26 July 2018
EMA/595848/2018

Assessment report

Pelgraz

International non-proprietary name: pegfilgrastim

Procedure No. EMEA/H/C/003961/0000

Note

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

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Administrative information

Name of the medicinal product:	Pelgraz
Applicant:	Accord Healthcare Limited Sage House 319 Pinner Road North Harrow Middlesex HA1 4HF United Kingdom
Active substance:	PEGFILGRASTIM
International Non-proprietary Name:	pegfilgrastim
Pharmaco-therapeutic group (ATC Code):	immunostimulants, colony stimulating factors (L03AA13)
Therapeutic indication(s):	Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes).
Pharmaceutical form(s):	Solution for injection
Strength(s):	6 mg
Route(s) of administration:	Subcutaneous use
Packaging:	pre-filled syringe (glass) with needle guard
Package size(s):	1 pre-filled syringe with needle guard + 1 alcohol swab

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List of abbreviations

Ab	Antibody
ADA	Anti-drug antibodies
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANC AUC_{0-960}	area under the plasma concentration-time curve from time 0 to 960 hours
ANC AUC_{0-last}	area under the plasma concentration-time curve from time 0 to last timepoint
ANC Tmax	time to peak of absolute neutrophil count
ANC_{max}	maximum absolute neutrophil count
ARDS	acute respiratory distress syndrome
AST	aspartate aminotransferase
ATC	anatomical therapeutic chemical
ATP	adenosine triphosphate
AUC	area under the plasma concentration-time curve
AUC_{0-288}	area under the plasma concentration-time curve from time 0 to 288 hours
AUC_{0-480}	area under the plasma concentration-time curve from time 0 to 480 hours
AUC_{0-960}	area under the plasma concentration-time curve from time 0 to 960 hours
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time 0 to infinity
AUC_{0-last}	area under the plasma concentration-time curve from time 0 to last time point
$AUEC_{0-last}$	area under the effect curve measured from the time of dosing to the last measurable concentration
BE	biosimilarity
BMI	body mass index
CD34+	Cluster of differentiation 34 positive
CI	confidence interval
CL/F	apparent systemic clearance
C_{max}	maximum plasma concentration
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
CV%	Coefficient of variation as percentage
ECG	Electrocardiogram
ECL	electrochemiluminescence
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
E_{max}	Maximum effect attributable to the study drug
FAS set	Full analysis set
G-CSF	granulocyte colony-stimulating factor
G-CSFR	granulocyte colony-stimulating factor receptor
GeoMean	Geometric mean
GLSM	geometric least square mean
GMR	geometric mean ratio
HMWP	High molecular weight protein
ICH	International Conference on Harmonisation
IL-3	interleukin-3

IMP	Investigational medicinal product
IPC	in-process control
IRB	Institutional Review Board
ISR	injection site reaction
LDH	Lactate dehydrogenase
LLOQ	Lower limit of quantitation
MAA	Marketing authorization application
MDD	Medical Device Directive
MedDRA	Medical Dictionary for Regulatory Activities
MRD	minimum required dilution
MTT	Thiazolyl Blue Tetrazolium Bromide
NAB	neutralizing antibodies
NOAEL	no observed adverse effect level
PD	pharmacodynamics
PEG	polyethylene glycol
PFS	prefilled syringe
Ph. Eur.	European Pharmacopoeia
PK	Pharmacokinetic
PMN	polymorphonuclear leukocyte (neutrophil)
PP	Per protocol
PPQ	Process performance qualification
PT	preferred term
PVDF	polyvinylidene difluoride
QC	quality control
RBC	red blood cell
r-met-Hu-G-CSF	recombinant human granulocyte colony stimulating factor, or filgrastim
RPC	Reverse phase chromatography
s.c.	Subcutaneous(ly)
SAE	Serious adverse event
SAF set	Safety analysis set
SAP	Statistical Analysis Plan
SC	subcutaneous(ly)
SCP	screening assay cutpoint
SD	standard deviation
SDS-PAGE	Sodium dodecyl sulfate – polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
SFU	Safety follow-up
SOC	System Organ Class (MedDRA dictionary)
SPR	surface plasmon resonance
t½	terminal elimination half-life
TEAE	treatment-emergent adverse event
TK	Toxicokinetics
T_{max}	time to maximum plasma concentration
ULN	upper limit of normal
ULOQ	upper limit of quantitation
US	United States
USP	United States Pharmacopeia

Vz/F	apparent volume of distribution
WBC	white blood cells
λz	apparent first-order terminal elimination rate constant

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Accord Healthcare Limited submitted on 27 April 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Pelgraz, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 February 2014.

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 6mg/0.6mL, solution for injection in pre-filled syringe,
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22-08-2002
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/02/227/001-005

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

- Product name, strength, pharmaceutical form: Neulasta 6mg/0.6mL, solution for injection in pre-filled syringe,
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22-08-2002
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/02/227/001-005

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 6mg/0.6mL, solution for injection in pre-filled syringe,
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22-08-2002
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/02/227/001-005

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

The applicant received scientific advice from the CHMP:

Scientific advice	**date**	**Area**
EMEA/H/SA/2938/1/2014/III	20 November 2014	the scientific advice pertained to quality, non-clinical and clinical aspects

1.2. Steps taken for the assessment of the product

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

Rapporteur: Sol Ruiz Co-Rapporteur: Ondřej Slanař

The application was received by the EMA on	27 April 2017
The procedure started on	18 May 2017
The Rapporteur's first Assessment Report was circulated to all CHMP members on	10 August 2017
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	4 August 2017
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	18 August 2017
The CHMP agreed on the consolidated List of Questions to be sent to	14 September 2017

the applicant during the meeting on	
The applicant submitted the responses to the CHMP consolidated List of Questions on	3 April 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	4 May 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	17 May 2018
The CHMP agreed on a list of outstanding issues to be addressed in writing to be sent to the applicant on	31 May 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	26 June 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	13 July 2018
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 Pelgraz on	26 July 2018

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Pelgraz is indented to be used for the 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 Applicant claims the authorisation for Pelgraz as a similar product to Neulasta (EU) which was granted a marketing authorisation in the EU on 22 of August 2002. The proposed indication for CHS-1701 is the same as for the reference product Neulasta (EU).

2.1.2. Epidemiology

Chemotherapy-induced neutropenia and its subsequent infectious complications represent the most common dose-limiting toxicity of cancer therapy. Febrile neutropenia, FN, develops in 25% to 40% of treatment-naïve patients during common chemotherapy regimens depending on the patient population; the dosage, timing and type of chemotherapy used (Dinan 2015). The severity of febrile neutropenia depends on the dose intensity of the chemotherapy regimen, the patient's prior history of either radiation therapy or use of cytotoxic treatment, and comorbidities.

2.1.3. Biologic features

The principal regulator of physiological granulopoiesis human G-CSF is a glycoprotein that has been shown to regulate the production and release of neutrophils from the bone marrow, mediated via a single affinity extracellular receptor. By binding and signalling through granulocyte colony-stimulating factor receptor (G-CSFR), G-CSF has multiple effects on circulating neutrophils and on neutrophil precursors in bone marrow (Roberts, 2005).

Stimulation of precursor cell proliferation in the bone marrow leads to an increase in the total mass of G-CSFR-expressing cells, which serves as a negative regulator of G-CSF levels through accelerated clearance of G-CSF (Anderlini, 2008).

2.1.4. Clinical presentation

Chemotherapy-induced neutropenia is a significant dose-limiting toxicity in cancer treatment and a major risk factor for infection-related morbidity and mortality. Febrile neutropenia, FN, develops in 25% to 40% of treatment-naïve patients during common chemotherapy regimens depending on the patient population; the dosage, timing and type of chemotherapy used (Dinan 2015). The occurrence of febrile neutropenia often necessitates chemotherapy delays or dose reductions. It may also lengthen hospital stay; increase monitoring, diagnostic, and treatment costs; and reduce patient quality of life.

2.1.5. Management

Primary prophylaxis with colony-stimulating factors, CSFs, reduces the frequency of chemotherapy induced neutropenia, all-cause mortality during chemotherapy, and need for hospital care e.g. in breast cancer (Renner 2012, Cochrane Systematic Review). The administration of G-CSF can accelerate the development of neutrophils from committed progenitors, thereby reducing the incidence, duration, and severity of neutropenia (Dale, 2002). Forms of G-CSF such as filgrastim and lenograstim including biosimilars, are administered by a course of daily injections, whereas pegfilgrastim allows once-per-cycle administration and may avoid suboptimal daily dosing.

EORTC 2010 guidelines cover use of granulocyte-colony stimulating factor, G-CSF, to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. Prophylaxis with a CSF is recommended for:

- Specified chemotherapy regimens with >20% risk of FN
- Specified chemotherapy regimens with 10% to 20% risk of FN, subject to patient specific risk factors such as elderly age (≥65 years) and neutrophil count
- Patients with a previous episode of FN

Pegfilgrastim and filgrastim can accelerate neutrophil recovery, leading to a reduced duration of the neutropenic phase in patients receiving cytotoxic chemotherapy. Filgrastim was initially approved for the prevention of infection as manifested by febrile neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive chemotherapy. The pivotal study in patients with small cell lung carcinoma receiving cyclophosphamide, etoposide, and doxorubicin chemotherapy demonstrated an approximately 50% reduction in the incidence of febrile neutropenia and duration of Grade 4 neutropenia, as well as statistically significant reductions in the incidence of hospitalizations and IV antibiotic usage (Crawford, 1991). Subsequent indications for filgrastim included engraftment following bone marrow transplantation, mobilization of peripheral blood progenitor cells and engraftment following transplantation, induction or consolidation chemotherapy for acute myeloid leukemia, and

severe chronic neutropenia. Because of its relatively short half-life of 3.5 hours, filgrastim is administered once daily by SC administration no less than 24 hours after chemotherapy and continuing until absolute neutrophil count (ANC) recovery within each cycle of treatment. Shortcomings of filgrastim include the requirement for either daily visits to the clinic or home injections by the patient during the period of administration, frequent ANC monitoring, the possibility of missed doses, and suboptimal duration of treatment (either too short or too long). Efforts to overcome these limitations led to the PEGylation of the G-CSF protein. The subsequent PEGylation of the G-CSF protein filgrastim altered the pharmacokinetic (PK) profile, resulting in slower clearance and a prolonged half-life (between 15 and 80 hours), thus permitting a single injection per cycle of chemotherapy (Foley, 2009). Pegylation of filgrastim increases the size of filgrastim so that it becomes too large for renal clearance. Due to its high molecular weight, pegfilgrastim exhibits limited transport into the blood capillaries after SC administration and enters the systemic circulation via an indirect route, through the lymphatics.

With a long half-life and target-mediated clearance, pegfilgrastim remains in the circulation until the bone marrow neutrophil precursors start to come back after chemotherapy. Pegfilgrastim (Neulasta) was first authorized for marketing in the EU and US in 2002.

About the product

Pelgraz has been developed as a proposed biosimilar medicinal product to the reference product Neulasta licensed by Amgen Inc. in different jurisdictions including EU, Canada and the USA.

Pelgraz (pegfilgrastim) belongs to the family of medications known as Granulocyte Colony Stimulating Factors (G-CSF). It is derived from recombinant methionyl human granulocyte colony-stimulating factor (rHu-met-G-CSF), a 175 amino acid protein, by conjugating methoxy polyethylene glycol (PEG)-propionaldehyde (20 kDa) to the N-terminal methionine residue of G-CSF. Pegfilgrastim acts on hematopoietic cells by binding to specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation.

The indications claimed are as for the reference product (Neulasta):

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

Type of Application and aspects on development

As part of the global development program, Pelgraz has been developed as a proposed biosimilar to both the EU-approved and US-licensed Neulasta. Consistent with relevant guidances which were current at the time of product development, as follows:

- EMEA/CHMP/BMWP/42832/2005, February 2006
- EMEA/CHMP/BMWP/31329/2005, February 2006
- FDA Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product, February 2012
- FDA Guidance for Industry: Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product, February 2012

- FDA Draft Guidance for Industry: Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product, May 2014
- Health Canada Guidance For Sponsors: Information and Submission Requirements for Subsequent Entry Biologics (SEBs), March 2010
- EMA - 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. 2015; 1-7.

EMA - Annex to Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Non-clinical and Clinical Issues. Guidance on Similar Medicinal Products Containing Recombinant Granulocyte-Colony Stimulating Factor. EMEA/CHMP/BMWP/31329/2005. 2006; 1-4.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as a solution for injection containing 6 mg of pegfilgrastim (INN) as active substance. Other ingredients are: sodium acetate, sorbitol (E420), polysorbate 20 and water for injections. The product is available in a pre-filled syringe (type I glass) with a permanently attached stainless steel injection needle, with a needle safety guard and one alcohol swab.

Pelgraz has been developed as a biosimilar medicinal product to the reference product Neulasta. Multivariate analysis has been used to determine the proven acceptance ranges (PARs) for the manufacturing steps but no design space has been claimed. The name Pelgraz AS is used to describe the active substance (AS) in this application.

2.2.2. Active Substance

General information

Pelgraz active substance is an N-terminally pegylated form of the recombinant human granulocyte colony stimulating factor or filgrastim (rHu-met-G-CSF) expressed in *E. coli*.

Filgrastim has an α-helical structure with two intra-molecular disulfide bonds formed between cysteine residues at amino acids Cys37 – Cys43 and Cys65 – Cys75, with a single free cysteine at position 18. The disulfide bonds form loop-like structures that maintain the biologically active conformation of the protein. rHu-met-G-CSF is a protein of 175 amino acids which is identical to natural human G-CSF except for the presence of an additional methionine at the N-terminal end and the absence of glycosylation.

Pelgraz AS is formed from rHu-met-G-CSF by the covalent attachment of one linear PEG (polyethylene glycol) molecule with an approximate molecular weight of 20 kDa to the N-terminus (terminal methionine) by a secondary amine linkage. The structural properties of filgrastim with respect to its amino acid sequence, secondary structure etc. remains largely unaltered in mono-pegylated Pelgraz AS.

Manufacture, process controls and characterisation

Intas Pharmaceuticals Ltd (IPL). Biopharma Division, Plot no: 423/P/A, Sarkhej Bavla Highway, Village Moraiya, Taluka Sanand, Ahmedabad, Gujarat, 382213, India is responsible for AS manufacture and release, including cell bank production, testing and storage. An additional site of master cell bank (MCB) storage has been specified.

Description of manufacturing process and process controls

Cells from one vial of the working cell bank (WCB) are expanded in the seed media to prepare the culture which is used to inoculate the fermenter. Post fermentation the cells are lysed and inclusion bodies (IB) are prepared.

During downstream manufacturing, IBs are solubilised, refolded and processed through multiple chromatographic and filtration steps to purify filgrastim critical intermediate. The product stream is diluted to the desired concentration and 0.2 micron filter-sterilised into a USP Type I glass bottle.

Filgrastim critical intermediate (CI) is subjected to a pegylation reaction with a 20 kDa monomethoxy polyethylene glycol propionaldehyde (mPEGPAL). The pegylation reaction mixture after filtration is further purified through chromatographic steps to result in purified active substance (AS). The expected quantity of AS per batch has been defined.

The process is adequately described with the operating parameters (OP) - input variables and performance parameters (PP) - output variables, for each process step. Operating and performance parameters are suitably defined with their acceptance criteria. Sampling for in-process testing is sufficiently described. There have been questions during the procedure regarding the control of bioburden/endotoxin and the control strategy is now considered acceptable to avoid microbial contamination. However the applicant should review acceptance criteria/ action limits after the production of a specified number of additional commercial batches (recommendation). No reprocessing is applied during any stage of the AS manufacture.

The components of the container closure system for AS are described. The integrity of this container is established based on the results of a container closure integrity test and from AS stability data. A suitable leachables and extractables risk assessment was performed. The three components of the container closure system fulfil the requirements for these types of materials.

Control of materials

Compendial raw materials are tested in accordance with the specified pharmacopoeial monographs. Raw materials are categorised as critical or non-critical. All critical materials are compendial with the exception of one of the media components used for fermentation. All incoming materials are subjected to review by quality assurance prior to release for use. Vendor information for resins, filters, and containers used is provided.

Raw materials of biological origin that are used in cell bank and filgrastim intermediate material are discussed. See adventitious agents safety for discussion on TSE risk.

The filgrastim critical intermediate is produced from a G-CSF producing clone. The cDNA was synthesised based on human mRNA coding for G-CSF. During cloning and optimisation laboratory experiments, the pET11a expression vector and *E. coli* DH5α strain were used. *E. coli* BL21 DE3 host cells were transfected using a heat shock method and kanamycin was used as a selection agent.

A two-tiered cell bank system was prepared comprising the master cell bank (MCB) and a working cell bank (WCB) in line with ICH Q5D. Cell banks are stored at -80°C. MCBs and WCBs are characterised to

ensure identity, purity, viability and stability of the cell bank for its intended use. Periodic testing is done for the MCB and the WCB to check suitability of cell bank for commercial manufacturing. Characterisation results for each of the produced cell banks are provided. The panel of tested parameters is adequate and observed results are within specifications. Also end of production cells (EOPCs) have been tested and genetic stability was confirmed for cells at the limit of *in vitro* cell age. A protocol for establishment of new working cell banks has been provided.

Control of critical steps and intermediates

The AS manufacturing process performance and product quality control strategy includes: controls on material attributes, controls on the design of the manufacturing process, in-process controls, key/critical process parameters (inputs), performance parameters: key performance parameters (KPPs) and critical performance parameters (CPPs) (outputs) and controls on the AS. All unit operations in the filgrastim intermediate manufacturing process and the Pelgraz AS manufacturing process are considered to be critical steps.

A Failure Modes and Effects Analysis (FMEA) risk assessment was performed for the manufacturing process to identify any parameter that could impact product quality or process failure. Process parameters were classified from there. The in-process manufacturing controls (critical and key process parameters) are summarised for each unit operation. The control strategy designed by the applicant is considered adequate (See AS manufacturing process also for bioburden/endotoxin control).

Filgrastim is considered a critical intermediate and the information about its manufacture provided is acceptable.

The structural and functional characteristics of filgrastim have been investigated using a wide variety of analytical tools, including N-terminal sequencing, SDS-PAGE, Isoelectric Focusing (IEF), Western Blot, peptide mapping, mass spectrometry, and biological activity. A number of state-of-the-art techniques have been used to assess higher-order structure, including circular dichroism (CD), FTIR (fourier transformed infrared spectroscopy), analytical ultracentrifugation (AUC) and thermal stability by differential scanning calorimetry (DSC). In addition, the biological activity was assessed using two orthogonal procedures: an in vitro cell proliferation assay and a receptor binding assay. Five main impurities were identified by RP-HPLC and SE-HPLC during development of the filgrastim CI (oxidised forms 1 and 2, deamidated forms, dimers and aggregates). Filgrastim quality is suitably controlled via the specifications (release and stability). Appropriate tests for identity, purity, content and potency are included. Analytical procedures have been validated according to ICH guidelines or the relevant compendial references and have been demonstrated to be suitable for their intended use. Batch analysis and stability data of filgrastim CI are acceptable. None of the identified impurities in filgrastim CI has been observed to increase over the shelf life of filgrastim CI. The proposed storage condition for the filgrastim CI when stored in specified containers has been defined.

