proportionally (Molineux, 1999; Roskos, 2006; Yang, 2011).

The PK and PD profiles of Grasustek and Neulasta have been compared in two trials performed in healthy volunteers and using the same design (single dose crossover with a wash-out period of approximately 4 weeks) at two different doses: the therapeutic dose of 6 mg (equivalent to 100 µg/kg in a 60-kg individual) and a sub-therapeutic dose of 2 mg. The 6 mg dose is considered to be sufficiently sensitive for both PK and PD comparison as it lies in the steep part of the dose-concentration and dose-response curve in healthy volunteers (HVs). The additional dose of 2 mg was recommended by the CHMP as it might be more sensitive if G-CSF receptors are not all saturated at this level.

Pharmacokinetics

The pegfilgrastim analytical assay has been appropriately validated; it shows potential interference of anti-pegfilgrastim antibodies, but not anti-PEG antibodies.

The pivotal study PEGF/USV/P1/001 demonstrates PK comparability of the 6 mg dose in 142 evaluable subjects based on the three key PK parameters of AUC_{0-t}, C_{max}, and $AUC_{0-\infty}$. The 90% CI of the geometric mean ratio for these three parameters is contained within the standard bioequivalence range of 80.00-125.00%. For the terminal half-life, its estimate was not considered reliable in approximately half the subjects based on very strict criteria; however, comparable half-lives are shown in the subset of selected subjects as well as in the whole PK population.

The second study PEGF/USV/P1/003, which was not powered to establish PK equivalence, provides supporting (descriptive) evidence of comparability at the dose of 2 mg although formal equivalence testing fails to show PK equivalence.

There is a concern that the wash-out period between the two administrations may not be sufficient as a PD carry-over effect may occur due to expansion of neutrophil and neutrophil precursor mass in the second period and that results could be compounded by the PD effect on PK (increased drug clearance). Presentation of the data by period shows a significant period effect at the higher dose with drug exposure lower in the first period compared to the second period. Mean pegfilgrastim concentrations appear lower after Grasustek than Neulasta in Period 1 while the opposite is observed in Period 2. The magnitude of the decrease within each sequence is comparable, suggesting that the period effect is similar for both products. Additional analyses also suggest that differences within each period are chance findings and likely to be due to differences between subjects of the two sequences. In addition, further evaluation of the discrepancy between PK and PD results in some individuals (i.e. apparent different PK exposure between the two products with similar PD response) was requested but no specific reason for this observation could be identified.

Since PK constitute a secondary objective of study PEGF/USV/P1/003, no formal statistical analysis of the PEG G-CSF (PK parameters (e.g. Cmax, AUC(0-last) and AUC(0-inf))) was required and descriptive summaries were provided as per the EMA SA (EMA/CHMP/SAWP/561213/2012), Corrigendum EMA/CHMP/SAWP/517803/2016. In line with the scientific advice (EMA/CHMP/SAWP/561213/2012) received from the CHMP to enrol only male subjects, to possibly decrease the variability of responses, especially since the dose is not adjusted to body weight, healthy male subjects 18 to 55 years of age were enrolled in this study.

Pharmacodynamics

The validation of flow cytometry assays and external quality assurance data are acceptable.

The scientific advice (EMEA/CHMP/SAWP/37301/2009) received from the CHMP recommended to establish PD equivalence at more than 1 dose level; at the therapeutic dose of 6 mg of Grasustek and EU-approved Neulasta® and a second dose, preferably lower than 6 mg and lying on the steep part of the dose-response curve, was observed by conducting two PD trials with such doses. The primary endpoints were chosen as per EMA advice (EMA/CHMP/SAWP/561213/2012).

In both trials, formal testing of all four PD parameters (AUEC and Emax for ANC as well as CD34+ cell count) shows that the 95% CI of the geometric mean ratio lies within the pre-defined equivalence interval of 90.00-111.11% in the pivotal trial and 80.00-125.00% in the supportive trial. Given the concern about the wash-out period, presentation of the data by treatment and period was requested by CHMP. A significant period effect is observed at the higher dose in the pivotal trial but not at the low dose, as could be anticipated. The differences between Grasustek and Neulasta are consistent with those observed in the PK results and further investigation of this carry-over effect was requested by the CHMP. Extensive analyses performed by the applicant suggest that the magnitude of this effect is comparable between the two products and that the observed differences are likely to be due to a different distribution of the subjects' responsiveness between the two sequences.

Immunogenicity

Anti-pegfilgrastim immune response can potentially target either moiety (PEG or G-CSF) or the linker. Antibodies against G-CSF appear to develop infrequently and have not been associated with relevant consequences for efficacy or safety. In contrast, anti-PEG antibodies have been found in healthy subjects at variable rates and may potentially impact on the PK and PD profile of pegfilgrastim; in addition, they may potentially be induced by pegfilgrastim administration. Safety issues related to anti-PEG antibodies have not been described.

The analytical method used in the pivotal PK/PD trial has poor sensitivity and is highly variable, which precludes any conclusion on the results generated.

The binding assay used in the supportive PK/PD trial is able to detect anti-G-CSF and anti-pegfilgrastim but not anti-PEG antibodies, which is considered acceptable as this might not constitute a clinical concern.

Re-testing of the pivotal trial samples following CHMP request did not detect any confirmed anti-pegfilgrastim ADA in any subject. The results from the supportive PK/PD trial show the development of binding ADAs after administration of Grasustek in 3/32 subjects (9%) versus none after administration of Neulasta. Despite their low titre and no apparent impact on PD response, these are associated with very low pegfilgrastim concentration in the second period (Neulasta), which is consistent with interference shown in the additional validation of the PK assay.

Importantly, there appears to be no difference between the rates of positive screened samples after Grasustek and Neulasta as well as baseline (off-drug) rates, which suggests that all these rates are within the range of the assay variability.

Overall, this very low level of ADA incidence does not raise concern when interpreted in the light of the totality of evidence provided by the comparability exercise and does not preclude a conclusion of comparable immunogenicity profile of the test and reference products.

2.4.5. Conclusions on clinical pharmacology

The PK, PD and immunogenicity profiles of Grasustek and Neulasta are considered comparable.

2.5. Clinical efficacy

2.5.1. Main study(ies)

PEGF/USV/P3/003: A randomized, multicentre, double-blind, active controlled, parallel group, equivalence phase III study comparing the safety and efficacy of Grasustek and Neulasta EU in breast cancer patients undergoing myelosuppressive chemotherapy

Methods

Study Participants

The trial enrolled women ≥ 18 years of age, with a bodyweight within 40 and 120 kg and Eastern cooperative oncology group (ECOG) performance status ≤ 2; they were chemotherapy-naïve with histologically proven breast cancer (Stage IIA, IIB, or IIIA) eligible for six chemotherapy cycles with the TAC regimen (docetaxel, doxorubicin and cyclophosphamide) as an adjuvant treatment within 60 days of complete surgical resection of the primary breast tumour. The study was conducted in 30 centres in seven EU and non-EU Eastern countries. The majority of treated patients were enrolled in Georgia (52%), with one centre accounting for 71 patients (29%).

Treatments

Patients received up to 6 three-week cycles of TAC chemotherapy consisting of the following sequence: 50 mg/m^2 doxorubicin, 500 mg/m^2 cyclophosphamide, and 75 mg/m^2 docetaxel. A subcutaneous injection of 6 mg pegfilgrastim (Grasustek or Neulasta) was administered at least 24 hours after chemotherapy. Patients were randomised in a ratio of 2:1 to receive either Grasustek or Neulasta with stratification by country.

Primary prophylactic antibiotic therapy was not allowed; only secondary antibiotic prophylaxis was allowed and was initiated upon development of episode of febrile neutropenia in accordance with the NCCN Practice Guidelines in Oncology).

Objectives

Primary objective:

The primary objective of this trial was to demonstrate the equivalent efficacy of Grasustek compared to Neulasta EU with respect to the duration of severe neutropenia (DSN), which was defined as Grade 4 neutropenia (ANC < 0.5 x 10^9/L), during Cycle 1 of chemotherapy.