Activated PEG that is used for pegylation of filgrastim was originally presented as a starting material by the applicant. However, this approach was considered unacceptable during the procedure (major objection) and it was re-classified as a critical intermediate. The activated PEG (mPEG-PAL) is manufactured under GMP conditions in compliance with ICH Q7. The starting material is proposed as mPEG-OH. The manufacturing process has been elaborated in sufficient detail. The commercial scale and storage containers have been defined.

Data on specifications of the activated mPEG and the starting material, analytical methods for control of the starting material and intermediate and their validation, batch release and stability data of starting material and intermediate and storage conditions (-20°C ± 5°C) have been provided. The re-test period for mPEG-PAL has been defined. The activated PEG is released as per the accepted

specifications (release and stability), by SunBio (the mPEG-PAL manufacturer). The specifications include tests for identification, molecular weight, polydispersity, purity and impurities including volatile organic compounds and heavy metals.

Process validation

Appropriate process validation studies were performed during the development of the filgrastim CI manufacturing process to provide an understanding of the process and unit step operations and establishment of a control strategy.

For the manufacture of the Pelgraz AS from filgrastim CI, studies were performed for the establishment of normal operating ranges (NOR) for all parameters and multivariate analysis was used to determine the PARs for all parameters that could potentially affect product quality, which were evaluated in a risk assessment. The process performance qualification consists of three steps: process design, process validation and ongoing process verification.

Process design: Using process knowledge a strategy for process control was designed. A risk assessment by Failure Modes and Effects Analysis (FMEA) was then conducted which facilitated process characterisation studies.

Process validation for the pegylated AS was performed at the intended commercial scale at IPL and was designed to achieve initial qualification of the routine production process. The results indicate that all unit operations performed during manufacturing are qualified to ensure consistent quality of the product produced.

As part of ongoing process verification, additional validation studies were conducted post PPQ e.g. validation to support the inclusion of a bioburden reduction filtration step. Process validation studies comprised suitable evaluation of manufacturing component compatibility and the risk of leachables and extractables. The ability of the manufacturing process to control process related impurities, bioburden and endotoxin were determined.

Studies have been conducted to demonstrate that specified process intermediates and process solutions do not have significant changes in the desired quality attributes over an extended hold period and do not have any adverse impact on the performance of subsequent process steps and final product quality.

Appropriate resin reusability studies have been carried out at the proposed commercial scale. The applicant indicates that on-going validation will be performed to ensure acceptability of resin/membrane lifetime, cleaning/regeneration procedures and sanitisation and storage procedures. No shipping validation was considered necessary since the Pelgraz AS and finished product (FP) are manufactured at the same site.

Manufacturing process development

The Pelgraz AS manufacturing process development has encompassed changes in both scale and process at the manufacturing site. The commercial product manufacturing process was developed in parallel with clinical development programme. Several changes have been introduced during the development of the manufacturing process. Analytical comparability of AS obtained from different processes has been demonstrated. The details of the batches manufactured to date are provided.

The applicant has conducted multivariate analysis to determine the PARs for each of the manufacturing steps and confirmed that no formal design space is claimed.

Characterisation

The characterisation of Pelgraz AS was performed using various batches of AS/FP and Neulasta. A series of orthogonal methods were used to elucidate the structure and other characteristics of the product, including: primary structure (N-terminal sequence, amino acid composition, peptide map ultra-violet mass spectrometry (UV/MS), pegylation site and linkage, electrospray ionisation mass spectrometry (ESI-MS), western blot, SDS-PAGE (non-reduced silver and iodine stain), higher order structure (circular dichroism (CD), fourier-transform infrared spectroscopy (FTIR), intrinsic fluorescence, free cysteine estimation), purity (size exclusion high-performance liquid chromatography (SE-HPLC), analytical ultracentrifugation (AUC), size exclusion chromatography - multi-angle laser light scattering (SEC-MALS), reverse-phase high-performance liquid chromatography (RP-HPLC), cation exchange chromatography HPLC (CEx-HPLC), and biological activity (*in vitro* biological activity, receptor binding assay).

The primary structure analysis revealed that the correct amino acids are present in the correct sequence and a single PEG molecule is attached to the N-terminal methionine by a secondary amine linkage. The higher order structure of the AS was evaluated confirming a three dimensional structure similar to the reference product Neulasta, with predominant α-helical content and a free cysteine at position 18. The overall size, charge, and hydrophobicity of the molecule was also confirmed. The level of impurities in the AS is low. The functional assays used to assess the biological activity of Pelgraz AS include an *in vitro* murine myeloblastic cell line based proliferation assay and a receptor binding assay (see AS analytical methods section).

Product related impurities of Pelgraz AS include those related to the filgrastim CI (formylated-methionine filgrastim, partially-reduced forms, oxidised forms, norleucine forms, N and C-terminal-clipped species). Pelgraz AS product related impurities included di-pegylated forms, oxidised forms, non-pegylated forms, high molecular weight (HMW) forms (dimers and aggregates) and deamidated forms. In general, the level of these impurities in the AS is very low, controlled, and only a (within specification) increase is observed during real-time storage of the FP over the 36 month proposed shelf life.

The applicant provides data indicative of the removal of the process-related impurities (see process validation section). Regarding host cell protein (HCP) and host cell DNA, they are also controlled during production of filgrastim CI, and validation clearance studies were performed. In conclusion, process and product related impurities are adequately discussed. All specified impurities have been present in product used in trials.

Specification

The proposed release specification for Pelgraz AS has been provided with information on the analytical methods used for control of AS. This includes general tests, biological activity, identity tests, quantitation, tests for purity and impurity and safety tests.

Shelf-life specifications include the same tests and specifications as for release except certain tests are omitted. The proposed AS release and stability specifications were established based on information from the entire process history from early development as well as data generated on US-licensed and EU-approved Neulasta.

In general, the specifications initially proposed had complex acceptance criteria (particularly for the chromatographic methods) but, upon request, some were modified. In addition, specifications for detection of sodium cyanoborohydride and free PEG content were implemented.

The specification for potency is broader than expected from the batch release results available and the applicant was requested to narrow the acceptance criteria or justify. The justification provided for the specification has been accepted although a review of the specification, if possible, after the analysis of a specified number of additional batches has been requested (recommendation).

The applicant has justified the current specifications with appropriate statistical evaluation during the procedure and additionally committed to re-evaluate the specifications for potency and purity when more manufacturing experience is reached and commits to submit a variation if changes are implemented.

Analytical methods

The same methods are used for release, stability and in-process analysis of AS and suitable descriptions have been provided.

Compendial methods are considered validated and only qualification for their application in the context of the AS was performed. Validation data (in line with ICH guidance) for non-compendial analytical methods developed in-house and used for release, stability and in-process control are provided. Overall, qualification and validation data provided are deemed acceptable.

The functional assays used to assess the biological activity of Pelgraz AS include an *in vitro* cell based proliferation assay and a receptor binding assay.

- *In vitro* cell based proliferation assay: The potency was determined by an in-house procedure similar to the specific activity procedure from the filgrastim concentrated solution (2206) monograph in the current edition of the Ph.Eur. The method is a cell proliferation assay. It utilises the ability of a murine myeloblastic cell line to proliferate in the presence of pegylated filgrastim in a dose dependent manner. The cells are incubated with varying concentrations of pegylated filgrastim reference standard and test sample. The dehydrogenase enzyme of viable cells reduces an added reagent into a coloured formazan product that is soluble in tissue culture medium and can be measured spectrophotometrically. The absorbance value is directly proportional to the number of viable cells in the culture and hence, to the concentration of pegylated filgrastim applied. Biological activity of test sample is determined by comparing its response to that of reference standard.
- *Receptor binding assay:* This procedure evaluates the receptor binding of Pelgraz AS to the G-CSF receptor.

Batch analysis

Batch analysis data of the active substance were provided. The results were within the specifications and confirm consistency of the manufacturing process. Batch analysis data for an appropriate number of AS batches manufactured according to the commercial process and batches made from the previous manufacturing process are provided. All batches comply with the specification in place at the time of release.

Reference materials

The previously used and current reference standards are described. The stability of the used reference standards is monitored and shelf-life was provided.

Two in-house standards have been used for the analysis of Pelgraz AS at different development stages. A primary reference standard (PRS) and a secondary reference standard (SRS) which have been used to support release and stability testing of the AS and FP. The quality attributes of the PRS and the SRS

have been evaluated by a comprehensive analytical comparability package that includes release, characterisation analysis and stability data.

The specification for release and characterisation for qualification of new reference standards and the plan for testing stability of new reference standards is provided. Overall the strategy followed for determination of suitability of reference standards is considered adequate.

Stability

Shelf-life and storage conditions have been specified for the AS. The stability data arising from samples stored at the intended storage, accelerated and stressed conditions have been assessed in compliance with ICH Q5C and ICH Q1E to support the proposed shelf-life of the AS (commercial process).

Justification for the shelf life is provided by a stability study with a suitable number of PPQ batches which indicate that each attribute remained within its pre-specified acceptance criterion at the proposed storage conditions in the commercial container closure system. In addition, stability testing was carried out under accelerated storage conditions (25 ± 2 °C) for 6 months and stressed storage conditions (40 ± 2 °C) for up to 7 days. There were no significant changes observed for any attribute.

A photostability assessment was also performed on Pelgraz AS in which samples were exposed to conditions per ICH Q1B and satisfactory data was provided in the application recommending its storage in a dark place thereby protecting from direct light exposure. The stability results indicate that the active substance is sufficiently stable and justify the proposed shelf life in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The FP is comprises 6 mg of Pelgraz AS in an acetate buffered isotonic solution for injection, filled in a 1 mL glass (Ph.Eur Type I), single-use, pre-filled syringe (PFS) with stainless-steel needle with a needle-guard for administration via subcutaneous (s.c.) injection and one alcohol swab (a declaration of conformity to the Medical Device Council Directive 93/42/EEC has been provided). The safety device has been assessed as suitable for the intended purpose. The container-closure components comply with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. Container usage is supported by results of the performed compatibility, safety (including extractable and leachable) and container-closure integrity studies.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are neither novel excipients used in the finished product formulation nor any excipients of human or animal origin.

The quantitative composition of the FP is shown in Table 1. There is no overage and the PFS is filled with a 0.65 mL target fill volume to ensure an extractable volume of 0.6 mL at the time of administration.

Table 1 Quantitative Composition of Pelgraz Finished Product

Ingredients	Quantity	Function	Supplier Reference to Quality Standard[1]
Pelgraz AS	6 mg	Active Pharmaceutical Ingredient (API)	IPL's (Intas Pharmaceutical Limited) In-house Specification
Glacial Acetic Acid	0.35 mg	Buffering Agent	Multi-Compendial:USP[2], BP[3]/ Ph.Eur.[4], JP[5]
Sorbitol	30 mg	Tonicity Agent	Multi-Compendial: NF[6], BP[3]/ Ph.Eur.[4], JP[5]
Polysorbate-20	0.024 mg	Stabiliser	Multi-Compendial: NF[6], Ph.Eur.[4], JP[5]
Sodium Hydroxide	0.034 mg	Buffering Agent	Multi-Compendial: NF[6], BP[3]/ Ph.Eur.[4], JP[5]
Water for Injection	0.6 mL	Diluent	Multi-Compendial: USP[2], Ph.Eur.[4]

Note that the SmPC lists sodium acetate as an excipient. Sodium acetate is formed by titrating glacial acetic acid with sodium hydroxide (shown in the table above).

The FP formulation was established based on knowledge of Neulasta's formulation excipients and concentrations. The applicant has presented published information on formulation development from Neulasta, including forced degradation studies. The applicant has also performed some formulation development studies. The final formulation mimics the one published for Neulasta.

During its development, the FP underwent one designated process change to the proposed commercial process. The current process resulted from changes that occurred in the manufacturing process, including changes related to scale up, formulation buffer constituents, filtration of the formulation buffer, changes to the filter vendor and filter priming.

The commercial process was used to manufacture the FP used for clinical trial studies, biosimilarity, stability and method validation. Comparability data including process performance parameter data show that the commercial process is comparable to the earlier process.

Manufacture of the product and process controls

Intas Pharmaceuticals Ltd. Biopharma Division, Plot no: 423/P/A, Sarkhej Bavla Highway, Village Moraiya, Taluka Sanand, Ahmedabad, Gujarat, 382213, India is responsible for FP manufacture. Additional sites for FP storage and secondary packaging, and importation into the EU are specified. Accord Healthcare Ltd, 319 Pinner Rd, Harrow, UK is specified for EU batch release.

The FP manufacturing process includes: preparation of filtered formulation buffer; preparation of formulation bulk solution; sterile filtration of formulated bulk solution; filling; visual inspection;

[1] These references to quality standards are reflective of the suppliers' certificate of analysis. In addition, Intas Pharmaceuticals Ltd., performs testing to meet both USP and Ph.Eur. requirements.
[2] USP: United States Pharmacopoeia
[3] BP: British Pharmacopoeia
[4] Ph. Eur.: European Pharmacopoeia
[5] JP: Japanese Pharmacopoeia
[6] NF: National Formulary

labelling; needle safety device and plunger rod fixing and packaging. No reprocessing is claimed by the applicant.

The FP control strategy is based on a planned set of controls, derived from current product and process understanding that assures process performance and product quality. The manufacturing process is well controlled at all levels. Process parameters (both operating and performance parameters) are well defined and their categorisation is considered acceptable. The maximum batch size has been specified.

A written protocol that specifies the manufacturing conditions, controls, testing, and expected outcomes was generated, approved, and executed for the Process Performance Qualification (PPQ) component of the Process Validation for the FP. The PPQ was designed to achieve initial qualification of the proposed commercial production process using three consecutive FP batches. Other validation support studies included: manufacturing component compatibility evaluation, validation of buffer and product intermediate hold times, filter validation, media fill simulation, validation of sterilisation processes and shipping validation / cold chain validation. For shipping, a qualification master plan has been prepared but the performance qualification will only be performed with product shipments under the normal supply chain. This is acceptable.

The FP manufacturing process does not increase or modify any product related degradants. Potential contaminants that might be introduced in the FP manufacturing process are microorganisms, bacterial endotoxins, and adulterating particles. All of these are controlled by the manufacturing environment and filling regime employed and are monitored by testing prior to release of the FP.

A risk assessment has been conducted to identify the potential sources in the process where elemental impurities can be potentially introduced. A risk assessment for elemental impurities in line with ICH Q3D guidance was conducted. Overall the risk of exposure to elemental impurities to the patient is anticipated to be negligible.

Product specification

The proposed release and stability specification for finished product has been provided. The panel of tests proposed covers the main characteristics of the product and includes appropriate tests for purity, identity and potency. Shelf-life specifications include stability-indicating methods and are the same tests and specifications as for release except certain tests are omitted (extractable volume, osmolality, peptide-mapping, SDS-PAGE with iodine stain, immunoblotting) and an additional test, i.e. dye ingress test, included. In general, the panel of tests originally proposed covered most of the main characteristics of the product. However, a specific test to measure impurities of non-protein origin (i.e. PEG) was considered critical for the control of FP and was asked to be included, unless appropriately justified. A method to test free PEG has now been developed and the validation data presented with the day 180 response. The applicant has been recommended to review the specifications with additional manufacturing experience and stability (recommendation).

Regarding acceptance limits, the applicant claims to have set them based on manufacturing experience. However, for some tests, the originally proposed limits did not seem to be justified based on experience since they did not cover the observed variation reflected by either the minimum/maximum values or any statistical approach. Thus, the applicant was requested to refine the acceptance limits for a number of tests or further justify the proposals. The applicant used the data from all lots employed in biosimilarity studies and the specifications for SDS-PAGE, RP-HPLC, SE-HPLC, and CEx-HPLC have been revised accordingly.

Current specifications for protein concentration and potency are justified considering the available manufacturing experience.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines. Aside from the tests for physical characteristics, sterility and polysorbate 20, all other tests are used for both AS and FP.

Batch analysis

A suitable number of batches produced by the current manufacturing process have been successfully released. Results are well within acceptance criteria and confirm consistency of the manufactured material.

Reference materials

FP is released against the same reference standards and control materials described for AS.

Stability of the product

The proposed FP shelf-life in the proposed container-closure system is 36 months when stored at 5 ± 3 °C. The stability data arising from samples stored at the intended storage, accelerated and stressed storage conditions have been assessed in compliance with ICH Q5C and ICH Q1E and justify the proposed shelf-life of the FP using the proposed commercial manufacturing process of up to 36 months when stored at 5 ± 3 °C. Supporting data of an appropriate number of lots stored in the proposed commercial container, include

i. 36 months of real time stability of developmental process (as supportive data); reproducibility batches,

ii. clinical batches and–PPQ batches.

The results indicate that each attribute remains within its pre-specified acceptance criterion.

Stability data for FP manufactured using the current commercial manufacturing process at the accelerated condition of 25 ± 2 °C for up to 6 months and at the stress condition of 40 ± 2 °C for either 7 or 28 days are also provided. Thus, within its shelf-life and for the purpose of ambulatory use, it is proposed that Pelgraz may be removed from the refrigerator and stored at room temperature (not above 25°C) for one single period of up to 72 hours. After 72 hours the product should be discarded (see SmPC).

Three PPQ batches of FP were included in a photostability study which as for AS, require the FP to be protected from light. FP is stored in secondary packaging (blister-packaged and then packaged in a carton). The applicant was requested to provide supportive data for the claim in the SmPC “Accidental exposure to freezing temperatures for a single period of less than 24 hours does not adversely affect the stability of Pelgraz.” and “Excessive shaking may aggregate pegfilgrastim, rendering it biologically inactive”. Data to support both statements have been provided.

The data support the proposed shelf-life and storage conditions.

Adventitious agents

One specified material of biological origin is used in the production of the filgrastim CI MCB and WCB. The manufacture of filgrastim CI utilises three specified raw materials of biological origin. Copies of the

certificates of origin are provided. The manufacture of AS from filgrastim CI does not utilise any excipient of biological origin.

The manufacture of FP utilises one excipient of biological origin, polysorbate 20, which is not animal derived. A copy of the statement from supplier confirming polysorbate is manufactured from materials of non-animal origin is provided.

Although few materials of animal origin are used, based on the information provided by the raw material suppliers (certificate of analysis), these raw materials have no detectable infectivity with regards to BSE (Bovine Serum Encephalopathy) and TSE (Transmissible Spongiform Encephalopathy) risk and are therefore regarded as TSE/BSE free. These raw materials also do not pose a viral safety risk.

Biosimilarity

Studies have been conducted to confirm biosimilarity of Pelgraz to its reference medicinal product, EU-approved Neulasta. Additionally, these studies are used as bridging studies comparing Pelgraz, EU-approved Neulasta and US-licensed Neulasta products. US authorised Neulasta was used in the non-clinical and clinical development programme.

Initial analytical similarity assessments were performed on AS and FP against Neulasta (EU authorised and US authorised) as a part of product characterisation prior to the initiation of the clinical studies; Further similarity studies were conducted with the clinical batches that are representative of the proposed commercial process.