Secondary objectives:

The main secondary outcomes included DSN during the other cycles, and in all cycles, the depth of ANC nadir (lowest ANC), febrile neutropenia episodes (defined as single temperature ≥ 38.3 °C measured orally or ≥ 38.0 °C for over 1h; neutropenia: ANC <0.5 x 10^9/L or <1 x 10^9/L with a

predicted decline to ≤0.5 x 10^9/L over the next 48h, ANC and temperature being measured on the same day); hospitalisation and time in intensive care unit (ICU) due to neutropenia complications; clinically documented infections and use of intravenous antibiotics.

Outcomes/endpoints

Primary efficacy endpoint:

DSN defined as the number of days with Grade 4 neutropenia (ANC < 0.5 x 10^9/L) during Cycle 1 of chemotherapy.

Secondary efficacy endpoints:

- DSN during Cycles 2 to 6;
- Depth of ANC nadir, defined as the lowest ANC in Cycles 1 to 6;
- Febrile neutropenia episodes as previously defined (incidence) in all cycles (Note: oral temperature measurements twice daily for D1-14, once daily D15-21, and whenever the subject felt feverish);
- Time to neutrophil recovery, defined as the time in days from the chemotherapy administration until the subject's ANC increases to > 2.0 × 10^9/L after the nadir in Cycle 1 (Note: in all cycles, full panel of haematology analysis on D4, D5, D6, D7, D8, and daily until recovery of ANC to >2 × 10^9/L after expected ANC nadir);
- Hospitalisation (frequency and duration) and time in intensive care unit (ICU) due to neutropenia complications by cycle;
- Clinically documented infections (incidence) by cycle;
- Use of intravenous antibiotics during each cycle.

Sample size

For the sample size determination, the following assumptions were made: expected difference in the means of DSN: 0.1 day; mean DSN in the reference arm: 1.8 day; common SD of DSN in both arms: 1.4 day; one-sided significance level: 2.5%; randomisation ratio: 2:1 (Grasustek: Neulasta).

The primary efficacy endpoint was assumed to have a negative binomial distribution. The LS Means for both treatment arms and differences (95% CIs) between LS Means were estimated within a general linear model framework, accounting for treatment arm, and applying a log link.

Equivalence would be concluded if the 95% CI of the ratio of LS Means was entirely contained in the interval [0.65, 1.55]. The upper limit of that acceptance range corresponded to a 1-day difference on the additive scale, assuming a reference mean of 1.8 days. The lower limit was calculated to be symmetrical around 1 on a multiplicative scale.

The power of the analysis was estimated within a simulation study. Data for the two treatment arms were generated from two negative binomial distributions, according to the assumptions. 30000 sets of data were generated and the proportion of cases where equivalence was concluded was considered to be the power estimate. With 30000 simulated cases the standard error of the power estimate was 0.17% for an underlying true power of 90%.

Therefore, based on the above assumptions 216 evaluable subjects (144 in Grasustek arm, 72 in Neulasta arm) were considered to be sufficient to achieve a 90% power at a 2.5% level of significance to reject both one-sided null hypotheses. The PP and FAS populations were the primary analysis populations. To acquire sufficient evaluable subjects for the PP population, an exclusion/dropout rate of 15% was estimated and taken into account. Hence, a sample size of 255 patients (170 in Grasustek arm, 85 in Neulasta arm) was required to be randomised. However, 254 subjects were randomised as

one subject was assigned with two randomisation codes. Nevertheless, both PP and FAS populations included more than 216 subjects (considered to be sufficient to achieve a 90% power at a 2.5% significance level to reject both one-sided null hypotheses).

Randomisation

Patients were randomised in a ratio of 2:1 to receive either Grasustek or Neulasta in a country stratified manner. There was a slight deviation from this planned proportion (2.097:1 instead of 2:1) due to usage of permuted block randomisation stratified by country.

Blinding (masking)

The study was assessor-blinded. Unblinded staff was responsible for the preparation and administration of study drug. All efforts were made to conceal the identity of the study drug to the subject.

Statistical methods

The main efficacy endpoint (DSN) was obtained by adding up the days with ANC below 0.5 x 10^9/L during the first treatment cycle; in case of days with missing ANC measurement, imputation rules were pre-specified. DSN was assumed to have a negative binomial distribution in order to allow for skewness and over dispersion. The least-square means (LSMs) were estimated within a general linear model framework, accounting for treatment arm, and applying a log link. LS mean difference and 95% CI between the two treatment arms was then back-transformed by exponentiation, resulting in a ratio of means and its 95% CI. The primary analysis of DSN in Cycle 1 consisted in testing equivalence of Grasustek and Neulasta; it was performed both on the full analysis set (FAS) and the per protocol (PP) population. The FAS included all randomised subjects who received the study drug and had at least one post-baseline ANC measurement in Cycle 1. The PP set included all randomised subjects who received the study drug, had baseline ANC value and sufficient ANC measurements to allow for estimation of DSN, if present, and its subsequent recovery, and had no major deviations from the protocol in Cycle 1. Equivalence was concluded if the 95% CI of the ratio of means was entirely contained in the interval [0.65, 1.55].

Results

Participant flow

A total of 254 patients (out of 296 screened) were randomised at 30 centres and 248 patients received at least one treatment dose: 166 Grasustek and 82 Neulasta. Overall, 230 patients completed treatment, 152 (91.6%) and 78 (95.1%) patients in the Grasustek and Neulasta treatment arm, respectively.

A total of 18 patients discontinued treatment for the following reasons: subject’s withdrawal of consent (10 [6.0%] and 3 (3.7%) patients from the Grasustek and Neulasta treatment arms, respectively); adverse event for 3 patients (1.8%) in the Grasustek arm; physician’s decision for 2 patients (poor protocol adherence and no need for further TAC cycles, respectively).

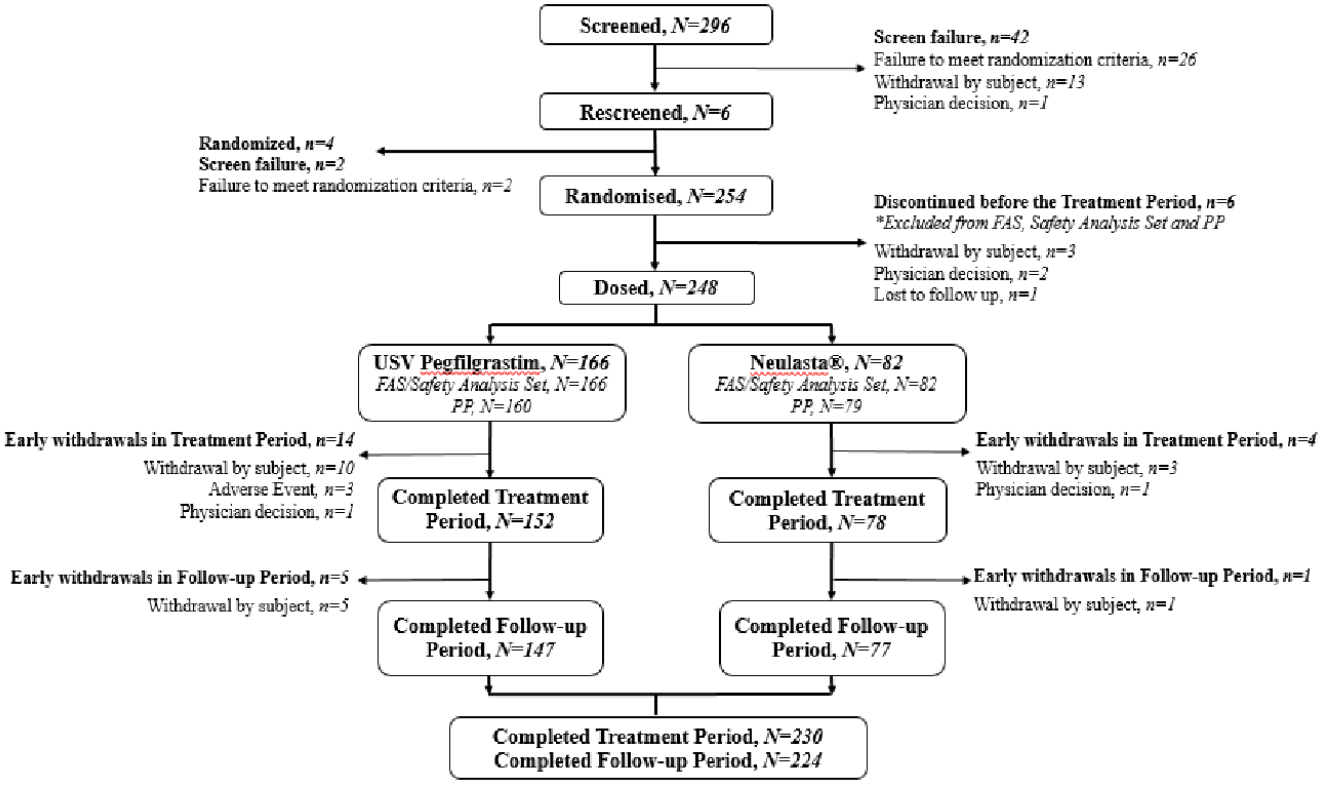


Figure 15: Participant flow

Recruitment

In total, 254 patients (out of 296 screened) were randomised at 30 centres and 248 patients received at least one treatment dose: 166 Grasustek and 82 Neulasta. The trial was conducted in Eastern EU and non-EU countries; more than half of the study

Conduct of the study

There was no major protocol amendment.

Major deviations occurred in the same proportion of patients in both arms (~14.5%).

Baseline data

The study population had a mean age of approximately 53 years and included chemotherapy naïve patients with early stage IIA (44%), IIB (22%) or IIIA (33%) breast cancer. In all cycles, compliance levels with the calculated chemotherapy dose were > 99% for all three TAC components in both treatment arms.

Numbers analysed

Patient disposition

A total of 254 patients were randomised and 248 patients received at least one treatment dose: 166 Grasustek and 82 Neulasta. Overall, 230 patients completed treatment, 152 (91.6%) and 78 (95.1%) patients in the Grasustek and Neulasta treatment arm, respectively.

A total of 18 patients discontinued treatment for the following reasons: subject's withdrawal of consent (10 [6.0%] and 3 (3.7%) patients from the Grasustek and Neulasta treatment arms, respectively);

adverse event for 3 patients (1.8%) in the Grasustek arm; physician's decision for 2 patients (poor protocol adherence and no need for further TAC cycles, respectively).

Major deviations occurred in the same proportion of patients in both arms (~14.5%). While the FAS includes all patients having received at least one dose of Grasustek (166) or Neulasta (82), the PP population includes 160 and 79 patients, respectively; the proportion of exclusions was low (< 4%) and the same in both arms.

Outcomes and estimation

Primary endpoint

Equivalence was concluded since the 95% CI of the ratio of means was entirely contained in the pre-defined interval [0.65, 1.55] in both FAS and PP set analyses.

Table 19: Primary endpoint results

Treatments groups	USV		Grasustek s.c. injection of 6 mg, up to 6 cycles; N=172
	NEU		Neulasta s.c. injection of 6 mg, up to 6 cycles; N=82
Primary endpoint	Duration of severe neutropenia	DSN C1	Number of days of Grade 4 neutropenia (ANC below 0.5 x 10^9/L) during cycle 1

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	FAS & Per protocol			
Descriptive statistics and estimate variability	Treatment group		USV	NEU
	Number of subject FAS		166	82
	DSN C1 (days) LS mean		1.58	1.65
	95% CI		1.40 – 1.79	1.39 – 1.95
	Number of subject PP		160	79
	DSN C1 (days) LS mean		1.54	1.65
	95% CI		1.36 – 1.74	1.39 – 1.95
Effect estimate per comparison	Primary endpoint DSN C1 FAS	Comparison groups	USV/NEU	
		LSM ratio	0.96	

		95% CI	0.78 – 1.18
		Pre-defined margin	0.65 – 1.55
	Primary endpoint DSN C1 PP	Comparison groups	USV/NEU
		LSM ratio	0.93
		95% CI	0.76 – 1.16
		Pre-defined margin	0.65 – 1.55

Secondary endpoints

Overall, there were 11 subjects with FN during all cycles out of which 9 (5.4%) were in Grasustek treatment arm and 2 (2.4%) were in Neulasta treatment arm.

The descriptive secondary endpoint results are detailed in Table 20.

Table 20: Secondary endpoint results

Treatments groups	USV		Grasustek s.c. injection of 6 mg, up to 6 cycles; N=172
	NEU		Neulasta s.c. injection of 6 mg, up to 6 cycles; N=82
Secondary endpoints	Depth of ANC nadir	ANC nadir C1	Lowest ANC in cycle 1
	Febrile neutropenia	FN C1	Single oral temperature ≥ 38.3 °C or ≥ 38.0 °C for over 1h; with ANC <0.5 x 10^9/L or <1 x 10^9/L with a predicted decline to ≤0.5 x 10^9/L over the next 48h – in cycle 1

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	FAS & Per protocol			
Descriptive statistics and estimate variability	Treatment group	USV	NEU	
	Number of subject FAS	166	82	
	ANC nadir C1 (x 10^9/L) Mean (±SD)	0.51 (0.96)	0.47 (0.80)	
	FN C1 (n; %) 95% CI	6 (3.6%) 1.34 -7.70	1 (1.2%) 0.03 – 6.61	

Ancillary analyses

DSN had means ≤ 1 day in most cycles and 95% CIs for their ratios ranging between 0.64 and 1.50. Descriptive summary statistics showed ANC nadir in the two treatment arms with a median of 0.155 x 10^9/L in Cycle 1 and around 0.5 x 10^9/L in the subsequent cycles. Time to recovery during Cycle 1 was 7.5 ± 4.6 days on Grasustek and 8.0 ± 5.2 days with Neulasta.

Five patients required hospitalisation, 4 (2.4%) from Grasustek treatment arm and 1 (1.2%) from Neulasta treatment arm). Overall, 10 patients, all from Grasustek treatment arm, had infections, and likewise, 5 patients, all from Grasustek treatment arm, required i.v. antibiotic use.

Summary of main study(ies)

The following table summarises the main efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the biosimilarity assessment (see later sections).

Table 21: Summary of efficacy for trial PEGF/USV/P3/003

Title: A Randomised, Multi-Centre, Assessor-Blinded, Active-Controlled, Parallel Group, Equivalence Phase III Study Comparing the Safety and Efficacy of Grasustek and Neulasta in Breast Cancer Patients Undergoing Myelosuppressive Chemotherapy			
Study identifier	PEGF/USV/P3/003		
Design	Randomised, comparative, parallel-arm, assessor-blinded		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Up to 6 chemotherapy cycles not applicable not applicable
Hypothesis	Equivalence		
Treatments groups	USV		Grasustek s.c. injection of 6 mg, up to 6 cycles; N=172
	NEU		Neulasta s.c. injection of 6 mg, up to 6 cycles; N=82
Endpoints and definitions	Primary: Duration of severe neutropenia	DSN C1	Number of days of Grade 4 neutropenia (ANC below 0.5 x 10^9/L) during cycle 1
	Secondary: Depth of ANC nadir	ANC nadir C1	Lowest ANC in cycle 1
	Secondary: Febrile neutropenia	FN C1	Single oral temperature ≥ 38.3 °C or ≥ 38.0 °C for over 1h; with ANC <0.5 x 10^9/L or <1 x 10^9/L with a predicted decline to ≤0.5 x 10^9/L over the next 48h – in cycle 1
Database lock	Final: 13-10-2017		
Results and Analysis			

Analysis description	**Primary Analysis**			
Analysis population and time point description	FAS & Per protocol			
Descriptive statistics and estimate variability	Treatment group		USV	NEU
	Number of subject FAS		166	82
	DSN C1 (days) LS mean		1.58	1.65
	95% CI		1.40 – 1.79	1.39 – 1.95
	Number of subject PP		160	79
	DSN C1 (days) LS mean		1.54	1.65
	95% CI		1.36 – 1.74	1.39 – 1.95
	ANC nadir C1 (x 10^9/L) Mean (±SD)		0.51 (0.96)	0.47 (0.80)
	FN C1 (n; %) 95% CI		6 (3.6%) 1.34 -7.70	1 (1.2%) 0.03 – 6.61
Effect estimate per comparison	Primary endpoint DSN C1 FAS	Comparison groups	USV/NEU	
		LSM ratio	0.96	
		95% CI	0.78 – 1.18	
		Pre-defined margin	0.65 – 1.55	
	Primary endpoint DSN C1 PP	Comparison groups	USV/NEU	
		LSM ratio	0.93	
		95% CI	0.76 – 1.16	
		Pre-defined margin	0.65 – 1.55	

Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable.