Several biosimilarity studies have been conducted to evaluate a variety of attributes of EU-approved and US-licensed Neulasta and Pelgraz, including identity (N-terminal sequencing, amino acid composition, peptide map analysis, PEG linkage analysis, SDS-PAGE non-reducing (silver and iodine stain) and western blot), structural characterisation (extinction coefficient, polydispersity, CD, FTIR, 1D and 2D NMR, DSC, fluorescence spectroscopy, free cysteine estimation, disulphide bond analysis, LC/ESI-MS, SEC-MALS, HDX, and biophysical analysis), purity and impurity profiles (SE-HPLC, AUC, SEC-MALS/FFF-MALS, RP-HPLC and CEx-HPLC), biological activity (in vitro biological activity assay and receptor binding assays, flow cytometry and SPR based), particle analysis, pharmaceutical property analysis and biosimilarity of the stability profiles.

Details of the specific lots of Neulasta (from EU and USA) and Pelgraz FP used in the biosimilarity studies are provided in the dossier. The age of the lots at the time of use is variable.

The Pelgraz, EU-approved and US-licensed Neulasta were generally found comparable in terms of identity, structural features and biological activity. Regarding the purity and impurity profiles, a higher percentage of impurities were observed in Neulasta as compared to the Pelgraz FP, however, the nature and type of impurities were similar. The number of batches used for the biosimilarity studies was initially very limited and after the assessment of the information provided at the time of the MAA submission, a major objection concerning biosimilarity was raised concerning the issues described below.

To address questions concerning the bridge between the reference product (EU-approved Neulasta) to the non-EU comparator (US-licensed Neulasta) used in clinical studies and the numbers of batches studied, the applicant included additional similarity studies including additional EU-approved Neulasta lots and conducted a comprehensive similarity assessment. Furthermore, data have been included within these assessments to appropriately establish a scientific bridge between EU-approved Neulasta and US-licensed Neulasta. The data evaluation was based on criticality ranking. For the attributes with

"high" criticality ranking, a high number of lots were used and statistical approaches to demonstrate equivalence of Pelgraz and Neulasta were employed. For attributes categorised as "lower/lowest", similarity was established for quantitative attributes by comparison of minimum to maximum range observed for the test products and for qualitative attributes based on overlays or profiles. The results of all tests demonstrate that Pelgraz is highly similar to EU-approved Neulasta and US-licensed Neulasta in all attributes studied.

Comparative stability studies were performed with Pelgraz FP, EU-approved Neulasta and US-licensed Neulasta at accelerated (25 ± 2 °C, up to 6 months) and stressed conditions (40 ± 2 °C, up to 28 days). Based on results from these studies and trend analysis, it was concluded that the stability profiles of Pelgraz and both the US-licensed and EU-approved Neulasta are comparable.

To address the issue of the suitability of analytical methods, the applicant provided method qualification and validation exercises for all analytical methods used in the biosimilarity studies to demonstrate the appropriateness for intended use.

In conclusion, from a quality point of view, Pelgraz is considered similar to Neulasta (EU). In addition, the non-EU comparator (Neulasta (US)), used in pivotal preclinical and clinical studies, has been shown to be representative of the EU reference medicinal product. Biosimilarity has been demonstrated on an adequate number of batches and using a comprehensive panel of analytical methods (see Table 2-5).

Table 2 Methods used to characterise and compare Pelgraz and Neulasta

Molecular parameter	**Attribute**	**Methods for control and characterisation**	**Key findings**
Primary structure	N terminal sequence	Edman degradation technique	Identical amino acids
	Amino acid composition	Amino acid analysis	Molar ratios of amino acids are comparable
	Extinction coefficient determination		Experimentally determined values are comparable
	Amino acid sequence	Peptide map analysis – UV detection	Comparable peptide map profiles
		Peptide map analysis-MS/MS	Identical amino acid sequence
	PEG Linkage analysis	NMR	Identical linkage between the N terminal methionine and PEG
		Upon acid and base treatment using SDS-PAGE with Iodine staining	Comparable stability of the Peg linkage
	Identity	Western Blot	Comparable profiles
	Intact mass	LC-MS	Comparable molecular mass
	Protein/PEG	LC-MS	Comparable molecular mass and

	molecular weight and polydispersity		polydispersity
		SEC-MALS	Comparable molecular mass and polydispersity
Higher order structure	Disulfide bond analysis	Reducing and non-reducing peptide map analysis- MS/MS	Identical disulphide bridging pattern
	Free Cysteine Estimation	Ellman's assay	Comparable moles of cysteine present per mole of Pegfilgrastim
	Secondary and tertiary structure	Circular Dichroism (CD) Spectroscopy – far UV	Comparable CD spectra
		FTIR	Comparable FTIR spectra and ratio of Amide I / Amide II
		1D and 2D NMR	Comparable NMR profiles
		Differential Scanning Calorimetry (DSC)	Comparable Tm values
		Fluorescence Spectroscopy	Comparable emission spectra
		Hydrogen-Deuterium Exchange (HDX)	Comparable 'fingerprint' like pattern of incorporation of deuterium over the length of amino acid sequence
	Biophysical testing	Viscosimetry, ATR-FTIR, URT, UV-Vis absorbance, Nile red microscopy and particle flow imaging	Comparable
Content	Protein Concentration	UV/ Vis spectroscopy	Comparable Protein Concentration

Table 3 Physico-chemical characterization of heterogeneity and stability indicating degradation products

Molecular parameter	Attribute	Methods for control and characterisation	Key findings
Size	Sedimentation coefficient	Analytical Ultracentrifugation	Comparable monomer (S) and high molecular weight species
	Mass and aggregates	SEC-MALS and FFF-MALS	Comparable molecular weight distribution of protein and PEG+protein
	Aggregates and	SDS PAGE (Non-Reducing; silver stain)	Comparable molecular weight profiles

	fragmentation		
	HMW and LMW	SE HPLC	Comparable HMW and LMW contents
	Free m-PEG	SDS-PAGE (non-reducing; iodine stain)	Comparable free m-PEG
Hydrophobicity	Hydrophobic Variants	RP HPLC	Comparable impurity contents
Charge	Charged variants	CEx-HPLC	Comparable charged variants contents
Amino acid modifications	Deamidation	LC -ESI-MS and MS/MS	Comparable deamidated species.

Table 4 Functional characterization

	Test	**Method / cell line**	**Key findings**
In vitro Biological Activity	Proliferation assay	M-NFS-60 cell based assay	Equivalent potency
Binding assay	Cell based receptor binding assay	Flow cytometry	Comparable binding to G-CSFR present on human granulocytes
	Soluble receptor binding assay	SPR	Comparable KD

Table 5 Particle analysis and pharmaceutical properties

Molecular parameter	**Test**	**Key findings**
Particle analysis and pharmaceutical properties	PFI	No relevant differences
	DLS	No relevant differences
	Sub visible particles	No relevant differences
	Visual appearance	No relevant differences
	Extractable volume	No relevant differences
	pH	No relevant differences
	Osmolality	No relevant differences

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Active Substance

The description of the manufacturing process is adequate and questions raised during the procedure have been suitably resolved. Filgrastim is considered a critical intermediate and is the AS of an authorised product in the EU. There were questions during the procedure regarding bioburden and endotoxin control and although suitable action/acceptance limits have been agreed, the applicant should review these with additional manufacturing experience of filgrastim CI and Pelgraz AS (recommendation).

Activated PEG that is used for pegylation of filgrastim was presented as a starting material by the applicant. However, re-classification of this material as an intermediate was required and was raised as a major objection. In response to this MO, the applicant provided new information and all the issues concerning mPEG-PAL have been resolved.

The ability of the manufacturing process to remove mPEG-PAL and sodium cyanoborohydride has been tested in a scale-down-model and was confirmed in PPQ batches. Specification for free PEG has been implemented for AS and FP release.

In general, the specifications proposed had complex acceptance criteria but now the applicant has modified some, upon request. The free PEG content specification will be reviewed when additional stability data are available from a specified number of lots (recommendation).

The specification for potency is broader than expected from the batch release results available and the applicant was requested to narrow the acceptance criteria or otherwise justify (See AS specification section). The applicant has provided justification to support approval of this limit although has been asked to revise and restrict the specification if possible, when data of additional batches are available (recommendation).

Finished Product

Two manufacturing processes have been used for the FP during development but the changes between them were not critical. The applicant was asked to further justify the acceptance criterion proposed for free PEG content. This specification should be reviewed when additional data are available (see recommendation and also FP specification section for more details). In relation to the FP stability studies, several questions were raised. These issues have been satisfactorily resolved.

Biosimilarity

A MO was raised on biosimilarity. To address issues concerning the non-EEA comparator and number of batches needed in the study, the applicant performed additional biosimilarity studies, with additional lots of Pelgraz, EU-approved Neulasta and US-licensed Neulasta and co

nducted a comprehensive similarity assessment. Data also permitted revision of some FP specifications and data supporting adequate qualification/ validation for methods used were submitted. The results of all tests demonstrate that Pelgraz is highly similar to EU-approved Neulasta and US-licensed Neulasta in all attributes studied and established a scientific bridge of EU-approved Neulasta to US-licensed Neulasta. In conclusion, from a quality point of view, Pelgraz is considered similar to Neulasta (EU). In addition, the non-EU comparator (Neulasta (US)), used in pivotal preclinical and clinical studies, has been shown to be representative of the EU reference medicinal product.

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. Data 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 has recommended three points for investigation as stated in the report.

2.3. Non-clinical aspects

2.3.1. Introduction

Pelgraz has been evaluated in non-clinical in vitro and in vivo studies in order to show comparability of the test and the reference product Neulasta EU and Neulasta US. Biological potency was evaluated in proliferative biological assay and receptor affinity in 2 receptor binding assays (flow cytometry and surface plasmon resonance). Apart from the in vitro data, the similarity of both medicinal products was evaluated in vivo studies using neutropenic rodent model. The repeat-dose toxicology with toxicokinetics study (Study 410.120.1797), comparative local tolerance study in New Zealand White rabbits (Study 410.542.4251), and comparative sensitization study in Guinea Pigs (Study 410.552.4252), as well as the pharmacology study (Study BIO EF 697) were conducted in accordance to Good Laboratory Practice (GLP).

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro studies

Biological potency

A cell proliferation assay that utilises the ability of a murine myeloblastic cell line to proliferate in the presence of pegylated filgrastim in a dose dependent manner was used.

Pelgraz (test product) had the relative potency of 101 ± 5.18 % (average ± SD) to reference standard. EU-approved and US-licensed Neulasta had similar values of 99 ± 5.22 % and 100 ± 5.50 % respectively. Considering that the assay is representing mechanism of action and is highly critical for assessment, an equivalence approach i.e. similarity is concluded when the 90 % confidence interval for the difference in means between the products is contained within an equivalence acceptance criterion of ±1.5 times the standard deviation of the dataset for the reference product lots tested (-1.5*σR, 1.5*σR) was adopted. For the statistical analysis, upper and lower biosimilarity limits are set using the EU-approved Neulasta and US –licensed Neulasta as shown in table below.

Table 6 Equivalence testing results for the relative potency by in vitro bioassay

Comparison	Equivalence margin, %	Difference in Mean	90% confidence interval for mean difference, %	Equivalent
Pelgraz and EU-approved Neulasta	(-7.83, +7.83)	-1.63	(-5.13, 1.88)	Yes
Pelgraz and US-licensed Neulasta	(-8.25, +8.25)	0.24	(-2.84, 3.31)	Yes
EU- approved Neulasta and US-licensed Neulasta	(-7.83, +7.83)	-1.39	(-4.81, 2.03)	Yes

Therefore, based on these data Pelgraz is highly similar to EU-approved Neulasta and US-licensed Neulasta in terms of *in vitro* potency.

Receptor binding assay (Suface Plasmon Resonance)

Surface Plasmon resonance was used as an alternative receptor binding assay to confirm the binding affinity as well as to probe the receptor kinetics (k_{on} and k_{off}, and binding constant (K_D)).

For assessment of binding affinity to G-CSFR using SPR, equilibrium dissociation constant (KD) was considered. The KD values (mean ± σ) for Pelgraz , EU-approved Neulasta and US-licensed Neulasta are 1.5E-10 ± 5.5E-11 M, 2 .9E-10 ± 7.3E-11 M and 1.8E-10 ± 6.3E-11 M respectively. Based on mean and population standard deviation of Reference product Pelgraz showed highly similar binding affinity to Neulasta.

Receptor binding assay using human granulocytes

To access the binding of Pelgraz and reference product to GCSF receptor present on human granulocytes a flow cytometry based method was used. The % relative binding activity values (mean ± σ) for Pelgraz, EU-approved Neulasta and US-licensed Neulasta are 101 ± 3.61 %, 98 ± 6.31 % and 100 ± 3.01 % respectively. Based on mean and population standard deviation of Reference product Pelgraz showed highly similar binding affinity to Neulasta.

In vivo studies

The applicant performed two comparative pharmacodynamic studies using the neutropenic rodent model.

Comparative in vivo Efficacy Study of Pegfilgrastim Drug products following subcutaneous administration to neutropenic swiss albino mice, Study BIO-EF 697

The study was performed with products intended for commercial use. The study was performed in accordance with OECD principles of GLP.

Swiss Albino mice were divided into 23 groups (10 animals per group). Two batches of each reference product (Neulasta EU and Neulasta US) and 3 batches of the test product were used in dosing schedule. Study contained untreated control (saline, no CPA) and vehicle (treated with CPA on D1) control groups. Neutropenia was induced by injecting CPA at the dose of 100 mg/kg BW intraperitoneally. 24 hours after the induction of neutropenia the test items (or vehicle) were

administrated through the subcutaneous route. All analyses and comparisons were evaluated at the 95% level of confidence.

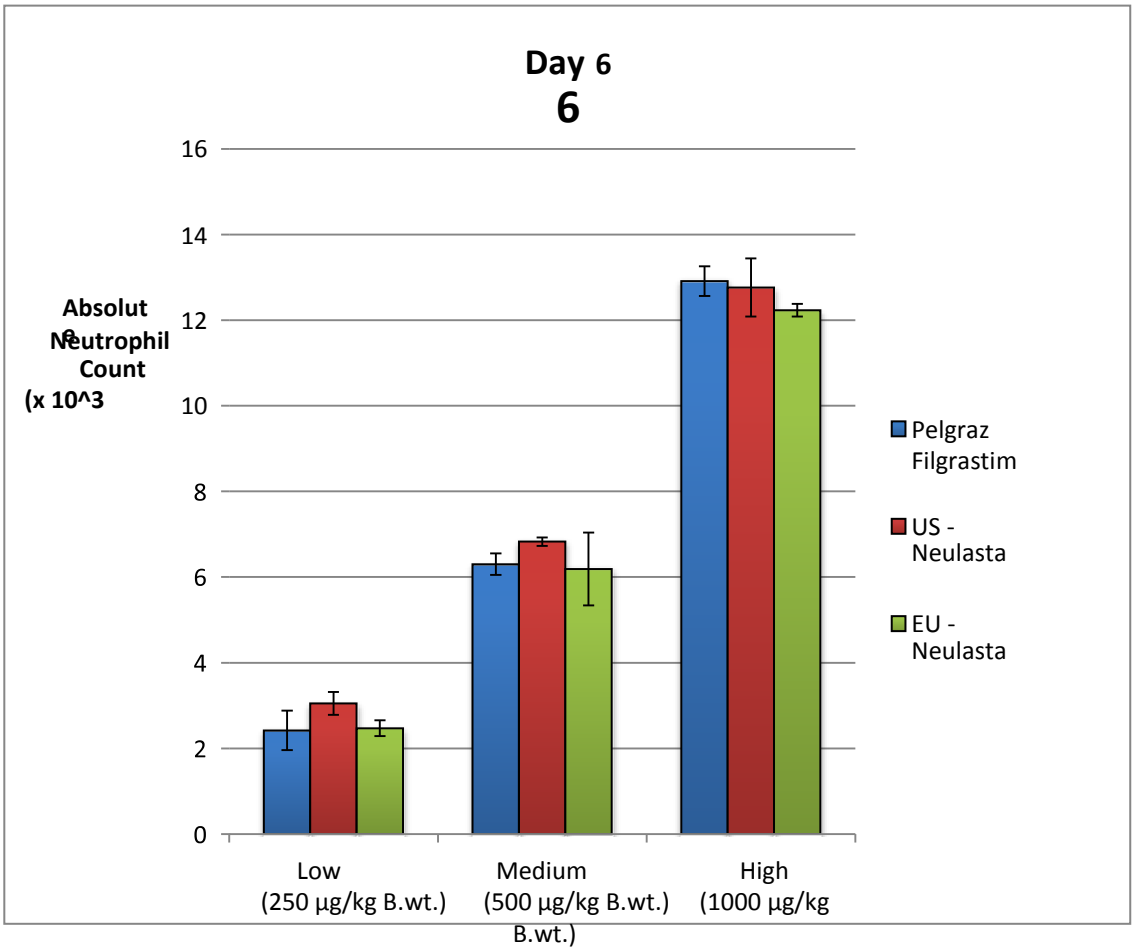


Figure 1 Absolute neutrophil count (x 10^3 cells/µL) on study day 6 (5 days after the neutropenia induction and 4 days after the treatment items administration)

Parallel-Line Analysis summary for the assessment of potency on day 6 demonstrated that the potency of the three Pelgraz batches in relation to pooled results from the two US licensed Neulasta batches and two EU-approved Neulasta batches are shown in Table 7.

Table 7 Study BIO-EF-697 Potency of Pelgraz Relative to Neulasta

Pelgraz	Relative potency to US-licensed Neulasta	Relative potency to EU-approved Neulasta
Batch No. G5030001	0.953	1.032
Batch No. G503CT0002	0.942	1.024
Batch No. P05FC-0003	0.967	1.048
Mean	0.954	1.035

A study evaluating the restoration of neutrophil blood cell counts by Pelgraz vs. Neulasta EU and Neulasta US in neutropenic female Balb/C mice (Study report 410.419.1796) showed similar results.

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 studies evaluating pharmacodynamic drug interactions (see non-clinical discussion).

2.3.3. Pharmacokinetics

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

2.3.4. Toxicology

Single dose toxicity

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

Repeat dose toxicity

Table 8 Repeat-dose toxicity studies

Study ID	Species/Sex/ Number/Group	Dose/Route	Duration	NOEL/ NOAEL (mg/kg/day)	Major findings
410.120.1797, GLP	Rats 10/sex/group	30, 180, 1100 µg/kg every 3 days, SC	28 days, 14 days recovery		Similar to reference product

Repeated dose toxicity study in rats (410.120.1797, GLP)

Wistar rats were treated with Pelgraz (30, 180, 1100 µg/kg) or EU-approved Neulasta (30, 1100 µg/kg). Animals were injected SC once every three days for a period of 28 days (a total of 10 doses). A recovery period of 14 days was established.

The endpoints of this study were mortality, clinical signs, body weight, food consumption, haematology, coagulation parameters, clinical chemistry, TK, anti-drug antibodies, serum IgG and IgM, urinalysis, ophthalmology, gross pathology, organ weights, histopathology.

No treatment-related mortality occurred during the study. Two animals died during anesthesia for blood sampling.