Clinical studies in special populations

Not applicable.

Supportive study(ies)

Not applicable.

2.5.2. Discussion on clinical efficacy

Design and conduct of clinical studies

The efficacy trial PEGF/USV/P3/003 compared the test and reference products in a population of chemotherapy-naïve women with early breast cancer receiving a TAC adjuvant chemotherapy regimen (doxorubicin + docetaxel + cyclophosphamide) known to be associated with significant myelosuppression and high probability of febrile neutropenia (> 20%). The assessor-blinded, parallel design with unbalanced 2:1 randomisation but stratification by country, and the trial duration of up to 6 cycles are acceptable. The primary efficacy outcome (duration of severe neutropenia (DSN) in the first cycle) and pre-defined equivalence margin for the means ratio of [0.65, 1.55], corresponding to ≤ ±1 day on the additive scale, were endorsed by the CHMP. Secondary outcomes are all related to ANC, including febrile neutropenia.

The trial was conducted in Eastern EU and non-EU countries; more than half of the study population was enrolled in Georgia. ANC measurements were performed locally and, based on the information provided on the assays; this is acceptable since it is only a supportive study. Information related to temperature, adverse events, and concomitant medications was collected in patient diaries and reported by the investigators in the CRF.

The study design, study population and primary/secondary efficacy and safety endpoints were among others based on the Scientific Advices (EMEA/CHMP/SAWP/357301/2009 and EMA/CHMP/SAWP/343876/2014) received from the CHMP on the development of biosimilar Grasustek.

Efficacy data and additional analyses

Overall, 254 patients were randomised and 248 received at least one subcutaneous injection of 6 mg pegfilgrastim: 166 Grasustek and 82 Neulasta; they all provided data for the primary efficacy analysis in the first cycle. Both FAS and PP analyses show 95% CIs for the mean DSN ratios well contained within the pre-specified equivalence interval. Based on these data, therapeutic equivalence between the test and reference products is demonstrated.

Febrile neutropenia (FN) and associated outcomes (fever, infections, hospitalisations, use of antipyretics, oral and intravenous antibiotics) occurred more frequently after Grasustek than Neulasta. Given the low incidence of these events, the unequal treatment allocation (2:1) may have been a factor and, in the absence of obvious reason, it can be accepted that it is likely a chance finding. The overall occurrence of FN is much lower than anticipated on the basis of historical Neulasta trials (9-13% over 4 chemotherapy cycles – Neulasta EPAR) and its level of reporting across centres/countries appears extremely variable. No explanation could be found for this observation.

While FN is an important efficacy outcome, which is mentioned in the indication of Neulasta, its anticipated occurrence is much lower than SN and the trial was not powered for this comparison, with the additional caveat of unbalanced randomisation. Therefore, the uncertainty around the FN results is such that they are not considered to preclude efficacy equivalence between the test and reference products.

2.5.3. Conclusions on the clinical efficacy

For the purpose of demonstrating comparable efficacy for G-CSF products, PK/PD trials are more sensitive than any efficacy trial in cancer patients undergoing myelosuppressive therapy. Notwithstanding, the efficacy data from the trial in breast cancer patients are considered supportive of the biosimilarity of Grasustek and Neulasta.

2.6. Clinical safety

Patient exposure

Healthy volunteers

- Study PEGF/USV/P1/001: 150 subjects received one dose (6 mg) of Grasustek and 149 subjects one dose (6 mg) of Neulasta
- Study PEGF/USV/P1/003: 62 subjects received one dose (2 mg) of Grasustek and 62 subjects one dose (2 mg) of Neulasta

Breast cancer patients

In study PEGF/USV/P3/003, most patients received 6 doses of study drug as shown in Table 22.

Table 22: Drug exposure summary in breast cancer patients

	Cycle	USV Pegfilgrastim (N=166)	EU-licensed Neulasta® (N=82)
No of Doses (6mg) administered per cycle, n (%)	1	166 (100.0%)	82 (100.0%)
	2	162 (97.6%)	82 (100.0%)
	3	160 (96.4%)	82 (100.0%)
	4	159 (95.8%)	82 (100.0%)
	5	159 (95.8%)	79 (96.3%)
	6	153 (92.2%)	79 (96.3%)

A total of 230 patients entered the safety follow-up and 224 patients (> 97%) completed it.

Adverse events

The overall summary of adverse events (AEs) reported in the three trials is presented in Tables 23 to 25.

Table 23: Overall AE summary - PEGF/USV/P1/001

	USV pegfilgrastim (N = 150) n (%)	Neulasta® (N = 149) n (%)	Overall (N = 156) n (%)
Number (%) of subjects reporting AEs	129 (86.0)	123 (82.6)	148 (94.9)
Number (%) of subjects reporting severe AEs	0	0	0
Number (%) of subjects reporting SAEs	0	0	0
Number (%) of subjects reporting IMP-related AEs	120 (80.0)	114 (76.5)	142 (91.0)
Number (%) of subjects reporting AEs leading to IMP withdrawal	2 (1.3)	2 (1.3)	4 (2.6)

Table 24: Overall AE summary - PEGF/USV/P1/003

	TREATMENT A (N=62)		TREATMENT B (N=62)	
	n (%)	Event	n (%)	Event
Subjects reporting: at least 1 TEAE	35 (56.5)	54	37 (59.7)	60
IMP-related TEAEs	23 (37.1)	29	28 (45.2)	37
TEAEs leading to IMP withdrawal	1 (1.6)	1	1 (1.6)	1
severe TEAEs	0	0	1 (1.6)	1
serious TEAEs	0	0	0	0
TEAEs leading to death	0	0	0	0

IMP-related AEs are events with a relationship of possibly related or related.
Treatment A: 2 mg (0.2 mL) USV Pegfilgrastim solution for SC injection into the abdomen
Treatment B: 2 mg (0.2 mL) Neulasta® solution for SC injection into the abdomen

Table 25: Overall AE summary - PEGF/USV/P3/003

Study period	Overview	USV Pegfilgrastim (N=166)	EU-licensed Neulasta® (N=82)
Treatment	Subjects Not Reporting Any Adverse Events, n (%)	2 (1.2%)	0 (0.0%)
	Subjects Reporting at Least One Adverse Event, n (%)	164 (98.8%)	82 (100.0%)
	Death, n (%)	1 (0.6%)	0 (0.0%)
	Subjects with Life-threatening Adverse Events, n (%)	3 (1.8%)	2 (2.4%)
	Subjects with Serious Adverse Events, n (%)	8 (4.8%)	3 (3.7%)
	Subjects with Febrile Neutropenia, n (%)	9 (5.4%)	2 (2.4%)
	Subjects with Adverse Events Leading to Early Withdrawal, n (%)	3 (1.8%)	0 (0.0%)
	Subjects with IMP/Comparator Related Adverse Events, n (%)**	52 (31.3%)	30 (36.6%)
	Subjects with TAC Related Adverse Events, n (%)**	163 (98.2%)	81 (98.8%)
	Subjects with IMP/Comparator Related Bone Pain, n (%)	44 (26.5%)	23 (28.0%)
	Subjects with Injection Site Reactions, n (%)	22 (13.3%)	8 (9.8%)

*** An AE is related if the recorded relationship to IMP/Comparator is 'possibly', 'probably' or 'definitely'*

Three patients treated with Grasustek reported an AE in the follow-up period; 2 were serious and one chemotherapy-related.

The most common treatment-emergent adverse events (TEAEs) in study PEGF/USV/P1/001 are presented in Table 26.

Table 26: Summary of TEAEs reported by ≥ 5% subjects - PEGF/USV/P1/001 (Safety Analysis Set)

System Organ Class Preferred term	USV pegfilgrastim (N = 150) n (%)	Neulasta® (N = 149) n (%)	Overall (N = 156) n (%)
Any Adverse Event	**129 (86.0)**	**123 (82.6)**	**148 (94.9)**
Musculoskeletal and Connective Tissue Disorders	**108 (72.0)**	**97 (65.1)**	**132 (84.6)**
Back pain	87 (58.0)	84 (56.4)	117 (75.0)
Pain in extremity	27 (18.0)	22 (14.8)	37 (23.7)
Musculoskeletal chest pain	13 (8.7)	9 (6.0)	22 (14.1)
Arthralgia	15 (10.0)	10 (6.7)	19 (12.2)
Neck pain	8 (5.3)	5 (3.4)	13 (8.3)
Musculoskeletal pain	4 (2.7)	4 (2.7)	8 (5.1)
Nervous System Disorders	**56 (37.3)**	**63 (42.3)**	**87 (55.8)**
Headache	52 (34.7)	60 (40.3)	81 (51.9)
Infections and Infestations	**23 (15.3)**	**22 (14.8)**	**42 (26.9)**
Upper respiratory tract infection	12 (8.0)	13 (8.7)	25 (16.0)
Gastrointestinal Disorders	**15 (10.0)**	**23 (15.4)**	**36 (23.1)**
Nausea	5 (3.3)	6 (4.0)	10 (6.4)
Abdominal pain	2 (1.3)	6 (4.0)	8 (5.1)
Investigations	**8 (5.3)**	**8 (5.4)**	**13 (8.3)**
Increased alanine aminotransferase	5 (3.3)	7 (4.7)	10 (6.4)

The same type of TEAEs were observed in study PEGF/USV/P1/003, although at much lower frequencies, with the exception of upper respiratory tract infections, which were reported in about 10% of the subjects in both trials and are unlikely related to pegfilgrastim. The majority of TEAEs were mild in severity and their frequency was broadly comparable between treatment arms.

The most common TEAEs during the treatment period of study PEGF/USV/P3/003 are presented in Table 27. Most AEs were judged moderate or severe.

Table 27: Summary of TEAEs reported by ≥ 5% patients - PEGF/USV/P3/003 (Safety Analysis Set)

SOC	PT	USV Pegfilgrastim (N=166)	EU-licensed Neulasta® (N=82)
Any SOC	Any PT, n (%)	164 (98.8%)	82 (100.0%)
Blood and lymphatic system disorders	Any PT, n (%)	145 (87.3%)	70 (85.4%)
	Neutropenia, n (%)	130 (78.3%)	63 (76.8%)
	Leukopenia, n (%)	74 (44.6%)	36 (43.9%)
	Thrombocytopenia, n (%)	25 (15.1%)	8 (9.8%)
	Anaemia, n (%)	15 (9.0%)	9 (11.0%)
	Febrile neutropenia, n (%)	9 (5.4%)	2 (2.4%)
	Leukocytosis, n (%)	6 (3.6%)	5 (6.1%)
Gastrointestinal disorders	Any PT, n (%)	109 (65.7%)	54 (65.9%)
	Nausea, n (%)	79 (47.6%)	38 (46.3%)
	Diarrhoea, n (%)	33 (19.9%)	20 (24.4%)
	Vomiting, n (%)	17 (10.2%)	7 (8.5%)
	Abdominal pain upper, n (%)	12 (7.2%)	9 (11.0%)
	Abdominal pain, n (%)	9 (5.4%)	2 (2.4%)
General disorders and administration site conditions	Any PT, n (%)	80 (48.2%)	38 (46.3%)
	Asthenia, n (%)	35 (21.1%)	18 (22.0%)
	Fatigue, n (%)	21 (12.7%)	12 (14.6%)
	Injection site reaction, n (%)	16 (9.6%)	8 (9.8%)
Infections and infestations	Any PT, n (%)	10 (6.0%)	2 (2.4%)
Investigations	Any PT, n (%)	12 (7.2%)	3 (3.7%)
Musculoskeletal and connective tissue disorders	Any PT, n (%)	59 (35.5%)	31 (37.8%)
	Bone pain, n (%)	54 (32.5%)	27 (32.9%)
	Spinal pain, n (%)	13 (7.8%)	8 (9.8%)
Nervous system disorders	Any PT, n (%)	68 (41.0%)	33 (40.2%)
	Headache, n (%)	46 (27.7%)	18 (22.0%)
	Dizziness, n (%)	36 (21.7%)	15 (18.3%)
Respiratory, thoracic and mediastinal disorders	Any PT, n (%)	9 (5.4%)	3 (3.7%)
Skin and subcutaneous tissue disorders	Any PT, n (%)	64 (38.6%)	31 (37.8%)
	Alopecia, n (%)	62 (37.3%)	30 (36.6%)

*Most common AE are defined as AEs with incidence rate >=5% in any treatment arm

Bone pain was specifically investigated during the trial and the results of this evaluation are provided in Table 28.

Table 28: Bone pain - PEGF/USV/P3/003 (Safety Analysis Set)

	Category	Statistics	USV Pegfilgrastim (N=166)	EU-licensed Neulasta® (N=82)
Total	Subjects	n (%)	44 (26.5%)	23 (28.0%)
		95% CI of percentage	20.22 to 34.3	18.68 to 39.06
	Events	n	483	206
Severity	Mild	Number of Subjects with AEs, n (%)	37 (22.3%)	21 (25.6%)
		Number of AEs, n	209	84
	Moderate	Number of Subjects with AEs, n (%)	35 (21.1%)	15 (18.3%)
		Number of AEs, n	228	84
	Severe	Number of Subjects with AEs, n (%)	16 (9.6%)	6 (7.3%)
		Number of AEs, n	46	38

Each subject can be counted several times for each level of intensity.
The denominator for percentages was the number of subjects in the respective treatment arm.

Local tolerability was specifically investigated in the HV trials. Mild erythema was reported by about 20% of the subjects 0.5 h after administration regardless of dose. Mild pain was reported in a small proportion of subjects (2-8%). Overall, injection site reactions were comparable between the two products.

Serious adverse events/deaths/other significant events

No death or SAE was reported in the HV studies.

In the breast cancer study, a patient died 10 days after Grasustek administration due to bilateral pneumonia complicated with acute respiratory distress syndrome (ARDS).

There were 15 serious TEAEs reported by 11 subjects during the treatment period: 8 (4.8%) subjects from Grasustek treatment arm reported 12 serious TEAEs and 3 (3.7%) subjects from Neulasta treatment arm reported 3 serious TEAEs. The most frequent SAEs were febrile neutropenia and neutropenia. Agranulocytosis, systemic inflammatory response syndrome, breast abscess, cerebral infraction and ARDS were reported in 5 subjects from Grasustek treatment arm; none were considered related to pegfilgrastim.

There were 2 serious TEAEs reported during the safety follow-up period both from Grasustek treatment arm: peripheral T-cell lymphoma unspecified and metastasis to central nervous system assessed as unlikely related/unrelated to pegfilgrastim.

Laboratory findings

Haematology

Decrease in platelet count below reference value was reported by about 30% of HVs after the 6 mg dose and 15% after the 2 mg dose. In the 6 mg study, thrombocytopenia was reported as an AE after Neulasta (3 subjects; 2.0%). In addition, neutropenia was reported with the same frequency after both products (2 subjects; 1.3%) and one subject had high WBC counts (20 to 60 × 10^9/L) after both products.