Clinical findings attributable to treatment included a dose-related, partially reversible increase in the incidence, duration and severity of swelling of the joints of the animal's legs and/or paws. Associated findings were limping, assumption of a relieving posture and lying in a ventral position. The incidence and intensity of these findings also increased in a dose-dependent manner. In addition, piloerection, indicative of stress, and yellow urogenital staining, due to impaired mobility, increased in prevalence and intensity in a dose-dependent manner during the treatment period. The Pelgraz and the EU-approved Neulasta treatment groups exhibited these signs in a comparable manner at the low and high doses. At the end of the recovery period there was only partial recovery of joint swelling and associated difficulties in mobility.

Slight to moderate impairment of body weight gain and reduction in food consumption was observed at the highest dose, more prominently in males.

Haematology: treatment with Pelgraz resulted in a marked to severe and dose-dependent increase of white blood cells in male and female animals.

Table 9 Effects on neutrophils (x10^3/µL; mean ± SD)

	Vehicle		Pegylated Apo-Filgrastim						EU-approved Neulasta			
			30 µg/kg		180 µg/kg		1100 µg/kg		30 µg/kg		1100 µg/kg	
Day 4	**M:15**	**F:15**	**M:14**	**F:14**	**M:9**	**F:10**	**M:13**	**F:14**	**M:14**	**F:15**	**M:15**	**F:15**
	0.9 ± 0.3	0.5 ± 0.2	6.0*** ± 2.5	1.3*** ± 1.1	11.1** ± 3.5	7.8*** ± 3.1	17.2** ± 5.5	10.0*** ± 4.0	3.8*** ± 1.2	1.9*** ± 0.8	17.1** ± 7.2	10.8*** ± 3.6
Day 28	**M:8**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:5**	**F:10**	**M:5**	**F:10**
	0.8 ± 0.2	0.6 ± 0.2	8.2*** ± 1.7	5.6*** ± 2.1	24.9** ± 4.7	21.5*** ± 9.6	78.7** ± 28.1	71.8*** ± 26.0	6.8*** ± 2.1	3.8*** ± 2.0	75.7** ± 14.7	73.8*** ± 24.3

Asterisks indicate significant differences between the control group 2 and the dose groups with * p<0.05, ** p<0.01 and *** p<0.005. Source: Study Report 410.120.1797, Appendix V/A

After the first treatment with the test item, mean levels of neutrophils in male animals increased up to levels of 6 x10^3 cells/µl in the low dose group, up to 11 x10^3 cells/µl in the mid dose group and up to 17 x10^3 cells/µl in the high dose group (all differences statistically significant, p<0.005) when compared to the mean neutrophil levels of the vehicle group (1 x10^3 cells/µl). In female animals mean neutrophil levels were slightly below the mean neutrophil levels of the corresponding levels of male animals. When compared to the treatment with the reference item a similar increase in neutrophil

levels was observed in both genders (males: low dose group 4 $x10^3$ cells/µl, high dose group 18 $x10^3$ cells/µl). Mean levels of monocytes, eosinophils and basophils were also elevated but to a lesser extent (up to approximately 10-fold).

At the end of the treatment period, mean white blood cell counts were severely increased. Mean neutrophil levels increased in both genders up to 8 $x10^3$ cells/µl in the low dose group, up to 25 $x10^3$ cells/µl in the mid dose group and up to 79 $x10^3$ cells/µl in the high dose group (all differences were statistically significant, $p<0.005$) when compared to mean neutrophil levels of the vehicle group.

Mean levels of erythrocytes, haemoglobin and hematocrit were moderately dose-dependent reduced in animals at the mid and high dose. Mean reticulocyte counts were increased slightly to moderately in a dose-dependent manner after both the first dose and at the end of treatment. At the end of the recovery period, both white and red blood cell levels had returned to normal or near normal levels in all treatment groups.

Clinical chemistry: Dose-dependent moderate to marked elevation of the levels of alkaline phosphatase was observed in male and female animals. A partial recovery was observed at the end of the recovery period. Aspartate aminotransferase (females) and total bilirubin (males and females) were slightly elevated, while mean levels of calcium, total protein, albumin, alanine aminotransferase and creatinine were reduced in both sexes at the highest dose. All these changes were reversible.

Organ weights and necropsy: A moderate to severe enlargement in the spleen and a slight decrease in thymus weight were observed at the highest dose, these organ weight increase were totally or partially reversible. The main macroscopic treatment-related findings at the end of the exposure period were dose-dependent enlarged spleens, swollen joints and atrophy of the skeletal muscle of the hind legs. Swollen joints and muscle atrophy persisted at the end of the recovery period, predominantly in males.

The histopathology analysis showed treatment-related changes in the liver, spleen, bone marrow and hind paw (skeletal system and joint). A dose-dependent, exacerbated pharmacological effect of pegfilgrastim was observed, with extramedullary hematopoiesis in the liver, increased granulocytic hematopoiesis in the spleen and myeloid hyperplasia in the bone marrow. These findings were partially reversible. The bone marrow and the hind paw also showed osteoclasis and osteogenesis at the highest dose, accompanied by an arthrosynovitis and myelofibrosis. The osteogenesis and osteoclasis in the bone marrow of the sternum mostly resolved during the recovery period but changes in the hind paws were not reversible.

No harmful effects were observed in the injection sites of both the Pelgraz and Neulasta groups.

Genotoxicity

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

Carcinogenicity

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

Reproduction Toxicity

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

Toxicokinetic data

ELISA was used to determine filgrastim levels in rat serum (study 410.732.1820) as part of the TK analysis. The lower limit of quantification (LLOQ) was defined at 78.1 pg/mL and the upper limit (ULOQ) at 3000 pg/mL. Results are shown in Table 10.

Table 10 Comparative TK of Pelgraz and Neulasta

Parameter	Sex	Pegylated Apo-Filgrastim			Neulasta	
		30 µg/kg	180 µg/kg	1100 µg/kg	30 µg/kg	1100 µg/kg
Dose 1						
C_{max} (µg/mL)	M	0.03 ± 0.02	0.37 ± 0.07	5.14 ± 2.78	0.02 ± 0.01	5.28 ± 1.11
	F	0.05 ± 0.00	0.65 ± 0.01	7.75 ± 0.50	0.04 ± 0.01	6.73 ± 0.55
T_{max} (min)	M	360	1440	240	360	1440
	F	1440	1440	1440	360	1440
$AUC_{0\text{-last}}$ (µg·min/mL)	M	35.2 ± 10.1	768.4 ± 105.4	14385.1 ± 2062.3	27.0 ± 7.6	13671.6 ± 1574.9
	F	106.7 ± 54.8	1296.5 ± 58.5	18381.8 ± 956.5	62.0 ± 10.8	16053.0 ± 1398.7
$T_{1/2}$ (min)	M	736.4	428.4	n.a.	625.6	1307.3
	F	426.1	395.2	1187.0	400.9	1232.6
CL_{obs} (mL/min/kg)	M	0.84	0.23	n.a.	1.10	0.07
	F	0.28	0.14	0.05	0.48	0.06
Dose 5						
C_{max} (µg/mL)	M	0.01 ± 0.00	0.18 ± 0.03	4.96 ± 1.29	0.03 ± 0.01	3.58 ± 0.26
	F	0.02 ± 0.01	0.31 ± 0.03	4.84 ± 0.30	0.02 ± 0.00	4.74 ± 0.54
T_{max} (min)	M	720	360	1440	360	720
	F	240	720	720	360	1440
$AUC_{0\text{-last}}$ (µg·min/mL)	M	15.2 ± 0.9	201.4 ± 43.3	10713.5 ± 1635.7	18.2 ± 3.1	7729.5 ± 1122.4
	F	16.6 ± 1.7	315.3 ± 23.5	8642.0 ± 718.7	16.9 ± 1.9	9830.9 ± 695.9
$T_{1/2}$ (min)	M	885.7	409.6	329.7	936.3	265.1
	F	999.8	472.8	283.2	725.6	272.7
CL_{obs} (mL/min/kg)	M	1.94	0.89	0.10	1.63	0.14
	F	1.77	0.57	0.13	1.76	0.11

Source: Study Report 410.120.1797, Appendix XV

Regarding the terminal elimination half-life, it was shortened after repeated dosing in males at the highest dose, whereas the body clearance was increased in all treatment groups. After repeated dosing, a markedly increased volume of distribution was calculated in the low and mid dose groups in both genders, whereas it was decreased by at least 50% in the high dose groups.

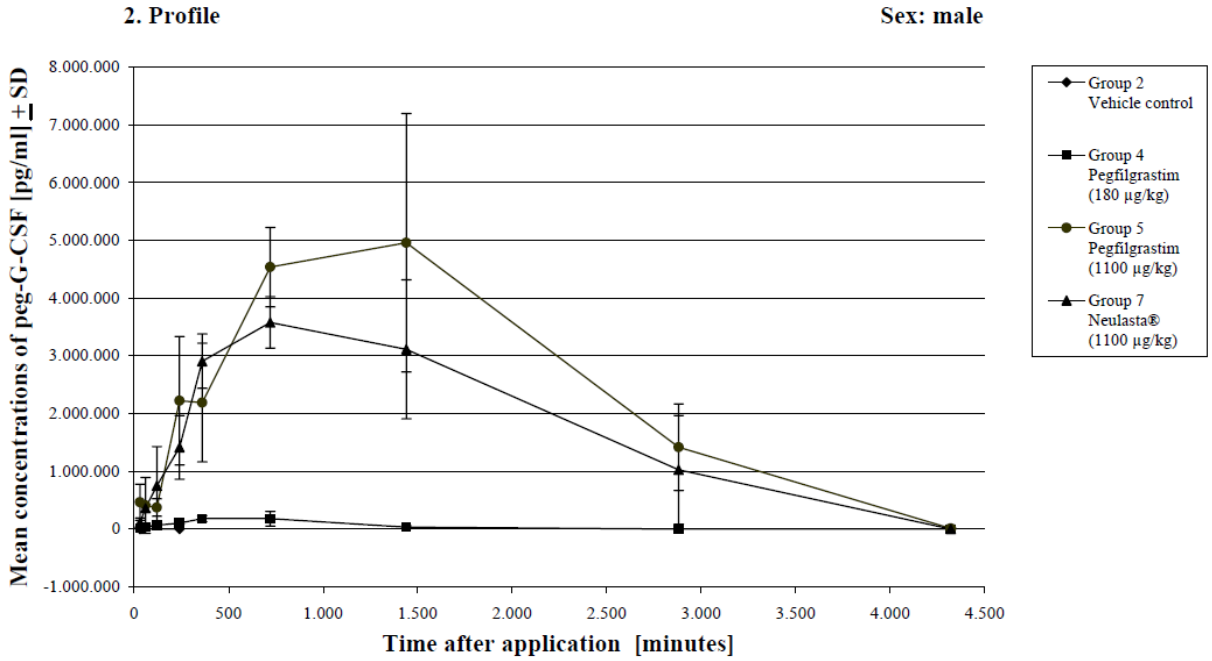


Figure 2 Mean plasma concentrations after repeated administration of Pelgraz and Neulasta at the highest dose of 1100 µg/kg.

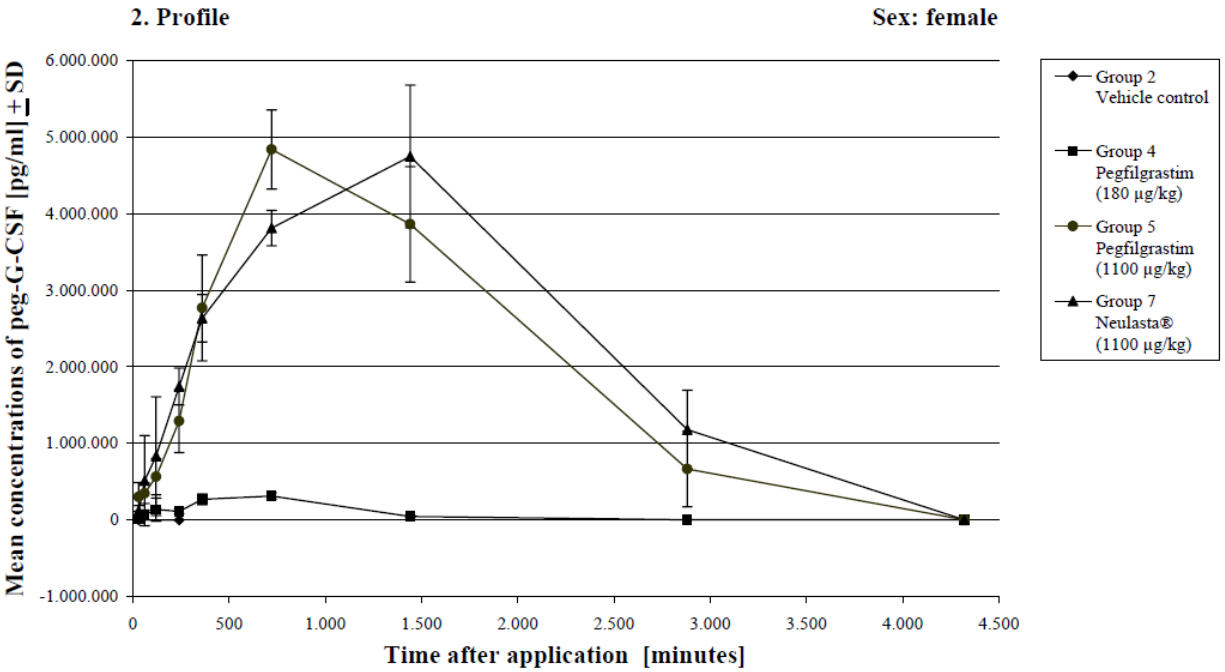


Figure 3 Mean plasma concentrations after repeated administration of Pelgraz and EU-approved Neulasta in high-dose male and female animals

Local Tolerance

Local tolerance testing of Pelgraz vs. EU-approved and US-licensed Neulasta in female New Zealand rabbits (410.542.4251, GLP)

The local tolerance of Pelgraz in comparison to Neulasta was tested in female New Zealand rabbits (6/group). The ears (right side) were injected by different routes of administration: subcutaneous (clinical route), intravenous, intra-arterial, paravenous or intramuscular with one of the three test articles. The Test item Pelgraz caused no adverse local skin reactions after intravenous, subcutaneous, intraarterial, paravenous and intramuscular injection.

Skin sensitization study of Pelgraz in guinea pigs (410.552.4252, GLP)

The skin sensitisation potential of Pelgraz was tested in Dunkin Hartley guinea pigs in comparison to US-licensed and EU-approved Neulasta, using the maximiazation test of Magnusson and Kligman (OECD406).

10 animals per treatment group were used. This study has two parts: Study Part I comprises an intra-dermal treatment and Study Part II comprises a challenge treatment in the form of topical dermal application. All three test products were used at a concentration of 50% for intra-dermal injections, formulated in a 1:1 (v/v) mixture of Freund's Adjuvant and physiological saline, and in undiluted state for dermal sensitisation treatment. Two weeks following the last induction exposure, a challenge dose (undiluted) was administered, by a 24-hour dermal application of the test item. When challenged, none of the treatment groups demonstrated visible dermal reactions at the challenge sites.

Other toxicity studies

Excipients

Pelgraz has been developed for registration in the North American and European markets in single-use prefilled syringes available in 6 mg/0.6 ml strength. The quantitative and qualitative formulations of US licensed Neulasta and Pelgraz are the same (Neulasta USPI). In addition, the qualitative formulations of EU-approved Neulasta and Pelgraz are the same (Neulasta SmPC).

Table 11 Summary of Composition of Pelgraz

Name of the Ingredient	Function	Quantity per Pre-filled Syringe
pegfilgrastim	Active Ingredient	6 mg
Acetate[#]	Buffering Agent	0.35 mg
Sorbitol	Tonicity Agent, Isotonicity adjuster	30 mg
Polysorbate 20	Stabilizer, nonionic surfactant	0.02 mg
Sodium	Buffering Agent	0.02 mg
Water for Injection	Vehicle/solvent	q.s. to 0.6 ml

2.3.5. Ecotoxicity / environmental risk assessment

The applicant submitted a justification for the lack of ERA. According to the "Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use", amino acid, peptides and proteins are unlikely to result in significant risk to the environment and no environmental risk assessment is required.

2.3.6. Discussion on non-clinical aspects

The performed *in vitro* bioassays are in line with the requirements stated in the "Guidance on biosimilar medicinal products containing recombinant granulocyte-colony stimulating factor" (EMEA/CHMP/BMWP/31329/2005).

Comparability testing of Pelgraz with the reference product Neulasta included cell binding and proliferation studies using a murine myeloblastic cell line. Pelgraz and Neulasta were able to bind to the murine cellular G-CSF receptors with apparent similar affinity and efficacy in inducing cellular proliferation. The binding ability in terms of the receptor affinity and biological potency is a crucial factor for decision on biosimilarity. In this sense, the design of studies evaluating biosimilarity was adequate to detect the differences of pharmacological activity. The number of batches used was acceptable. Biosimilarity of the to-be-marketed product and reference product (EU approved Neulasta) was considered demonstrated on the basis of *in vitro* data.

The Applicant submitted two *in vivo* studies evaluating the effect of test and reference products in the neutropenic mice model.

According to the Annex to Guideline on similar biological medicinal products containing biotechnology derived proteins as active substance - Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor, the comparability of the test and the reference product should be demonstrated at the receptor level in an appropriate in vitro cell based bioassay or receptor-binding assay. Apart from that, both non-neutropenic and neutropenic rodent models should be used to compare the pharmacodynamic effects of the test and reference product. Two in vivo pharmacodynamic studies using only neutropenic mice models were performed by the Applicant (see below). Nonetheless, it is appreciated to avoid unnecessary animal studies given that in vitro studies are usually more specific and sensitive to detect differences between the biosimilar and the reference product (EMA/CHMP/CVMP/JEG-3Rs/450091/2012, EMA/CHMP/BMWP/214262/2015). In vivo data are thus considered as sufficient for comparability exercise.

The in vivo potency studies conducted on neutropenic mice with Pelgraz, EU-approved and US-licensed Neulasta showed similar restoration of neutrophil counts. The relative potency values were within the conventional acceptance criterion of 80-125% for biosimilarity.

In addition, in the repeated-dose toxicity study, neutrophil values were measured in non-neutropenic rats treated with Pelgraz and Neulasta. A dose-dependent increase of white blood cells was observed, attributable principally to increased neutrophils.

No secondary pharmacology, safety pharmacology or pharmacodynamic drug interactions were conducted by the Applicant.

There were no nonclinical PK studies conducted with Pelgraz. According to the toxicokinetic data obtained in the general toxicity study in rats (410.120.1797), the mean serum exposure to pegfilgrastim increased dose-dependently but not linearly in both genders and for both the test item and the reference product. The disproportionality of the increases in Cmax and AUClast with dose could be caused by the saturation of the receptor-mediated clearance.

Although some differences were found between AUC and Cmax values for Pelgraz and Neulasta, this could be explained by the high inter-individual variability of these parameters and the low number of animals.

The toxic effects caused by Pelgraz or EU-approved Neulasta in rats were consistent with the known pharmacodynamic effects of pegfilgrastim, with an expected increase in neutrophil counts accompanied

by an increase in myeloid hyperplasia in bone marrow, an increase in alkaline phosphatase, spleen weight and hematopoiesis in liver and spleen. All these effects were dose-dependent and were observed for both Pelgraz and EU-approved Neulasta to the same extent.

Only a slight difference between Pelgraz and Neulasta was observed in the increase in prothrombin time, with partial recovery at the end of the treatment-free period in the EU-approved Neulasta group and total recovery in the Pelgraz group. This slight difference does not seem of any relevance for the safety of the product.

In addition, during evaluation of anti-drug antibody response indices for a mild induction of antidrug antibodies in the high dose of EU-approved Neulasta were found. However, the grade of the signal detected during ELISA measurement and number of animals impaired in relation to the number of animals tested does not allow consideration of an immunological difference of Pelgraz when compared to Neulasta.

Overall, no considerable differences were found between Pelgraz and EU-approved Neulasta regarding the parameters evaluated in this general toxicity study. Both items showed comparable effects.

Regarding toxicokinetics, the mean serum exposure to pegfilgrastim increased dose-dependently but not linearly in both genders and for both the test item and the reference product. The disproportionality of the increases in Cmax and AUClast with dose could be caused by the saturation of the receptor-mediated clearance.

Although some differences were found between AUC and Cmax values for Pelgraz and Neulasta, this could be explained by the high inter-individual variability of these parameters and the low number of animals.

Genotoxicity, carcinogenicity and reproductive toxicity studies were not conducted, as they are not required for biosimilar products.

Local tolerance studies showed that a single intravenous, subcutaneous, intraarterial, intramuscular and paravenous injection of 0.25 ml (p.v.) or 0.5 ml (all other routes) of Pelgraz is well tolerated in rabbits and comparable in local irritation potential to Neulasta.

In a skin sensitization study in guinea pigs, challenge with Pelgraz evoked no positive responses in the test animals sensitised previously. According to the net percentage value of positively response in animals and to the net score value of the skin reactions, Pelgraz was classified as a non-sensitiser. No sensitisation potential difference was found between Pelgraz and Neulasta. Pelgraz is being developed as biosimilar product of Neulasta; therefore its approval should not cause in increase of risk to the environment and in addition, according to Neulasta EPAR, pegfilgrastim is unlikely to be of environmental concern.

No ERA studies were provided with reference to the guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 Corr 2). Conjugated PEG component is commonly used, considered safe and represents no additional environmental risk. Regarding the fact that the active substance pegfilgrastim is a polypeptide which is expected to be largely metabolised after administration and easily biodegraded in the environment, the omission of ERA studies indeed can be accepted as described in above mentioned guideline. A biosimilar, pegfilgrastim is already used in existing marketed products and no significant increase in environmental exposure is anticipated. Therefore, pegfilgrastim is not expected to raise a risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

The biosimilarity of Pelgraz to Neulasta in terms of non-clinical data has been shown.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Pelgraz development program utilized a step-wise approach and included an analytical similarity exercise for Pelgraz with both EU-approved and US-licensed Neulasta to demonstrate similarity in the quality attributes of the proposed biosimilar and the reference biologic drug Neulasta, sourced from the two markets in order to generate data that supports the establishment of a scientific bridge between the two reference products.

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

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

- Tabular overview of clinical studies

Table 12 Clinical studies

Type of Study	Study No.	Location of Study Report	Study Objective	Study Design, Type of Control	Test Product; Dosage Regimen; Route of Administration	No. of Subjects	Healthy Subjects/ Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase I PK/PD study	APO-Peg-02	5.3.4.1	To assess and compare the proposed biosimilar recombinant Pegylated Apo-Filgrastim (pegylated granulocyte colony-stimulating factor; pegG-CSF) with Neulasta® (the US-licensed recombinant pegG-CSF) in healthy volunteers based on pharmacokinetic (PK) and pharmacodynamic (PD) parameters following subcutaneous administration of a single 6 mg dose.	Single-dose, randomized two-way crossover, assessor-blinded, active-controlled	**Test Product:** Pegylated Apo-Filgrastim **Reference Product:** US-licensed Neulasta Single dose (6mg/0.6mL); subcutaneous administration	66 Subjects (56 completed the study) (Male/Female ; 18-55 years old)	Healthy Subjects Subjects recruited from 1 study center in Canada	Approximately 85 days (28 days in each period with at least 8 weeks washout between periods)	Complete; Full

Type of Study	Study No.	Location of Study Report	Study Objective	Study Design, Type of Control	Test Product; Dosage Regimen; Route of Administration	No. of Subjects	Healthy Subjects/ Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Phase III Safety and Efficacy	APO-Peg-03	5.3.5.1	**Primary objective:** To demonstrate an equivalent efficacy of Pegylated Apo-Filgrastim as compared to each of the commercially available US and EU licensed Neulasta® in patients suffering from early breast cancer and receiving TAC (docetaxel, doxorubicin, cyclophosphamide) anticancer chemotherapy in adjuvant setting. **Secondary objectives:** • To assess the safety of Pegylated Apo-Filgrastim as compared to that of commercially available US and EU licensed Neulasta® when administered through 6 cycles of TAC anticancer chemotherapy • To assess the potential antigenicity of Pegylated Apo-Filgrastim during the chemotherapy and 30 weeks after the completion of chemotherapy	Multi-Center, Randomized, Active Controlled, Assessor-Blinded	**Test Product:** Pegylated Apo-Filgrastim **Reference Products:** US-Licensed Neulasta and EU-Approved Neulasta Multiple dose (6mg/0.6mL) for each administration on Day 2 of each chemotherapy cycle (at least 24 hours after chemotherapy); subcutaneous administration	589 female patients (randomized and treated) Pegylated Apo-Filgrastim: 294 patients, US-Licensed Neulasta: 148 patients, and EU-approved Neulasta: 147 patients	Cancer patients suffering from stage IIA, IIB or IIIA breast cancer Patients recruited from 56 study centers in 11 countries in Central and Eastern Europe	The study consisted of 3 periods: 1. Screening (up to 3 weeks). 2. Treatment period (6 cycles each of 3 weeks i.e. a total of 18 weeks). 3. Safety follow-up period (up to 30 weeks following the completion of TAC regimen).	Treatment and Safety Follow-up phases complete; Full

2.4.2. Pharmacokinetics

Absorption

- **Bioavailability**

No bioavailability study has been performed with pegfilgrastim.

On the basis of the studies with Neulasta, the absolute bioavailability of pegfilgrastim is estimated to be in the range of 20-50%. After SC administration, C_{max} and AUC values were approximately 3% and 4% respectively of the values obtained after IV administration. Pegfilgrastim exhibited first-order, delayed absorption with a mean absorption time of 1.84 days. The bioavailability of pegfilgrastim relative to the 30-µg/kg dose after subcutaneous administration was approximately 94%, 101%, and 80% for the 60-, 100-, and 300-µg/kg doses, respectively. The lymphatic system becomes an important absorption pathway for proteins after subcutaneous administration. The contribution increases with increasing molecular weight and appears to be the predominant absorption pathway for proteins that are larger than 16 kDa (Roskos, 2006).

BIOEQUIVALENCE STUDY Pelgraz-02

Comparative, randomized, single-dose, assessor-blinded, 2-way crossover pharmacokinetic and pharmacodynamic study of subcutaneously administered Pelgraz (Test) and Neulasta (Reference) (USA) in healthy volunteer subjects.

Study objectives

The objective of this study was to assess and compare the proposed biosimilar pegfilgrastim (pegylated granulocyte colony-stimulating factor; pegG-CSF) with Neulasta (US-licensed) reference comparator in healthy volunteers based on pharmacokinetic and pharmacodynamic parameters following subcutaneous administration of a single 6 mg dose.

Study design

All subjects enrolled in the study received a single dose of either one of the aforementioned drugs at time zero (0) for each period of the study, with the time interval between the consecutive doses (wash-out period) of 8 weeks. The subjects were confined in the clinical facility 10 hours pre-dose until 120 hours (day 5) post-dose. However, they had to return to the clinic for the 144 (Day 6), 168 (Day 7), 192 (Day 8), 216 (Day 9), 240 (Day 10), 264 (Day 11), 288 (Day 12), 312 (Day 13) and 360 (Day 15) hour sampling points and at 672 (Day 28) hour for immunogenicity and diagnostic sampling.

Investigational products

A single, 6 mg dose of pegfilgrastim (Pelgraz or Neulasta) was administered by injection subcutaneously in the upper arm to each subject at approximately the same time during each period (12 mg of pegfilgrastim in total to be administered over the course of the two periods of the study).

Subjects were to be required to fast for at least 10 hours before dosing and for 4 hours thereafter. Water was not to be permitted from 2 hours before until 2 hours after dosing at which time 240 mL of water was to be consumed. Water was to be allowed ad lib at all other times.

For pegfilgastrim plasma concentration measurements, 4 mL blood samples were taken via vacuum tubes containing K2EDTA by venipuncture or an indwelling catheter. Two 4 mL blood samples were taken 5–45 minutes prior to dosing time (0) followed by single 4 mL samples at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24 (Day 1), 28, 32, 36, 40, 48 (Day 2), 60, 72 (Day 3), 84, 96 (Day 4), 108, 120 (Day 5), 144 (Day 6), 168 (Day 7), 192 (Day 8), 216 (Day 9), 240 (Day 10),264 (Day 11) and 288 (Day 12) hours after the s.c. injection of the study drug(s) in each period.

Population studied and sample size calculation

A maximum of 66 subjects were to be entered in this study to ensure 52 subjects completed the study. The determination of sample size was based on the following assumptions: estimated intrasubject CV of 30% (expected variability of pegfilgrastim), a formulation difference of up to 5% between the two recombinant pegfilgrastim products, and statistical power of 90% to detect a 20% difference. The sample size also took into account the possibility of dropouts and subjects dismissed for medical or other reasons.

The randomized volunteers included 66 subjects (49 males and 17 females), mean age was 40 years, mean BMI was 26 kg/m^2. Thirty-five (35) subjects were white, 4 were black, 6 were Asians and 11 were of other origin.

Ten subjects (15.15%) in total discontinued from the study - 4 subjects Pelgraz AS vs. 6 subjects Neulasta. The main reasons for discontinuation were non-compliance with the study requirements, as subjects did not attend the clinic for scheduled visits (4 subjects; 1 subject Pelgraz vs. 3 subjects Neulasta). Other discontinuations included 1 withdrawal by subject for personal reasons in the Pelgraz group. Three subjects experienced adverse events, 1 subject Pelgraz with abnormal white blood cell count vs. 2 subjects Neulasta with 1 subject due to a right wrist fracture and 1 subject due to

hypersensitivity that was classified as a serious adverse event. Two subjects were withdrawn due to PK/PD reasons as subjects were administered IV fluids and the additional fluid volume which were assessed to have a potential impact on PK/PD measures (1 subject Pelgraz vs. 1 subject Neulasta).

Protocol deviations

The protocol deviations observed were related to sampling time and subject monitoring and were documented for all 66 subjects over the course of the study. Five (5) subjects (7.58%) had deviations related to subject monitoring however there was no impact on subject safety and on PK/PD assessments.

Conduct of the study

Additional protocol deviations occurred after completion of the clinical phase of the study, prior to or during the data analysis phase of the study such as: Adjustment of the concentration data for pegfilgrastim prior to the pharmacokinetic statistical analysis to account for the difference in pegfilgrastim content of greater than 5% between Pelgraz and US-Licensed Neulasta; an additional analysis population, Intention-to- Treat (ITT) for PD data, was included in this study; Additional gender-specific analyses were performed in this study for information purposes only. Prior to performing database lock and unblinding of the data, a supplementary Statistical Analysis Plan (SAP) was prepared in advance of data analysis to define the approach to be used to examine the gender effects within each treatment for pharmacokinetic and pharmacodynamics measures, and any differences in gender effects between treatments.

Pharmacokinetic variables

Table 13

AUCt	The area under the curve (AUC - calculated by the linear trapezoidal rule) from time zero up to the sampling time for which the last concentration was equal to or larger than LOQ.
AUCinf	The AUC from time zero to infinity estimated by adding to AUCt a value equal to Clast/Kel, where Clast is the last quantifiable concentration (occurring at time Tlast) and Kel is the apparent elimination rate constant (see below).
Cmax	The maximum concentration of pegfilgrastim over the sampling interval.
Tmax	The sampling time at which Cmax occurred.
Kel	Apparent first-order terminal elimination rate constant estimated as the absolute value of the slope of the least-squares regression line fitted through the ln(concentration)-time data pairs located on the terminal linear phase of the ln(concentration)-time profile.
Thalf	The apparent terminal elimination half-life of pegfilgrastim, calculated as $\left(\frac{\ln(2)}{Kel}\right)$.
Cl	The rate at which the pegfilgrastim is cleared from the body, measured as a volume per unit time, calculated as Dose/AUCt.
Vd	The apparent volume into which the pegfilgrastim is distributed, calculated as Cl/Kel or Dose/(AUCt*Kel).

Randomisation

Randomization was to be done according to the crossover design. Half of the volunteers were to receive the test product in period 1 followed by the reference product in period 2 and the other half were to receive the products in reverse order. Randomization was to be based on a computer generated balanced randomization scheme..

Blinding (masking)

This study used an assessor blinded design. To ensure blinding, the following measures were taken:

1) The test articles were placed inside brown envelopes, labelled according to randomization scheme.
2) Staff involved with packaging of test articles were not allowed to participate in other study activities.
3) Dosing took place in an office where only the staff dosing (two paramedics), staff verifying the dosing (two management staff) and two subjects to be dosed were present at the same time. Subjects were sitting against each other and were not able to see what syringe was used for dosing the other subject.
4) The Study Personnel in charge of recording adverse events/side effects were to be blinded to the dosing code.
5) The laboratory analysts were blinded since the samples were identified only by Study code, Subject Identification No.—Period No.—Sampling Time / Day and they were not allowed access to the dosing code until sample analysis had been complete.

Statistical methods: Analysis sets

-PK Statistical Analysis

Analysis of Variance (ANOVA) was to be performed on the logarithmic transformation of AUCt, AUCinf, Cmax, Cl and Vd and on the raw data for Tmax, Kel and Thalf parameters. The ANOVA model was to include sequence, subject within sequence, period, and treatment effects. The sequence effect was to be tested using the subject within sequence mean square from ANOVA as the error term. All other main effects were to be tested against the residual error (error mean square) from ANOVA to detect statistically significant differences (α=0.05).

Least squares means for the formulations (treatments) and the adjusted difference between formulation means together with the associated standard error were to be estimated using the SAS

GLM procedure. The two one-sided hypothesis at the α=0.05 level of significance was to be tested for AUCt and Cmax by constructing the 90% confidence interval for the ratio between the test and reference formulation means for pegfilgrastim.

-PD Statistical Analysis

For each of ANC and CD34+, ANOVA was to be performed on the logarithmic transformation of the AUECt and Emax and on the raw data for Tmax. The ANOVA model was to include sequence, subject within sequence, period, and treatment effects. The sequence effect was to be tested using the subject within sequence mean square from ANOVA as the error term. All other main effects were to be tested against the residual error (error mean square) from ANOVA to detect statistically significant differences (α=0.05).

Least squares means for the formulations (treatments) and the adjusted difference between formulation means together with the associated standard error were to be estimated using the SAS GLM procedure.

For ANC, the two one-sided hypothesis at the α=0.025 level of significance was to be tested for AUECt and Emax by constructing the 95% confidence interval for the ratio between the test and reference formulation means.

For CD34+, the 95% confidence interval for the ratio between the test and reference formulation means for PD endpoints of absolute CD34+ cell counts was to be constructed for presentation purposes.

Other statistical tests were to be performed, if necessary.

-Safety Analysis

Subjects who received at least one administration of pegfilgrastim were to be included in the safety population and hence, included in the safety data analysis. Safety was to be evaluated through the assessment of adverse events, laboratory tests, and vital signs throughout the duration of the study and up to 2weeks post-study. Safety analysis was to include immunogenicity testing. Safety data was to be analyzed by descriptive statistics.Adverse events were to be listed and tabulated by system organ class (SOC), severity, and relationship to study medication. Incidence rate and number of AEs and SAEs were to be presented in a frequency table. Number and percentage of out of range and clinically significant laboratory values were to be tabulated.

Results

Since the drug content of the batches of Pelgraz and Neulasta employed in this study differed by more than 5%, any differences in PK parameter between the two products could be significantly obscured by this difference in drug content. To avoid bias by ensuring that the concentration data for pegfilgrastim was accurately reflective of the drug content of the test and reference products, prior to conducting the pharmacokinetic and statistical analysis, the pegfilgrastim concentration data for Pelgraz and Neulasta was corrected for protein content and purity. The adjustment factor for Pelgraz and Neulasta was based on the actual Certificate of Analysis (CofA) for pegylated filgrastim protein concentration and purity data for each treatment. For Pelgraz the adjustment factor was 0.986, for Neulasta 1.066.

For the analysis of adjusted pegfilgrastim data, plasma concentrations from 56 subjects were included in the PK population. Two subjects had pre-dose concentrations in period 2, which corresponded to 0.216 % and 0.881 % of their Cmax. Since the pegfilgrastim levels were low, the data obtained from these subjects were included in the PK/statistical analysis, as stipulated in the study protocol. No pre-dose concentrations were detected in any other subject.

Table 14

Product	Lot #	Expiry	Label Claim	Pegylated Filgrastim Protein Concentration	CoA Purity (% SE-HPLC)#
Pegylated Apo-Filgrastim	G503CT0002	03/2014	6 mg/0.6 ml	10.2 mg/ml	99.45%
Neulasta®	1029211	04/2014	6 mg/0.6 ml	5.7 mg/0.6 ml (9.5 mg/ml)	98.7%

#SE-HPLC denotes Size Exclusion High Performance Liquid Chromatography; the purity values are obtained via subtraction (100% - Impurities) in order to obtain these numbers. The SE-HPLC method is the most capable of resolving the main peak from protein aggregates.