However, based on platelet measurements and using CTCAE classification, Grade 3/4 thrombocytopenia was more frequent with Grasustek (14%) than with Neulasta (4%). No case of hyperleucocytosis ≥ 100 x 10^9/L was reported. Finally, at the end of treatment visit, 51/149 patients (34%) had low haemoglobin level in the Grasustek treatment arm and 35/75 (47%) in the Neulasta treatment arm.

Clinical chemistry

After both treatments in HVs at the 6 mg dose, mean values for alkaline phosphatase (ALP) were outside the reference range at 144 h post-dose and returned to normal at 312 h post-dose. At the 2 mg dose, the mean ALP values increased but remained within the normal range.

Overall at the 6 mg dose, 12 (7.7%) subjects had abnormal values that were recorded as an AE. Increased liver enzymes were similarly reported after both products: increased ALT (5 [3.3%] vs 7 [4.7%]) subjects), increased AST (4 [2.7%] vs 2 [1.3%] subjects), after Grasustek and Neulasta, respectively. At the 2 mg dose, only 2 subjects had abnormal values reported as an AE: increased ALT, which led to withdrawal, one after each treatment.

Markers of liver function all increased during treatment as well as mean LDH level, similarly in both treatment arms. Clinically significant increases in ALT, AST, or GGT were reported as AEs in less than 2% of the patients in both treatment arms.

Safety in special populations

N/A

Immunological events

Out of 949 samples from randomised patients in the breast cancer study, two were confirmed positive for anti-pegfilgrastim ADA: one pre-dose sample (positive in the Grasustek assay but negative with the Neulasta assay) and one post-dose sample (positive in the two confirmatory assays) after the end of treatment in a patient treated with Grasustek; the sample was also positive in the Nab assay and the titre was low (=2) in both binding and neutralising assays. In this patient, all other samples (pre-dose, after 3 cycles and follow-up) were negative. The DSN appeared longer (6 days) in the last 3 cycles than in the first 3 cycles (3-4 days) with a trend for lower ANC nadir (0-0.01 $x10^9$/L) compared to 0.06 and 0.04 $x10^9$/L in the first 2 cycles. This patient also developed (non-serious) interstitial pneumonia at the end of Cycle 3 (cycle with the highest ANC peak at ~19 $x10^9$/L), which was considered unrelated.

Antibodies against the new impurities of the biosimilar candidate (His-filgrastim and bovine recombinant enterokinase) were mainly detected in pre-dose samples (anti-His-filgrastim in 13 (5%) patients; anti-enterokinase in 2 patients). One patient in each treatment arm had possible drug-induced ADAs against His-filgrastim and one patient in the Grasustek arm had possible drug-induced ADAs against enterokinase.

The immunogenicity results in HVs are summarised in the Pharmacology section (3.3.2).

Safety related to drug-drug interactions and other interactions

Not applicable.

Discontinuation due to AES

In study PEGF/USV/P1/001, 4 HVs discontinued the study due to AEs. Two subjects received Grasustek and were withdrawn due to neutropenia (and unrelated diarrhoea, vomiting) and increased ALT/AST, neutropenia and thrombocytopenia, respectively. Two subjects received Neulasta and were withdrawn due to abdominal pain (no splenomegaly) and tooth abscess (unrelated), respectively.

In study PEGF/USV/P1/003, 2 HVs discontinued the study due to increased ALT/AST after administration of Grasustek (1) and Neulasta (1).

No patient discontinued the study due to pegfilgrastim-related AE in study PEGF/USV/P3/003. Three patients (1.8%), all from Grasustek treatment arm, discontinued treatment due to diarrhoea (1), herpes zoster (1), and fatal pneumonia (1), all considered unrelated.

Post marketing experience

Not applicable.

2.6.1. Discussion on clinical safety

The safety database comprises healthy volunteers having received a single dose of the test and reference products (150 HVs exposed to 6 mg and 62 HVs to 2 mg of pegfilgrastim). In addition, 166 and 82 breast cancer patients were exposed to Grasustek and Neulasta, respectively, for up to 6 cycles in combination with doxorubicin, docetaxel and cyclophosphamide.

Provided the biosimilar and the reference product exhibit comparable physicochemical and functional characteristics as well as comparable pharmacokinetic and pharmacodynamic profiles, those adverse events that are related to exaggerated pharmacological effects (e.g., bone pain, leucocytosis, splenomegaly) can be expected to occur at similar frequencies. Therefore, the safety data from the breast cancer patient trial are mainly considered supportive, the more so because of the small size of the trial and its unbalanced randomisation.

Regarding immunogenicity, the population of immunosuppressed breast cancer patients is far from sensitive, especially since pegfilgrastim is known to have low immunogenicity. This holds also true for the investigation of antibodies against the new impurities of the biosimilar candidate (His-filgrastim and bovine recombinant enterokinase) even if the multiple dose regimen is more appropriate than the single dose crossover design in HVs. This investigation is further hampered by the lack of robustness of the developed assays.

The proportion of subjects reporting AEs, AEs judged by the investigator as related to pegfilgrastim, and AEs leading to trial discontinuation was broadly comparable between the test and reference products in the three clinical studies.

The most common listed ADRs of pegfilgrastim (musculoskeletal and bone pain, headache, nausea, injection site reactions, elevated liver enzymes) were reported with similar incidences after both products regardless of the population, although some were confounded by chemotherapy in the breast cancer patients. Of note, ADRs appeared more frequent after the 6 mg dose than the 2 mg dose, as expected. No splenomegaly was reported in any trial.

Substantial variations in the level of reporting occurred across centres/countries in the patient trial; in particular, a low incidence of ADRs (febrile neutropenia, thrombocytopenia, injection site reactions, and bone pain) was reported in Georgia, which contributed to more than half of the study population. No explanation could be found for this observation. There also seems to be a lack of standardisation in the definition of haematological toxicities (e.g., clusters of leucocytosis reports in 2 centres) but no case of hyperleucocytosis ≥ 100 x 10^9/L has been reported. Notwithstanding, this does not impact the relative ADR incidence given the randomisation stratification by country and therefore does not constitute a concern regarding the biosimilarity assessment.

Two listed uncommon ADRs of pegfilgrastim were reported in the Grasustek arm of the patient trial (one patient each) although not considered treatment-related by the investigator/sponsor: ARDS and interstitial pneumonia. However, this observation does not raise any concern on the biosimilarity of the products as similar rates of uncommon ADRs are not expected in such a small clinical trial, especially given its unequal randomisation.

In the breast cancer study, the changes in haematology clinical chemistry parameters were as expected in subjects undergoing chemotherapy, and were usually comparable between the treatment arms. This included decrease in WBC and platelet count, which had usually recovered by the next cycle, similarly in both treatment arms.

As far as immunogenicity results from the breast cancer patient trial are concerned, one patient treated with Grasustek was found to have developed neutralising ADAs (likely against the G-CSF moiety), which seem to have prolonged the duration of severe neutropenia during the last chemotherapy cycles. This finding is unexpected as anti-pegfilgrastim ADAs are usually reported to be non-neutralising. The same imbalance in favour of Neulasta is observed in HVs, i.e. no confirmed ADAs developed against Neulasta vs. 3 cases against Grasustek.

Analytical assays were also developed to detect antibodies against the impurities of the biosimilar candidate, namely His-filgrastim and bovine recombinant enterokinase. ADAs were detected mainly in pre-dose samples. Due to the lack of robustness of the assays developed, these data cannot contribute to the evaluation of potential immunogenicity of the impurities. However, in view of currently available evidence that these impurities are found at very low levels, this is not considered a concern anymore.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

The safety profile of the test and reference products appears comparable and broadly in line with the product information of Neulasta, therefore supporting biosimilarity.

The immunogenicity data show ADA development in a very small number of subjects (healthy volunteers and patients) only after Grasustek administration. With this very low level of ADA incidence, this result does not raise concern when interpreted in the light of the totality of evidence provided by the comparability exercise and does not preclude a conclusion of comparable immunogenicity profile of the test and reference products.