The following equation was used to determine the adjustment factor:

$$= \frac{Label Claim}{CoA Concentration} x \frac{100\%}{CoA Purity\%}$$

For Pegylated Apo-Filgrastim:

$$= \frac{10 mg/mL}{10.2 mg/mL} x \frac{100\%}{99.45\%} = 0.986$$

For Neulasta®:

$$= \frac{10 mg/mL}{9.5 mg/mL} x \frac{100\%}{98.7\%} = 1.066$$

Table 15 PK parameters following a fixed single subcutaneous injection of 6 mg / 0.6 ml Pelgraz or Neulasta to healthy subjects (adjusted pegfilgrastim data) (PK population)

Endpoint		Pegylated Apo-Filgrastim (N=56)	Neulasta® (N=56)	Ratio of Geometric Means[%]	90% CI [%]	Pr > [F]
AUCt [pg*h/mL]	Mean	8165681	8125513	103.2	91.7 – 116.1	Treatment 0.6549 Period 0.2072 Sequence 0.1225
	SD	5261409	6005813			
	Min	898669	1487894			
	Max	26009051	30054490			
AUCinf [1] [pg*h/mL]	Mean	8108814	8410220	100.8	88.3 – 115.0	Treatment 0.9205 Period 0.6567 Sequence 0.1264
	SD	5443322	6067565			
	Min	918122	1527847			
	Max	26027768	30080089			
Cmax [pg/mL]	Mean	190076	194909	97.7	86.7 – 110.2	Treatment 0.7523 Period 0.8055 Sequence 0.0980
	SD	113710	129021			
	Min	16762	41681			
	Max	458490	558584			
Tmax [h]	Mean	25.82	24.18	105.2	95.9 – 114.5	Treatment 0.3575 Period 0.0002 Sequence 0.3063
	SD	8.00	9.20			
	Min	12.00	10.11			
	Max	40.00	48.00			
Kel[1] [1/h]	Mean	0.01354	0.01398	97.4	89.2 – 105.6	Treatment 0.5992 Period 0.0413 Sequence 0.3327
	SD	0.00471	0.00536			
	Min	0.00491	0.00731			
	Max	0.02616	0.03529			
Thalf [1] [h]	Mean	58.03	55.09	103.2	96.1 – 110.3	Treatment 0.4566 Period 0.0210 Sequence 0.7981
	SD	22.46	16.41			
	Min	26.49	19.64			
	Max	141.07	94.81			
Cl [ml/h]	Mean	1185	1206	96.9	86.1 – 109.0	Treatment 0.6549 Period 0.2072 Sequence 0.1225
	SD	1072	858			
	Min	231	200			
	Max	6677	4033			
Vd[1] [ml]	Mean	105461	97139	101.6	89.0 – 116.0	Treatment 0.8441 Period 0.0760 Sequence 0.3051
	SD	103629	93230			
	Min	11868	11750			
	Max	575223	551588			

[1] N = 50 for test product and N = 53 for reference product; Kel, Thalf, AUCinf and Vd parameters were not determined if the log-linear terminal phase was not clearly defined.
[2] The ratio of the geometric means are based on LSE of the geometric means for the ln-transformed parameters and on arithmetic means for the untranformed parameters.

Table 16 PK parameters following a fixed single subcutaneous injection of 6 mg / 0.6 ml Pelgraz or Neulasta to healthy subjects (unadjusted (raw) pegfilgrastim data) (PK population)

Endpoint		Pegylated Apo-Filgrastim (N=56)	Neulasta® (N=56)	Ratio of Geometric Means[%]	90% CI [%]	Pr > [F]
AUCt [pg*h/mL]	Mean	8281624	7622433	111.6	99.2 - 125.5	Treatment 0.1252 Period 0.2072 Sequence 0.1225
	SD	5336115	5633971			
	Min	911429	1395773			
	Max	26378347	28193705			
AUCinf [1] [pg*h/mL]	Mean	8223949	7889512	109.0	95.5 - 124.3	Treatment 0.2801 Period 0.6567 Sequence 0.1264
	SD	5520610	5691899			
	Min	931158	1433252			
	Max	26397330	28217720			
Cmax [pg/mL]	Mean	192775	182841	105.7	93.7 - 119.2	Treatment 0.4456 Period 0.8055 Sequence 0.0980
	SD	115324	121033			
	Min	17000	39100			
	Max	465000	524000			
Tmax [h]	Mean	25.82	24.18	105.2	95.9 – 114.5	Treatment 0.3575 Period 0.0002 Sequence 0.3063
	SD	8.00	9.20			
	Min	12.00	10.11			
	Max	40.00	48.00			
Kel[1] [1/h]	Mean	0.01354	0.01398	97.4	89.2 – 105.6	Treatment 0.5992 Period 0.0413 Sequence 0.3327
	SD	0.00471	0.00536			
	Min	0.00491	0.00731			
	Max	0.02616	0.03529			
Thalf [1] [h]	Mean	58.03	55.09	103.2	96.1 – 110.3	Treatment 0.4566 Period 0.0210 Sequence 0.7981
	SD	22.46	16.41			
	Min	26.49	19.64			
	Max	141.07	94.81			
Cl [ml/h]	Mean	1168	1285	89.6	79.7 - 100.8	Treatment 0.1252 Period 0.2072 Sequence 0.1225
	SD	1057	914			
	Min	227	213			
	Max	6583	4299			
Vd[1] [ml]	Mean	103984	103550	94.0	82.3 - 107.3	Treatment 0.4334 Period 0.0760 Sequence 0.3051
	SD	102178	99384			
	Min	11701	12526			
	Max	567170	587993			

[1] N = 50 for test product and N = 53 for reference product; Kel, Thalf, AUCinf and Vd parameters were not determined if the log-linear terminal phase was not clearly defined.
[2] The ratio of the geometric means are based on LSE of the geometric means for the ln-transformed parameters and on arithmetic means for the untranformed parameters.

Table 17 Summary of equivalence results for various analyses of adjusted and raw Pelgraz and Neulasta concentration data (PK population)

Analysis Type	Cmax Ratio [90% CI]	AUCt Ratio [90% CI]	AUCinf Ratio [90% CI]
Adjusted Pegylated Apo-Filgrastim vs. Adjusted Neulasta®	97.7 [86.7 – 110.2]	103.2 [91.7 – 116.1]	100.8 [88.3 – 115.0]
Adjusted Pegylated Apo-Filgrastim vs. Raw Neulasta®	104.2 [92.4 – 117.5]	110.0 [97.8 – 123.8]	107.4 [94.2 – 122.6]
Raw Pegylated Apo-Filgrastim vs. Adjusted Neulasta®	99.1 [87.9 – 111.8]	104.7 [93.0 – 117.8]	102.2 [89.6 – 116.6]
Raw Pegylated Apo-Filgrastim vs. Raw Neulasta®	105.7 [93.7 - 119.2]	111.6 [99.2 - 125.5]	109.0 [95.5 - 124.3]

Sources: Appendix 16.1.9.1 (Adjusted Pegylated Apo-Filgrastim vs. Adjusted Neulasta®)
Appendix 16.6.1.3.2 (Adjusted Pegylated Apo-Filgrastim vs. Raw Neulasta®)
Appendix 16.61.4.2 (Raw Pegylated Apo-Filgrastim vs. Adjusted Neulasta®)
Appendix 16.6.1.2.5 (Raw Pegylated Apo-Filgrastim vs. Raw Neulasta®)

Distribution

In the study Pelgraz-02 the volume of distribution was calculated for both adjusted and unadjusted Apo-pegfilgrastim and Neulasta. Adjusted value was 105461 ml and 97139 ml for test pegfilgrastim and Neulasta, respectively. Unadjusted value 103984 ml and 103550 ml for test pegfilgrastim and Neulasta, respectively.

Elimination

The observed pharmacokinetics of pegfilgrastim are consistent with a predominantly neutrophil-mediated clearance mechanism (Neulasta EPAR). Renal clearance is minimal (Yang et al, 2008). In CIN (chemotherapeutic induced neutropenia) the clearance of pegfilgrastim is significantly reduced, until neutrophil granulocyte recovery.

Dose proportionality and time dependencies

Healthy subjects: After subcutaneous administration of pegfilgrastim, the peak of the serum concentration-time profile became broader and the time to maximum concentration (Tmax) occurred at a later time with increasing dose (from 8 to 24 hours after dosing), suggesting that one of the clearance pathways for pegfilgrastim was saturated at high serum concentrations of pegfilgrastim. A 10-fold increase in the dose resulted in an approximately 25-fold increase in the maximum observed concentration (Cmax) and an approximately 75-fold increase in the AUC.

Similar to observations in healthy subjects, the pharmacokinetics of pegfilgrastim appeared to be non-linear in patients with NSCLC before and after chemotherapy. After chemotherapy, pegfilgrastim reached a maximum concentration similar to that before chemotherapy for each dose level; however, the maximum concentration was sustained for a longer period of time and began to decline rapidly only at the onset of neutrophil recovery. The exposure to pegfilgrastim (AUC) after chemotherapy was

higher than that before chemotherapy, supporting the hypothesis that neutrophils and neutrophil precursors play an important role in the elimination of pegfilgrastim.

In a dose-ranging study, women with high-risk stage II, III or IV breast cancer received a single subcutaneous dose of pegfilgrastim at 30, 60 or 100 mg/kg 24 hours after completion of doxorubicin/docetaxel chemotherapy; treatment was repeated every 21 days for up to four cycles. The exposure to pegfilgrastim was lower in chemotherapy cycle 3 than in cycle 1, while an improvement in ANC nadir and a decrease in duration of neutropenia occurred in cycle 2 and subsequent cycles of treatment, suggesting that an expansion of neutrophil and neutrophil precursor mass in the later cycles resulted in an increase in drug clearance (Yang, 2011).

Special populations

As this concerns a biosimilar application, no separate investigation in special populations have been performed by the Applicant, and none are required. Information stated in this section is based on data of the reference product with the exception of gender subanalysis which was performed in the study Pelgraz-02.

- **Impaired renal function**

Renal impairment, including end-stage renal disease, appears to have no effect on the pharmacokinetics of pegfilgrastim and no dosage adjustment is required.

- **Gender**

Table 18 Significance of Gender by Treatment Interaction for Pegfilgrastim (ANOVA)

	Gender*Treatment		Error			
Parameter	DF	SS	DF	SS	F Test	Prob > F
lnAUCt	1	0.25274	53	7.20531	1.86	0.1785
lnCmax	1	0.26337	53	7.50381	1.86	0.1784
lnAUCinf	1	0.59551	44	5.90726	4.44	0.0409
Tmax	1	28.17629	53	2745.56729	0.54	0.4641
Kel	1	0.00001	44	0.00048	0.97	0.3308
Thalf	1	5.50565	44	5952.82039	0.04	0.8411
lnCL	1	0.25274	53	7.20531	1.86	0.1785
lnVD	1	0.43413	44	6.12743	3.12	0.0844

Pharmacokinetic interaction studies

As this concerns a biosimilar application, no new interaction studies were performed by the Applicant and none are required. Reference is made to the interaction studies of Neulasta.

Pharmacokinetics using human biomaterials

BIOEQUIVALENCE STUDY: A new PK/PD study (154-14). Protocol No. 154-14. Celerion Project No. CA21285

This was an assessor blind, balanced, randomized, 2-treatment, 2-period, single-dose, 2-waycrossover, comparative, SC, 2 dose levels [3 mg/0.3 mL and 6 mg/0.6 mL; INTP5 of Intas Pharmaceuticals Ltd., India and Neulasta of AMGEN (EU-licensed product)] PK/PD studyin healthy, normal, adult, human subjects under fasting conditions separated by a washout period of 8 weeks.

In each dose level group, after an overnight fast of at least 10 hours, a single dose of Pegfilgrastim 3 mg/0.3 mL (either of INTP5, Test product T1 or Neulasta, Reference product R1) or 6 mg/0.6 mL (either of INTP5, Test product T2 or Neulasta, Reference product R2) was administered subcutaneously to the outer area of the right upper arm to each subject in each period.

In this study, 6 mg and 3 mg doses were evaluated to determine the bioequivalence and safety.

The study was performed with the EU-approved reference product. Both batches were tested before expiry date and the CoAs show a similar content. Therefore, content correction is not necessary.

A total of 28 blood samples were collected for PK evaluation at the following times: Pre-dose(within 60 minutes prior to dosing) and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30,36, 42, 48, 60, 72, 96, 120, 144, 168, 192, 240, 288, and 336 hours post-dose.

As per the plan, a total of 344 subjects (172 subjects per dose level group) entered the study and were randomized to study treatment. A total of 299 subjects completed the study (there were 148 completers for the 3 mg/0.3 mL dose level group and 151 completers for the6 mg/0.6 mL dose level group).

In healthy volunteers, maximal pegfilgrastim concentrations are observed between 8-48 hours post-dosing, depending on the dose. Terminal elimination half-life is approximately 46-62 hours. Therefore, the proposed time points seem adequate to describe the pharmacokinetic profile of pegfilgrastim for evaluation of comparability of Pelgraz and Neulasta. The 14 day sampling interval would correspond to approximately 5 half-lives and would allow covering at least 80% of the AUC. Based on the results it is also noteworthy that a review of the AUC_{0-t} and AUC_{inf} data for both Pelgraz and Neulasta treatments demonstrates that the AUC_{0-t} parameter provides a reliable estimate of the extent of exposure for both the test and reference treatments as the AUC_{0-t} covered > 99% of AUC_{inf}.

The 56 days washout period between the two administrations seems sufficient to prevent carry-over and no pre-dose levels were detected.

The selection of $AUC_{0-tlast}$ as primary PK endpoint is in line with the Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor (EMEA/CHMP/BMWP/31329/2005). C_{max} is also evaluated as primary endpoint, which is in line with the Guidance mentioned above, and to the Biosimilar Guideline (EMEA/CHMP/BMWP/42832/2005 Rev1) in which, AUC_{0-inf} and C_{max} should be evaluated as co-primary endpoint for subcutaneously administered products. In conclusion, the PK endpoints are considered acceptable since the AUC_{0-inf}, AUC_{0-t} and C_{max} has been assessed.

AUC determination by standard non-compartmental linear trapezoidal rule is considered adequate.

The statistical method employed by the Applicant is PROC MIXED of SAS is considered acceptable as only subjects with observations in both treatment were used (there were no missing data).

Since the interaction terms group*sequence and treatment*group were not statistically significant the model was simplified for all PK parameters in both PK statistical comparisons (T1 versus R1 and T2 versus R2). The method including subjects (sequence), sequence, period and treatment was used to obtain the 90%CI for the ratios test/reference.

The statistical comparisons of serum Pegfilgrastim PK parameters with the interaction terms group*sequence and treatment*group removed from the model for all PK parameters are summarized per dose level in Table 19 and Table 20.

Table 19 Summary of Statistical Comparisons of Serum Pegfilgrastim Pharmacokinetic Parameters AUC0-t, AUC0-inf, and Cmax for Test Product T1 versus Reference Product R1

	Treatment T1 (Test)		Treatment R1 (Reference)					
Parameter	Geometric LSMs	n	Geometric LSMs	n	GMR (%)	90% Confidence Interval	Intra-subject CV%	Power (%)
AUC0-t (ng*hr/mL)	3772.4	139	3979.0	139	94.81	89.21 - 100.75	31.35	99.82
AUC0-inf (ng*hr/mL)	3840.6	133	4018.8	133	95.57	90.05 - 101.42	29.89	99.94
Cmax (ng/mL)	122.3	144	127.5	144	95.99	90.52 - 101.78	30.70	99.97

Treatment T1: A Single Subcutaneous Dose of INTP5 Administered at 3 mg/0.3 mL (Test Product T1)
Treatment R1: A Single Subcutaneous Dose of Neulasta® Administered at 3 mg/0.3 mL (Reference Product R1)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs derived from the ANOVA.
Geometric Mean Ratio (GMR) = 100*(test/reference)
Intra-subject CV% was calculated as 100 x square root(exp[MSE]-1), where MSE = Residual variance from ANOVA.
Source: Table 14.2.1.13
Program: /CA21285/sas_prg/pksas/intext-stats-tables-mixed.sas 31MAR2017 7:32

Table 20 Summary of Statistical Comparisons of Serum Pegfilgrastim Pharmacokinetic Parameters AUC0-t, AUC0-inf, and Cmax for Test Product T2 versus Reference Product R2

	Treatment T2 (Test)		Treatment R2 (Reference)					
Parameter	Geometric LSMs	n	Geometric LSMs	n	GMR (%)	90% Confidence Interval	Intra-subject CV%	Power (%)
AUC0-t (ng*hr/mL)	16276	146	15406	146	105.65	99.60 - 112.06	31.10	99.87
AUC0-inf (ng*hr/mL)	16330	142	15446	142	105.72	99.55 - 112.28	31.34	99.82
Cmax (ng/mL)	394.4	148	380.6	148	103.62	98.19 - 109.35	28.53	100.00

Treatment T2: A Single Subcutaneous Dose of INTP5 Administered at 6 mg/0.6 mL (Test Product T2)
Treatment R2: A Single Subcutaneous Dose of Neulasta® Administered at 6 mg/0.6 mL (Reference Product R2)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs derived from the ANOVA.
Geometric Mean Ratio (GMR) = 100*(test/reference)
Intra-subject CV% was calculated as 100 x square root(exp[MSE]-1), where MSE = Residual variance from ANOVA.
Source: Table 14.2.1.14
Program: /CA21285/sas_prg/pksas/intext-stats-tables-mixed2.sas 31MAR2017 7:34

2.4.3. Pharmacodynamics

Mechanism of action

G-CSF is a hematopoietic growth factor which regulates the production of neutrophils within the bone marrow; endogenous G-CSF is a glycoprotein produced by monocytes, fibroblasts, and endothelial cells. G-CSF promotes the growth, proliferation, differentiation, and maturation of neutrophil precursors. It induces their terminal differentiation and enhances the function of mature neutrophils by increasing phagocytic activity and antibody-dependent cell-mediated cytotoxicity.

The addition of a polyethylene glycol (PEG) moiety to filgrastim (rHu-met-G-CSF, with an identical sequence to the endogenous one, except for the addition of an N-terminal methionine necessary for expression in *E. coli*.) resulted in the development of pegfilgrastim. Pegfilgrastim is produced by conjugating methoxy polyethylene glycol (PEG)-propionaldehyde (20 kDa) to the N-terminal methionine residue of G-CSF. Pegfilgrastim is a long-acting form of filgrastim. Pegfilgrastim retains the same biological activity as filgrastim, and binds to the same G-CSF receptor, stimulating the proliferation, differentiation and activation of neutrophils (Curran and Goa, 2002; Neulasta CPM).

Primary and Secondary pharmacology

Primary pharmacology study Pelgraz-02

For the pharmacodynamic characterization of absolute neutrophil counts (ANC), blood samples were drawn prior to the dosing time (0 hours) and at 1, 2, 4, 8, 12, 18, 24, 28, 32, 36, 40, 48, 60, 72, 84, 96, 108, 120, 144, 168, 216, 264, 312 and 360 hours after dosing.

For the pharmacodynamic characterization of absolute CD34+ cell counts (CD34+), blood samples were drawn prior to the dosing time (0 hours) and at 24, 48, 72, 84, 96, 108, 120, 144 168, 192, 240 and 288 hours after dosing. Blood samples for immunogenicity assessment were taken prior to Period 1 dosing (0 hours) and at 672 hours after dosing in both periods 1 and 2.

Endpoints

Primary PD endpoints were the AUECt and Emax parameters of the absolute neutrophil count (ANC).

Secondary endpoints were: Tmax (for ANC endpoint), AUECt and Emax (for CD34+ endpoint).

The tertiary PD endpoint for CD34+ was the Tmax parameter.

Calculation of PD parameters was performed using the Enterprise Pharmacology (EP 2.5) software. The statistical analysis of the PD data was performed using SAS software version 8.2.

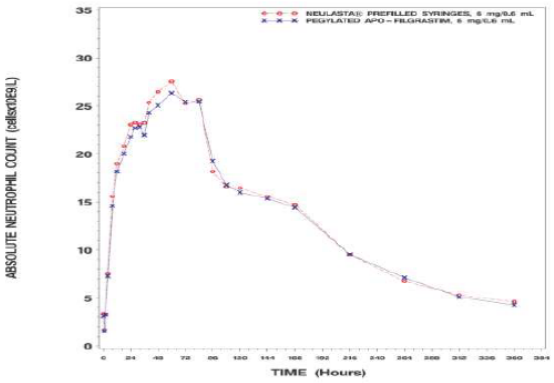
PD Statistical Analysis: For ANC and absolute CD34+ cell count data, ANOVA was performed on the log-transformed AUECt and Emax parameters and on the untransformed Tmax parameter (Proc GLM of SAS). The ANOVA included sequence, subjects nested within sequence, period and treatment as factors. The significance of the sequence effect was tested using the subjects nested within sequence as the error term. Based on the same statistical model, the least-squares means for the treatments, the adjusted differences between the treatment means and the standard error associated with these differences were estimated.