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table 29 Summary of safety concerns

Important identified risks	• Severe splenomegaly/splenic rupture • Cutaneous vasculitis • Sweet's syndrome (Acute Febrile Dermatosis) • Anaphylactic reaction • Capillary leak syndrome • Serious pulmonary adverse events (including Interstitial pneumonia and ARDS) • Sickle cell crisis in patients with sickle cell disease • Musculoskeletal pain-related symptoms • Leukocytosis • Thrombocytopenia • Glomerulonephritis
Important potential risks	• Acute myeloid leukaemia [AML] and myelodysplastic syndrome [MDS] • Cytokine release syndrome • Medication errors including overdose • Drug interaction with lithium • Off-label use • Immunogenicity (incidence and clinical implications of anti-G-CSF antibodies) • Extramedullary haematopoiesis
Missing information	• Risks in children <18 years of age • Risks during pregnancy and lactation

Discussion on safety specification

The important identified and potential risks and missing information are in line with the current safety concerns of Neulasta (reference product).

Conclusions on the safety specification

Having considered the data in the safety specification, the Rapporteur agrees that the safety concerns listed by the applicant are appropriate.

Pharmacovigilance plan

Routine pharmacovigilance activities

The applicant proposes to monitor all safety concerns via routine pharmacovigilance activities. As part of routine pharmacovigilance activities specific adverse reaction follow up questionnaires have been developed for the following safety concerns:

- Capillary leak syndrome
- Cytokine release syndrome
- Medication errors including overdose
- Drug interaction with lithium
- Off-label use
- Immunogenicity (incidence and clinical implications of anti-G-CSF antibodies)
- Risks in pregnancy and lactation

No additional pharmacovigilance activities are planned or ongoing. This is acceptable.

Overall conclusions on the PhV Plan

The CHMP and PRAC, having considered the data submitted, are of the opinion that routine pharmacovigilance is sufficient to identify and characterise the risks of the product. The proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

Risk minimisation measures

Routine Risk Minimisation Measures

The applicant proposes the below routine risk minimisation measures for Grasustek that are acceptable.

Table 30 Summary table of risk minimisation activities by safety concern

Important Identified Risks

Safety concern	Risk minimisation measures
Severe splenomegaly/splenic rupture	Routine risk minimisation measures: Section 4.4 and 4.8 of Pegfilgrastim SmPC has information on this safety concern. Section 2 and 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Cutaneous vasculitis	Routine risk minimisation measures: Section 4.8 of Pegfilgrastim SmPC has information on this safety concern Section 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Sweet's syndrome (Acute Febrile Dermatosis)	Routine risk minimisation measures: Section 4.8 of Pegfilgrastim SmPC has information on this safety concern Section 1 of Pegfilgrastim PIL has information on this safety concern.

Safety concern	Risk minimisation measures
	Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Anaphylactic reaction	Routine risk minimisation measures: Section 4.3, 4.4 and 4.8 of Pegfilgrastim SmPC has information on this safety concern. Section 2 and 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include prescription only status of the product. Additional risk minimisation measures: None
Capillary leak syndrome	Routine risk minimisation measures: Section 4.4 and 4.8 of Pegfilgrastim SmPC has information on this safety concern Section 2 and 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None

Safety concern	Risk minimisation measures
Serious pulmonary adverse events (including Interstitial pneumonia and ARDS)	Routine risk minimisation measures: Section 4.4 and 4.8 of Pegfilgrastim SmPC has information on this safety concern. Section 2 and 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Sickle cell crisis in patients with sickle cell disease	Routine risk minimisation measures: Section 4.4 and 4.8 of Pegfilgrastim SmPC has information on this safety concern. Section 2 and 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Musculoskeletal pain-related symptoms	Routine risk minimisation measures: Section 4.8 of Pegfilgrastim SmPC has information on this safety concern Section 4 of Pegfilgrastim PIL has information on this safety

Safety concern	Risk minimisation measures
	concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Leukocytosis	Routine risk minimisation measures: Section 4.4 and 4.8 of Pegfilgrastim SmPC has information on this safety concern Section 2 and 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Thrombocytopenia	Routine risk minimisation measures: Section 4.4 and 4.8 of Pegfilgrastim SmPC has information on this safety concern Section 2 and 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation

Safety concern	Risk minimisation measures
	measures: None
Glomerulonephritis	Routine risk minimisation measures: Section 4.4 and 4.8 of Pegfilgrastim SmPC has information on this safety concern. Section 2 and 4 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Important Potential Risks	
Acute myeloid leukaemia [AML] and myelodysplastic syndrome [MDS]	Routine risk minimisation measures: Section 4.4, 5.1 and 5.3 of Pegfilgrastim SmPC has information on this safety concern Section 2 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Cytokine release syndrome	Routine risk minimisation measures include the prescription only status of the product.

Safety concern	Risk minimisation measures
	Additional risk minimisation measures: None
Medication errors including overdose	Routine risk minimisation measures: Section 1, 2, 4.2, 4.5 and 4.9 of Pegfilgrastim SmPC has information on this safety concern. Section 1 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Drug interaction with lithium	Routine risk minimisation measures: Section 4.5 of Pegfilgrastim SmPC has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Off-label use	Routine risk minimisation measures: Section 4.1 and 4.4 of Pegfilgrastim SmPC has information on this safety concern. Other routine risk minimisation

Safety concern	Risk minimisation measures
	measures include the prescription only status of the product. Additional risk minimisation measures: None
Immunogenicity (incidence and clinical implications of anti-G-CSF antibodies)	Routine risk minimisation measures: Section 4.4 of Pegfilgrastim SmPC has information on this safety concern. Section 2 of Pegfilgrastim PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Extramedullary haematopoiesis	Routine risk minimisation measures: Section 5.3 of Pegfilgrastim SmPC has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Missing information	
Risks in children <18 years of age	Routine risk minimisation measures: Section 4.2 and 4.8 of Pegfilgrastim SmPC has

Safety concern	Risk minimisation measures
	information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None
Risks during pregnancy and lactation	Routine risk minimisation measures: Section 4.6 of Pegfilgrastim SmPC has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation measures: None

Overall conclusions on risk minimisation measures

The PRAC having considered the data submitted was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

Of note, for the reference product Neulasta prefilled syringes, no additional RMM are in place.

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.2 is acceptable.

2.8. Pharmacovigilance

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.

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.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use*.

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Grasustek (pegfilgrastim) is included in the additional monitoring list as it is a biological product that is not covered by the previous category and authorised after 1 January 2011.

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

The claimed indication for Grasustek is the same as for the reference product Neulasta:

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

The comparability exercise is based on:

- Quality data: an extensive comparability exercise (including side-by-side testing) which included 14 batches of EU Neulasta and 15 batches of Grasustek DP.
- Non-clinical studies comparing the biosimilar candidate to EU-sourced Neulasta:
    - an *in vitro* study with a murine myeloblastic cell line, comparing fifteen Grasustek batches (6 of the final commercial manufacturing process) versus 13 batches of Neulasta in a total of 6 studies
    - an *in vitro* Surface Plasmon Resonance receptor binding affinity study of fifteen batches of Grasustek versus 14 lots of Neulasta.
    - an *in vivo* PK/PD study of 3 different single doses in 72 normal and neutropenic rats.
- Clinical data comparing the biosimilar candidate to EU-sourced Neulasta:
    - two PK/PD trials of the same design (single dose, cross-over) conducted in healthy volunteers and using two different doses: 6 mg (therapeutic dose) for the pivotal PK/PD trial and 2 mg (sub-therapeutic dose) for a supportive PK/PD trial;
    - a supportive efficacy/safety trial conducted in breast cancer patients undergoing myelosuppressive chemotherapy for up to 6 cycles.

The development plan appears in line with CHMP guidance and Scientific Advices received.

3.2. Results supporting biosimilarity

3.2.1. From a quality perspective

For the biosimilarity analysis, the applicant has performed an extensive comparability exercise (including side-by-side testing) which included EU Neulasta and Grasustek DP. In general, all quality attributes analysed proved to be highly similar between Grasustek and EU Neulasta. The primary structure of Grasustek is identical to that of the reference medicinal product. Molecular weight and polydispersity indicated similar PEG moieties between Grasustek and the reference medicinal product. Product-related variants and impurities appeared to be somewhat higher in the reference medicinal product, but this is most likely due to the difference in age of reference medicinal product lots and Grasustek lots. In general, differences were small and not considered relevant. Importantly, relative potency and receptor binding kinetics were highly similar for Grasustek and the reference medicinal product. Comparative stability testing demonstrated that Grasustek and the reference medicinal product degrade in a comparable manner. In conclusion, from a quality point of view, Grasustek could be considered as biosimilar to EU Neulasta.

3.2.2. From a non-clinical perspective

Comparative assessment of the pharmacodynamic effect of Grasustek and Neulasta was addressed by *in vitro* and *in vivo* studies which are in accordance with the European biosimilar guideline for (peg)filgrastim (EMEA/CHMP/BMWP/31329/2005) and with the scientific advice given by EMA.

Biosimilarity of Grasustek and Neulasta could be demonstrated by comparative assessment of the binding affinity to the G-CSF receptor as well as of the potency to stimulate proliferation of myeloblastic cells.

The results of a comparative PD/PK study in healthy and neutropenic rats provided an increase and dose-response in overall leukocyte levels and in neutrophil granulocyte levels after treatment with Grasustek and Neulasta.

The selected methods and procedures appear appropriate. No relevant differences between Grasustek and Neulasta became obvious.

3.2.3. From a clinical perspective

PK comparability is considered established in the pivotal PK/PD trial based on the 90% CI of the geometric mean ratio for the three key parameters (AUC_{0-t}, C_{max}, and $AUC_{0-\infty}$) that is contained within the standard bioequivalence range of 80.00-125.00%. Descriptive results of the same parameters estimated for the 2 mg dose further support PK comparability.

PD comparability is considered established in both PK/PD trials based on the 95% CI of the geometric mean ratio for all four PD parameters (AUEC and Emax for ANC as well as CD34+ cell count) that is contained within the pre-specified equivalence interval of 90.00-111.11% in the pivotal trial and 80.00-125.00% in the supportive trial.

Comparable efficacy is considered established since both FAS and PP analyses show that the 95% CI for the ratio of mean duration of severe neutropenia (primary efficacy outcome) is well contained within the pre-specified equivalence interval [0.65, 1.55]. In the FAS analysis, the mean DSN was 1.58 days with Grasustek and 1.65 days for Neulasta; on the additive scale, the maximum possible difference would be less than half a day.

Safety data from the three trials show similar safety profile for the test and reference products, which is in line with the product information of Neulasta. The most common listed ADRs of pegfilgrastim (musculoskeletal and bone pain, headache, nausea, injection site reactions, elevated liver enzymes) were reported with similar incidences after both products regardless of the population, although some were confounded by chemotherapy in the breast cancer patients.

3.3. Uncertainties and limitations about biosimilarity

3.3.1. From a quality perspective

From a quality point of view, Grasustek can be considered as biosimilar to EU Neulasta.

3.3.2. From a non-clinical perspective

The additional requested raw data regarding both the Biacore receptor binding and the cell proliferation assays were submitted. This additional information confirmed the conclusions regarding biosimilarity.

The *in vivo* PD/PK study had some shortcomings such as limitations of the neutropenic animal model as well as small group size. Thus, the study was only evaluated descriptively and a definite conclusion on biosimilarity could not be drawn. This uncertainty is considered to be minor as quality, non-clinical *in vitro* and clinical data are regarded more relevant.

3.3.3. From a clinical perspective

A significant period effect is observed in the pivotal PK/PD crossover trial at the 6 mg dose, but not at the lower dose. The ANC response is higher after the second administration, and as a result, the drug exposure is lower, compared to the first administration. This is consistent with a carry-over effect of pegfilgrastim on the expansion of the neutrophil mass. As this could potentially confound the comparison between the two products, further investigation of this effect was requested, which suggested it is of similar magnitude for the two products.

In the breast cancer trial, the incidence of a number of adverse drug reactions appears lower than anticipated, which may be due to general issues in reporting, especially in the main recruiting country. However, this generally affected both treatment arms, and therefore, does not impact the comparability assessment. A few numerical differences regarding febrile neutropenia, infections and the isolated haematological finding of thrombocytopenia (while other cell lineages do not reveal differences) are likely to be chance findings, especially given the unequal randomisation.

As far as the evaluation of immunogenicity is concerned, confirmed anti-pegfilgrastim antibodies have only been detected in three HVs and one patient treated with Grasustek. As the screening and confirmatory cut points of the analytical assay are close, screened positive samples are also important to consider and their proportion is similar with both products. Given these small numbers, the numerical imbalance in confirmed ADAs does not preclude a conclusion of comparable immunogenicity profile of the test and reference products.

3.4. Discussion on biosimilarity

<u>From a quality perspective</u>

For a biosimilar, the benefit-risk balance is derived from the reference product provided the totality of evidence collected from the quality and clinical data package supports the comparability of both products; the animal study conducted does not contribute to the judgement about biosimilarity.

The quality data is in general supportive of comparable respective profiles.

From a non-clinical perspective

The non-clinical biosimilarity program comprised comparative assessment of *in vitro* PD effects as well as an *in vivo* PD/PK study. The results of the *in vitro* assays support biosimilarity. The *in vivo* study has however little additive value for the comparability exercise, and is regarded to have only supportive character.

In conclusion, from a non-clinical perspective the requirements for biosimilarity assessment have been met and sufficient evidence for the demonstration of biosimilarity has been provided.

From a clinical perspective

PK/PD trials are the cornerstone of the comparability exercise for biosimilar G-CSF products as they are more sensitive than any efficacy trial in patients to demonstrate comparable efficacy. The PK and PD profiles of Grasustek are comparable to those of Neulasta at two different doses, which makes the conclusion especially robust. A significant period effect is observed in the pivotal trial at the higher dose, consistent with the known PD carry-over effect of pegfilgrastim, but it appears to be of similar magnitude for both products.

This PK/PD results are further supported by comparable efficacy in breast cancer patients undergoing myelosuppressive chemotherapy, who showed very similar duration of severe neutropenia with the two products. The study was not powered for the secondary efficacy outcome of febrile neutropenia, an uncommon event, and therefore, the uncertainty around this result does not preclude therapeutic equivalence between Grasustek and Neulasta.

Provided the biosimilar and the reference product exhibit comparable physicochemical and functional characteristics as well as comparable pharmacokinetic and pharmacodynamic profiles, those adverse events that are related to exaggerated pharmacological effects (e.g., bone pain, leucocytosis, splenomegaly) can be expected to occur at similar frequencies. Therefore, the safety data from the breast cancer patient trial are mainly considered supportive, the more so because of the small size of the trial and its unbalanced randomisation. For this reason, the observation of two uncommon ADRs of pegfilgrastim (interstitial pneumonia and ARDS) after Grasustek does not raise any concern on the biosimilarity of the products.

Otherwise, the safety profiles of Grasustek and Neulasta appear broadly comparable. The uncertainty around the incidence of some ADRs due to inconsistencies across centres/countries does not impact the relative ADR incidence given the randomisation stratification by country and therefore is not a concern for the biosimilarity assessment.

Finally, the immunogenicity of pegfilgrastim is known to be low and immunosuppressed patients, the only target population of Neulasta, are not a sensitive population for the detection of potential differences between the test and reference products. Because of the very low level of ADA incidence, the numerical imbalance in confirmed ADAs does not preclude a conclusion of comparable immunogenicity profile of the test and reference products and has to be interpreted within the totality of evidence of the comparability exercise.

3.5. Extrapolation of safety and efficacy

Not applicable as comparable efficacy and safety have been shown in the indication of Neulasta.

3.6. Additional considerations

None.

3.7. Conclusions on biosimilarity and benefit risk balance

Based on the review of the submitted data, Grasustek 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 Grasustek is favourable in 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 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 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.

These conditions fully reflect the advice received from the PRAC.