For the ANC data, the two one-sided hypothesis was tested at the α=0.025 level of significance by constructing the 95% confidence interval (95% CI) for the ratio between the test and reference means.

For the absolute CD34+ cell count data, the 95% CI for the ratio between the test and reference means was constructed for information purposes.

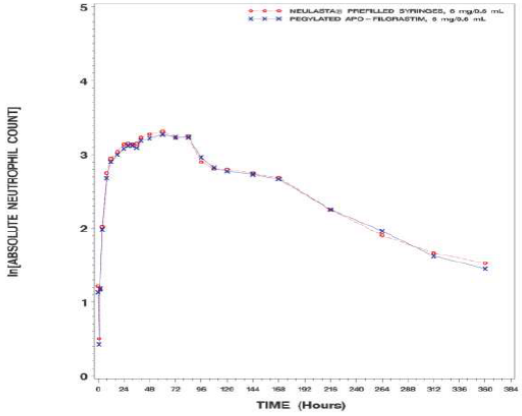
The PD statistical analyses included descriptive statistics across subjects and within treatments, periods and sequences.

Pharmacodynamic Results :



Source: APO-Peg-02, Section 11.4.6, Pharmacodynamic Population

Figure 2.7.3-1. APO-Peg-02: Mean Cell Count-Time Profile of Absolute Neutrophil Count (Linear Plot) Following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-Licensed Neulasta in Healthy Volunteers (PD Population)



Source: APO-Peg-02, Section 11.4.6, Pharmacodynamic Population

Figure 2.7.3-2. APO-Peg-02: Ln (Average Cell Count)-Time Profile of Absolute Neutrophil Count Following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-Licensed Neulasta in Healthy Volunteers (PD Population).

Figure 4 <u>1- ANC</u>

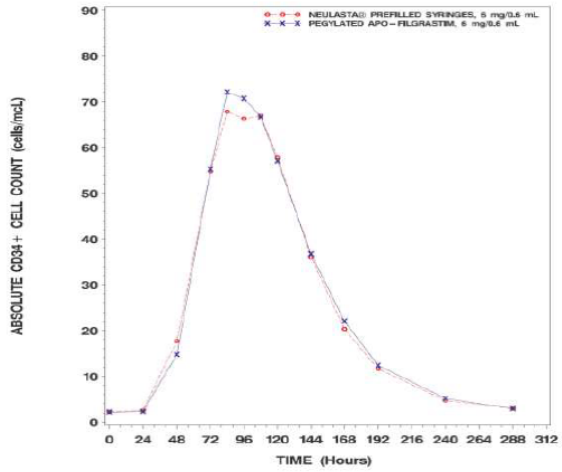
For both treatments, ANC increased as expected after single dose subcutaneous (SC) dose administration with peak levels occurring at approximately 64 hours and 61 hours post dose for Pelgraz and Neulasta respectively, and returning towards baseline levels by approximately 360 hours post-dose. A summary of the corresponding PD endpoint parameters is presented below.

Table 21 APO-Peg-02: Summary of ANC PD Endpoint Parameters following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-Licensed Neulasta to Healthy Subjects (PD Population)

ANC PD Parameter	Pegylated Apo-Filgrastim Mean[#] (CV %) N = 56	US-Licensed Neulasta Mean[#] (CV %) N=56	Relative Mean[##] Ratio [%]	95% CI [%]	Pr > [t][###]
AUECt (cells x 10^9*h/L)	4749.85 (26)	4817.55 (27)	98.8	96.0-101.6	0.3822
Emax (cells x 10^9)	29.75 (27)	30.94 (28)	96.3	92.6-100.1	0.0566
Tmax (h)	63.43 (26)	60.86 (31)	103.8	96.1 - 111.4	0.3251

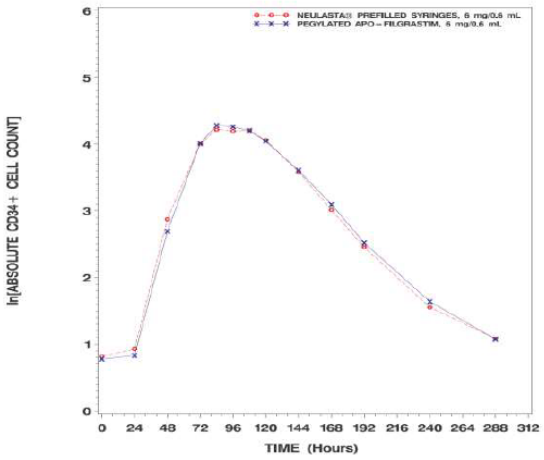
[#] of raw data
[##] Based on the least square estimates of the geometric means of AUECt and Emax, and based on the least square estimates of the arithmetic means for Tmax.
[###] P value is significance of treatment effect
Source: APO-Peg-02, Appendix 16.1.9.2

This pharmacodynamic similarity is further evidenced by the results for the additional PD marker, Absolute CD34+ cell count. The linear and log-linear plots for CD34+ count are presented below.



Source: APO-Peg-02, Section 11.4.6, Pharmacodynamic Population

Figure 2.7.3-3. APO-Peg-02: Mean Cell Count-Time Profile of CD34+ Count (Linear Plot) Following a Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-licensed Neulasta in Healthy Volunteers (PD Population).



Source: APO-Peg-02, Section 11.4.6, Pharmacodynamic Population

Figure 2.7.3-4. APO-Peg-02: Ln (Average Cell Count)-Time Profile of CD34+ Count Following a Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-licensed Neulasta in Healthy Volunteers (PD Population)

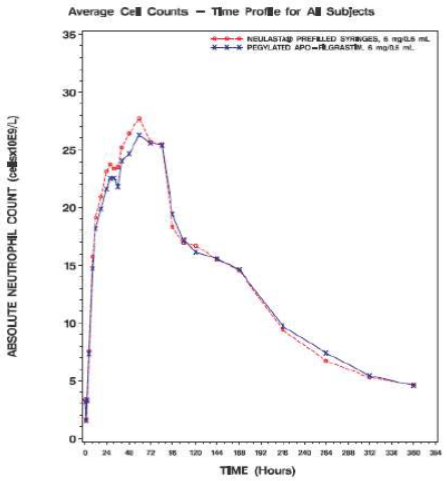
Figure 5 -Absolute CD34+ cell count

Table 22 APO-Peg-02: Summary Table for CD34+ Count Endpoint Parameters following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-Licensed Neulasta to Healthy Subjects (PD Population)

CD34+ Count PD Parameter	Pegylated Apo-Filgrastim Mean[#] (CV %) N=56	US-Licensed Neulasta Mean[#] (CV %) N=56	Relative Mean[##] Ratio [%]	95% CI [%]	Pr > [t][###]
AUECt (cells*h/mcL)	7153.34 (73)	6991.64 (97)	105.9	99.5-112.7	0.0693
Emax (cells/mcL)	78.82 (63)	76.03 (89)	106.8	98.7-115.5	0.0998
Tmax (h)	94.07 (13)	96.64 (15)	97.6	94.0-101.3	0.2019

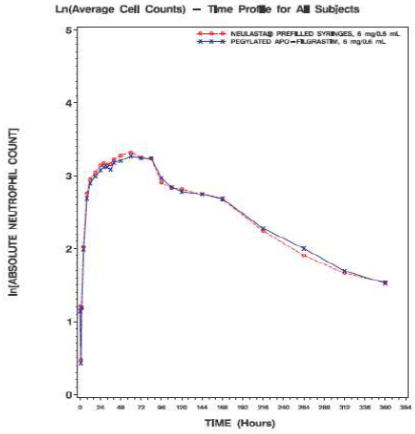
[#] of raw data
[##] Based on the least square estimates of the geometric means of AUECt and Emax, and based on the least square estimates of the arithmetic means for Tmax,
[###] P value is significance of treatment effect
Source: APO-Peg-02; Appendix 16.1.9.3

The pharmacodynamic endpoint parameters were also analyzed for the ITT population:



Source: APO-Peg-02, Appendix 16.6.2.2.2

Figure 2.7.3-5. APO-Peg-02: Mean Cell Count-Time Profile of Absolute Neutrophil Count (Linear Plot) Following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-Licensed Neulasta in Healthy Volunteers (ITT Population).



Source: APO-Peg-02, Appendix 16.6.2.2.3

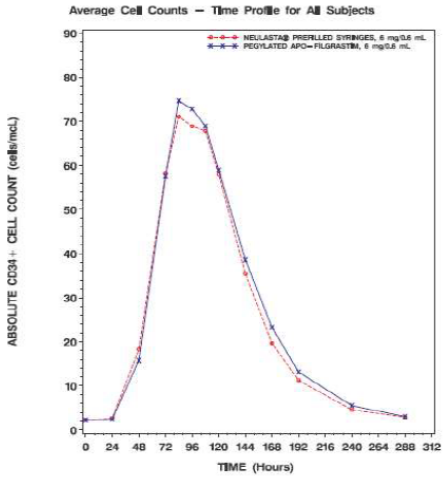
Figure 2.7.3-6. APO-Peg-02: Ln (Average Cell Count)-Time Profile of Absolute Neutrophil Count Following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-Licensed Neulasta in Healthy Volunteers (ITT Population).

Figure 6 1-ANC

Table 23 APO-Peg-02: Summary of Absolute Neutrophil Count PD Endpoint Parameters following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or Reference US-Licensed Neulasta to Heathy Subjects (PD Population)

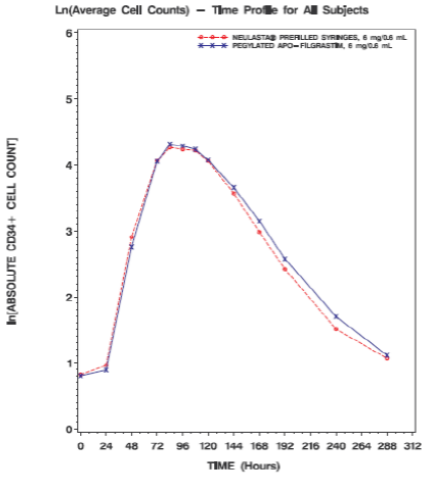
Endpoint	Pegylated Apo-Filgrastim Mean# (CV) (N=60)	US-Licensed Neulasta Mean# (CV) (N=63)	Ratio of Geometric Means## [%]	95% CI [%]	Pr > [F]###
AUECt [cellsx10^9*h/L]	4811.51	4823.41	99.7	96.6 – 102.8	0.8265
	28	27			
Emax [cellsx10^9/L]	29.64	31.37	95.6	91.8 – 99.5	0.0300
	28	28			
Tmax [h]	62.74	60.64	101.9	93.5 – 110.3	0.6514
	28	31			

Of raw data.
Based on the least squares estimates of the geometric means of AUCt and Cmax and based on the least square estimates of the arithmetic means for Tmax.
p value is significance of treatment effect
Source: APO-Peg-02, Appendix 16.6.2.2.5



Source: APO-Peg-02, Appendix 16.6.3.2.2

Figure 2.7.3-7. APO-Peg-02: Mean Cell Count-Time Profile of CD34+ Count (Linear Plot) Following a Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-Licensed Neulasta in Healthy Volunteers (ITT Population).



Source: APO-Peg-02, Appendix 16.6.3.2.3

Figure 2.7.3-8. APO-Peg-02: Ln (Average Cell Count)-Time Profile of CD34+ Count Following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or US-Licensed Neulasta in Healthy Volunteers (ITT Population).

Figure 7 2- Absolute CD34+ cell count

Table 24 APO-Peg-02: Summary of Absolute CD34+ Count PD Endpoint Parameters following a Fixed Single Subcutaneous Injection of 6 mg Pegylated Apo-Filgrastim or Reference US-Licensed Neulasta to Heathy Subjects (ITT Population)

Endpoint	Pegylated Apo-Filgrastim Mean# (CV)(N=59)	US-Licensed Neulasta Mean# (CV) (N=63)	Ratio of Geometric Means## [%]	95% CI [%]	Pr > [F]###
AUECt [cells*hr/mcl]	7458.89	7104.64	105.9	99.5 – 112.7	0.0723
	74	94			
Emax [cells/mcl]	81.42	78.74	106.8	98.7 – 115.5	0.0998
	63	87			
Tmax [h]	93.97	95.05	97.6	93.9 – 101.3	0.2019
	13	18			

Of raw data.
Based on the least squares estimates of the geometric means of AUCt and Cmax and based on the least squares estimates of the arithmetic means for Tmax.
p value is significance of treatment effect
Source: APO-Peg-02, Appendix 16.6.3.2.5

STUDY (154-14)

Criteria for PD Evaluation: A total of 20 blood samples were collected for PD evaluation from pre-dose (within 60 minutes prior to dosing) up to 672 hours post-dose. PD markers ANC and CD34+ were analysed using automated cell counter and flow cytometer, respectively, using validated methods at Lambda Therapeutic Research Ltd., India. The following PD parameters were calculated from the

baseline-adjusted and baseline non-adjusted ANC and CD34+ versus time profiles: AUEC0-t, Emax, and Tmax

Statistical Methods: Pharmacodynamics: Summary statistics, including sample size (n, arithmetic mean, SD, CV%, SEM, median, minimum, and maximum) were calculated for all nominal time points and ANC and CD34+ PD parameters. In addition, IQR, geometric means, and geometric CV% were presented for all PD parameters. A comparison of ln-transformed PD parameters Emax and AUEC0-t, for baseline-adjusted and baseline non-adjusted ANC and CD34+ was made to evaluate the relative response of T1 versus R1 and T2 versus R2, by performing an ANOVA model, similar to PK model for subjects in the PD evaluable population only. An additional statistical comparison that included excluded subjects was presented for supportive information only. Biosimilarity was concluded if the 95% CIs for the baseline-adjusted ANC Emax and AUEC0-t GMRs of T2 versus R2 lay within the 80.00% to 125.00% reference interval.

PD Results: The statistical comparisons of baseline adjusted/non-adjusted PD parameters are summarized below:

Table 25 Summary of Statistical Comparisons of Baseline Non-Adjusted Absolute Neutrophil Count Pharmacodynamic Parameters AUEC0-t and Emax for Test Product T1 versus Reference Product R1

	Treatment T1 (Test)		Treatment R1 (Reference)					
Parameter	Geometric LSMs	n	Geometric LSMs	n	GMR (%)	95% Confidence Interval	Intra-subject CV%	Power (%)
AUEC0-t (hr x 10^3/uL)	5730.8	135	5790.6	135	98.97	96.58 - 101.41	10.16	100.00
Emax (x 10^3/uL)	30.44	144	29.96	144	101.59	98.91 - 104.35	11.54	100.00

Treatment T1: A Single Subcutaneous Dose of INTP5 Administered at 3 mg/0.3 mL (Test Product T1)
Treatment R1: A Single Subcutaneous Dose of Neulasta® Administered at 3 mg/0.3 mL (Reference Product R1)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the ANOVA.
Geometric Mean Ratio (GMR) = 100*(test/reference)
Intra-subject CV% was calculated as 100 x square root(exp[residual variance]-1).
Source: Table 14.2.2.11
Program: /CA21285/sas_prg/pksas/pd/intext-pd-stats-tables.sas 07APR2017 10:43

Table 26 Summary of Statistical Comparisons of Baseline Non-Adjusted Absolute Neutrophil Count Pharmacodynamic Parameters AUEC0-t and Emax for Test Product T2 versus Reference Product R2

	Treatment T2 (Test)		Treatment R2 (Reference)					
Parameter	Geometric LSMs	n	Geometric LSMs	n	GMR (%)	95% Confidence Interval	Intra-subject CV%	Power (%)
AUEC0-t (hr x 10^3/uL)	7149.0	144	7191.7	144	99.41	97.22 - 101.64	9.54	100.00
Emax (x 10^3/uL)	34.93	148	35.29	148	98.98	96.05 - 102.01	13.07	100.00

Treatment T2: A Single Subcutaneous Dose of INTP5 Administered at 6 mg/0.6 mL (Test Product T2)
Treatment R2: A Single Subcutaneous Dose of Neulasta® Administered at 6 mg/0.6 mL (Reference Product R2)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the ANOVA.
Geometric Mean Ratio (GMR) = 100*(test/reference)
Intra-subject CV% was calculated as 100 x square root(exp[residual variance]-1).
Source: Table 14.2.2.12
Program: /CA21285/sas_prg/pksas/pd/intext-pd-stats-tables.sas 07APR2017 10:43

Table 27 Summary of Statistical Comparisons of Baseline Adjusted Absolute Neutrophil Count Pharmacodynamic Parameters AUEC0-t and Emax for Test Product T1 versus Reference Product R1

	Treatment T1 (Test)		Treatment R1 (Reference)					
Parameter	Geometric LSMs	n	Geometric LSMs	n	GMR (%)	95% Confidence Interval	Intra-subject CV%	Power (%)
AUEC0-t (hr x 10^3/uL)	3307.6	135	3398.5	135	97.32	93.41 - 101.41	17.19	100.00
Emax (x 10^3/uL)	26.65	144	26.24	144	101.56	98.33 - 104.89	13.92	100.00

Treatment T1: A Single Subcutaneous Dose of INTP5 Administered at 3 mg/0.3 mL (Test Product T1)
Treatment R1: A Single Subcutaneous Dose of Neulasta® Administered at 3 mg/0.3 mL (Reference Product R1)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the ANOVA.
Geometric Mean Ratio (GMR) = 100*(test/reference)
Intra-subject CV% was calculated as 100 x square root(exp[residual variance]-1).
Source: Table 14.2.3.11
Program: /CA21285/sas_prg/pksas/pd/intext-pd-stats-tables.sas 07APR2017 10:43

Table 28 Summary of Statistical Comparisons of Baseline Adjusted Absolute Neutrophil Count Pharmacodynamic Parameters AUEC0-t and Emax for Test Product T2 versus Reference Product R2

	Treatment T2 (Test)		Treatment R2 (Reference)					
Parameter	Geometric LSMs	n	Geometric LSMs	n	GMR (%)	95% Confidence Interval	Intra-subject CV%	Power (%)
AUEC0-t (hr x 10^3/uL)	4757.2	144	4720.0	144	100.79	97.75 - 103.92	13.17	100.00
Emax (x 10^3/uL)	31.20	148	31.61	148	98.70	95.52 - 101.98	14.23	100.00

Treatment T2: A Single Subcutaneous Dose of INTP5 Administered at 6 mg/0.6 mL (Test Product T2)
Treatment R2: A Single Subcutaneous Dose of Neulasta® Administered at 6 mg/0.6 mL (Reference Product R2)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the ANOVA.
Geometric Mean Ratio (GMR) = 100*(test/reference)
Intra-subject CV% was calculated as 100 x square root(exp[residual variance]-1).
Source: Table 14.2.3.12
Program: /CA21285/sas_prg/pksas/pd/intext-pd-stats-tables.sas 07APR2017 10:43

Secondary pharmacology

Immunogenicity:

A total of 190 samples were collected from subjects and analysed using a multi-tiered approach to screen, confirm, and report a relative ADA concentration (titer). Confirmed positive samples were also characterized for specificity and neutralizing activity.

Based on the test results of the 190 samples from 66 subjects, 16 samples from 10 subjects were reported as potential positive in the ADA screening assay and were subsequently analyzed in the confirmatory assay. Of the 16 samples analyzed, 10 samples from 6 subjects were confirmed positive in the ADA confirmatory assay and underwent further characterization for binding specificity and neutralizing activity.

None of the 66 subjects dosed in Period 1 had confirmed positive ADAs at baseline (pre- dose) samples. Therefore the prevalence of pre-existing antibodies in this study was 0% (0/66). In Period 1, three subjects (GC01, GC50 and GC66) had detectable treatment emergent ADA after receiving Pelgraz in Period 1, representing an incidence of 9% (3 out of 33). Titers at 672 hrs in Period 1 for these subjects ranged from 1- 8. Three subjects (GC24, GC34 and GC62) had detectable treatment

emergent ADA after receiving Neulasta in Period 1, representing an incidence of 9% (3 out of 33). Titers at 672 hrs in Period 1 for these subjects ranged from 1-14.

There was no apparent difference in induction or magnitude of ADA after exposure to either Pelgraz or Neulasta.

In Period 2 of this study, with a crossover design, 27 subjects received Pelgraz (after prior exposure to Neulasta) and 30 subjects received Neulasta (after prior exposure to Pelgraz). No additional subjects developed ADA in Period 2 of this study and no ADA positive subjects had increased ADA titers. Two subjects became ADA negative; therefore, 4 subjects were positive for ADA at the end of Period 2. Titers at Period 2 ranged from 1 to 12.

The results showed that the majority of the ADA positive subjects (5 of 6 subjects or 83%) were positive for anti-PEG antibodies (8 out of 10 samples or 80.0%). Antibodies were detected to Apo-Filgrastim in 4 subjects (67%; 6 of 10 samples or 60%) and to recombinant human granulocyte colony-stimulating factor (rhuGCSF) in 2 subjects (33%; 3 of 10 samples or 30%). The results of the confirmed ADA positive samples from the 6 subjects were tested in the cell-based assay to evaluate the presence of antibodies with neutralizing activity. All confirmed ADA positive samples tested negative for neutralizing antibodies.

The binding specificity evaluated in the characterization assays of ADAs can be summarized in 5 categories:

a. Binding specific to Pelgraz AS, rhuGCSF and PEG

b. Binding specific only to Pelgraz AS and PEG

c. Binding specific only to rhuGCSF and PEG

d. Binding specific only to Pelgraz AS

e. Binding specific only to PEG

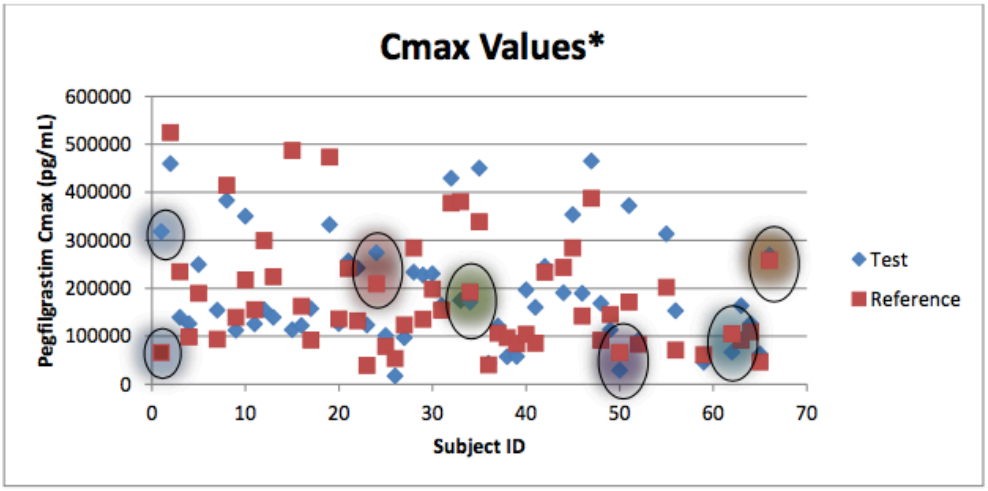
Table 29 Summary of Immunogenicity Results for ADA Confirmed Positive Subjects

Subject-ADA Confirmed Positive	Period	Treatment	Sample Timepoint (hr)	Characterization (Positive (+) or Negative (-))			ADA Titer	Final NAb Result
				PEG	Apo-Filgrastim	rhuGCSF		
GC01*	1	Pegylated Apo-Filgrastim	672	+	-	+	8	Negative
	2	US-Neulasta	672	+	+	+	3	Negative
GC24*	1	US-Neulasta	672	+	+	+	3	Negative
	2	Pegylated Apo-Filgrastim	672	+	-	-	1	Negative
GC34*	1	US-Neulasta	672	+	+	-	14	Negative
	2	Pegylated Apo-Filgrastim	672	+	+	-	12	Negative
GC50	1	Pegylated Apo-Filgrastim	672	+	-	-	1	Negative
GC62*	1	US-Neulasta	672	-	+	-	1	Negative
	2	Pegylated Apo-Filgrastim	672	-	+	-	1	Negative
GC66	1	Pegylated Apo-Filgrastim	672	+	-	-	1	Negative

Source: APO-Peg-02, Table 18

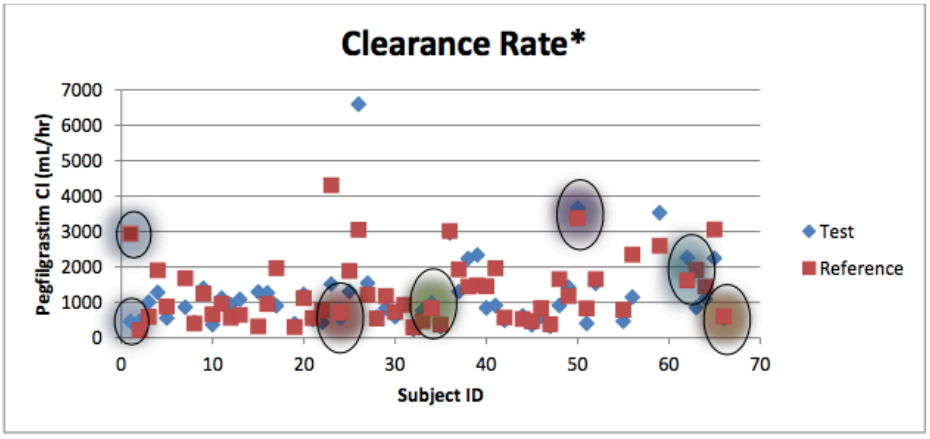
To more closely examine any potential impact of ADA on the data, the rate and extent of drug exposure (i.e., AUCt, Cmax) and clearance rate (Cl) of Pelgraz and Neulasta (Reference), parameters which had the potential of being influenced by ADA, were presented using scatter plots for all subjects included in the PK population (n=56).

The Cmax, AUCt and Clearance values for subjects GC01, GC24, GC34, GC50, GC62, and GC66 were consistent with the rest of the subjects in this study. For all ADA-positive subjects except GC01, the magnitude of Period 2 data was not substantially different from that of Period 1, and was in the range of other subjects in the study. Even though Subject GC01 was associated with relatively high P1/P2 ratios, the Cmax and AUCt values within both periods/treatments were in line with the values obtained for the rest of the subjects in the study. Therefore, it is concluded that ADA development did not have an attributable impact on the extent and rate of exposure or clearance of pegfilgrastim.



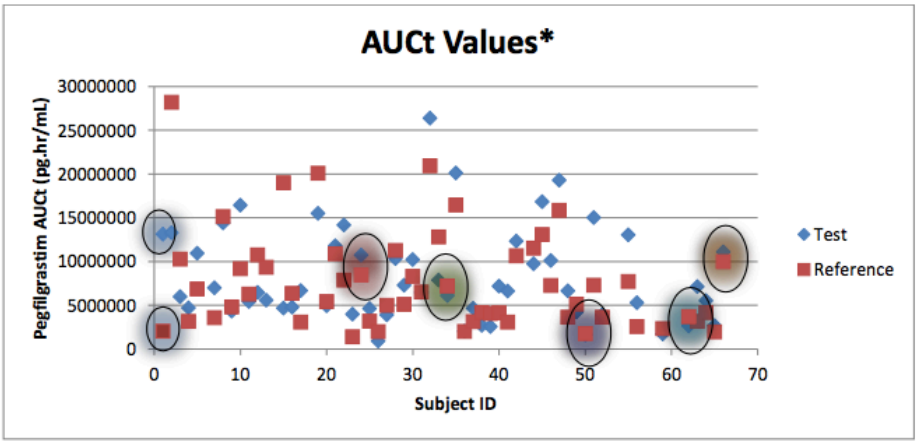
* Based on raw data. Similar results are obtained from the content-adjusted data.
Source: APO-Peg-02, Figure 12

Figure 8Cmax Values Analysis with ADA-positive population Identified



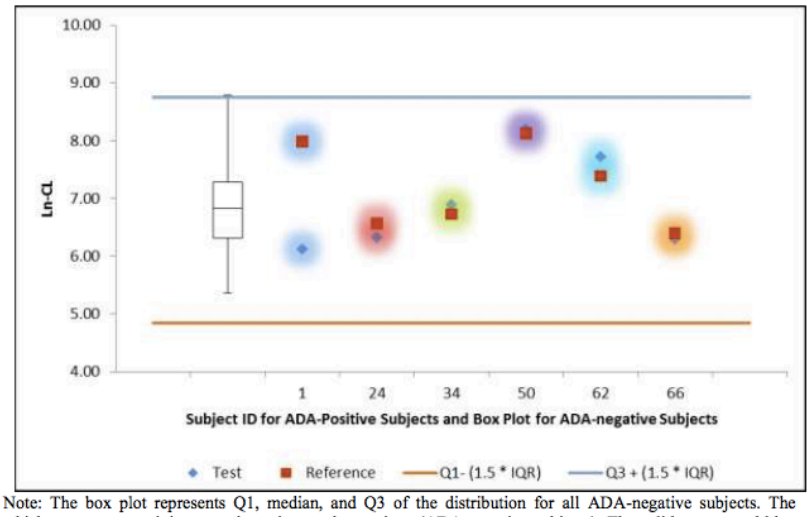
* Based on raw data. Similar results are obtained from the adjusted data.
Source: APO-Peg-02, Figure 14

Figure 9 Clearance rate Analysis with ADA-positive Population Identified



* Based on raw data. Similar results are obtained from the content-adjusted data.
Source: APO-Peg-02, Figure 13

Figure 10 AUC-t Values Analysis with ADA-positive Population Identified



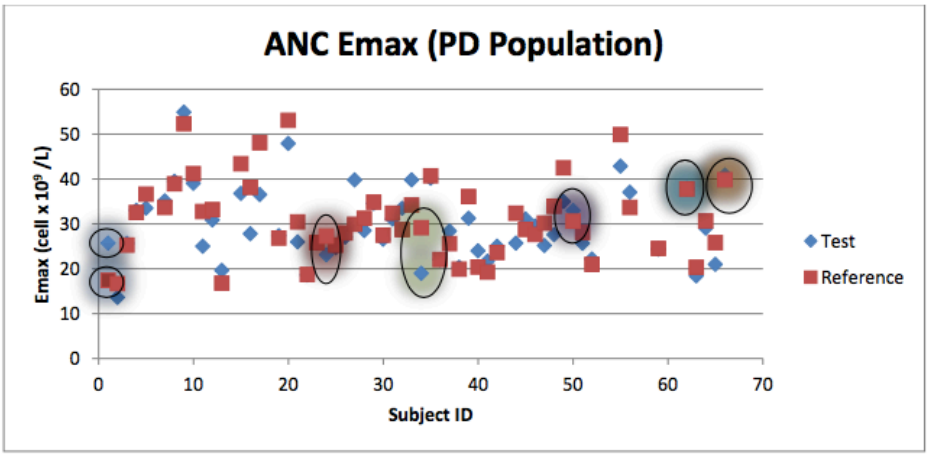
Note: The box plot represents Q1, median, and Q3 of the distribution for all ADA-negative subjects. The whiskers represent minimum and maximum observations (ADA-negative subjects). The solid orange and blue lines represent the lower and upper bound of the 1.5*IQR range (ADA-negative subjects), respectively. The scatter points represent the individual ln-Cl for ADA-positive subjects; all ADA-positive subjects are within the 1.5*IQR range.

Source: APO-Peg-02, Figure 15

Figure 11 In-transformed Cl Box Plot Analysis with ADA-positive Population identified

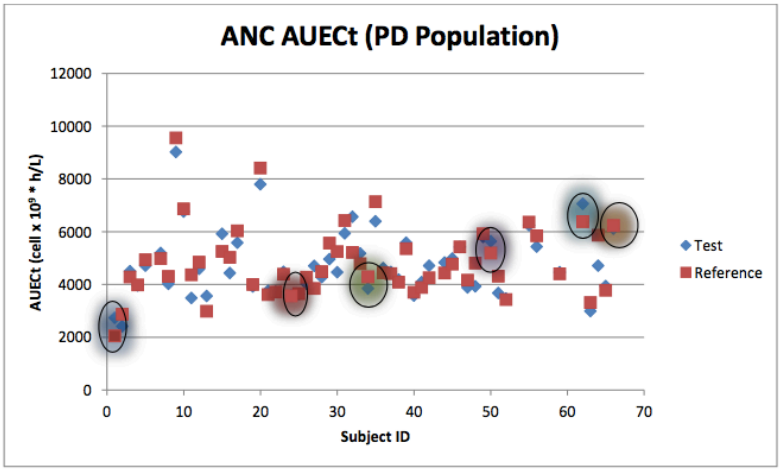
The titer levels observed in the ADA-positive subjects in this study are considered very low (range 1-14 after minimum required dilution of 1:20).

With respect to the PD parameters, the ANC and CD34+ Emax and AUECt values for the subjects who were confirmed ADA-positive (GC01, GC24, GC34, GC50, GC62, and GC66) were in line with the values obtained from the rest of the subjects in the study as illustrated in the plots below. Overall, there is no evidence to support a significant impact of ADA development on the pharmacodynamics of pegfilgrastim.



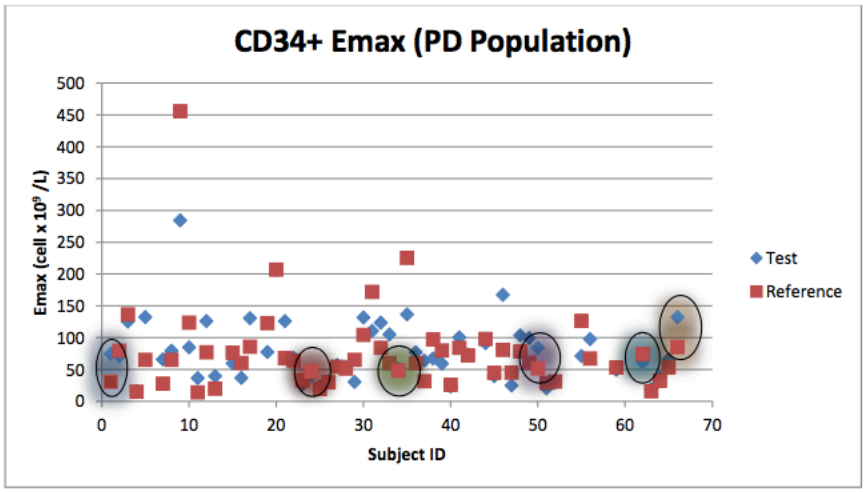
Source: APO-Peg-02, Figure 16

Figure 12 Absolute Neutrophil Count (ANC) Emax Analysis with ADA-positive Population Identified



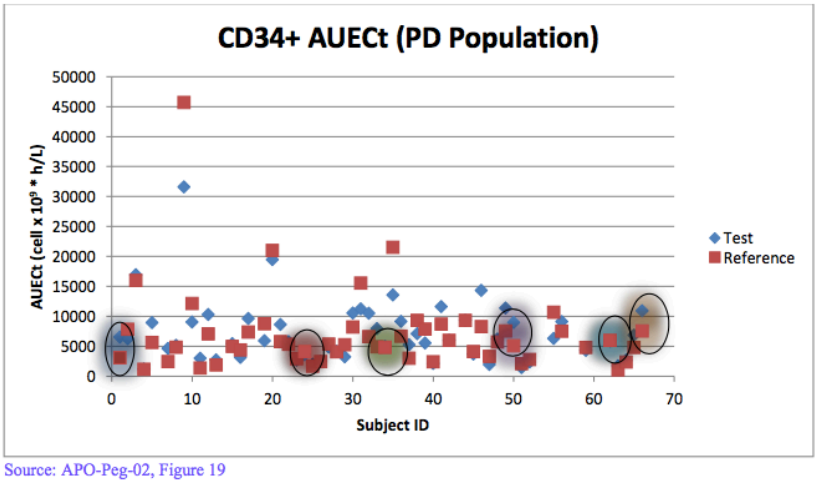
Source: APO-Peg-02, Figure 17

Figure 13 ANC AUECt Analysis with ADA-positive Population Identified



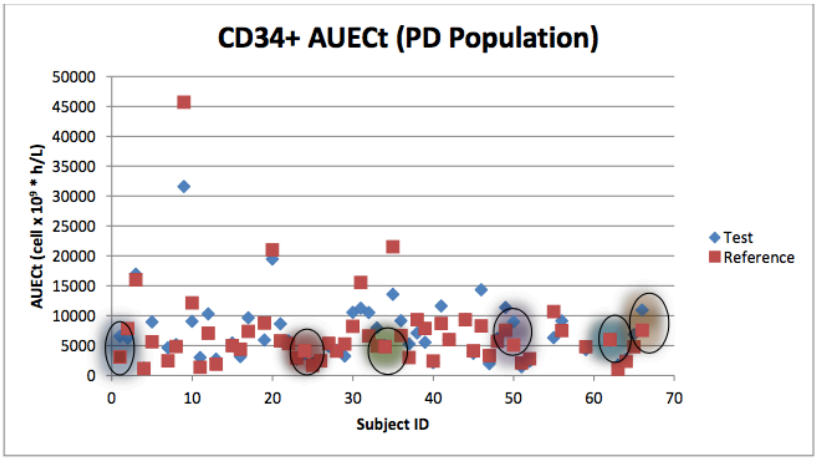
Source: APO-Peg-02, Figure 18

Figure 14 CD34+ Emax Analysis with ADA-positive Population Identified



Source: APO-Peg-02, Figure 19

Figure 15 CD34+ AUECt Analysis with ADA-positive Population identified



Source: APO-Peg-02, Figure 19

Figure 16 CD34+ AUECt Analysis with ADA-positive Population Identified

2.4.4. Discussion on clinical pharmacology

The Pelgraz development program utilized a step-wise approach and included an analytical similarity exercise for Pelgraz with both EU-approved and US-licensed Neulasta to demonstrate similarity in the quality attributes of the proposed biosimilar and the reference biologic drug Neulasta - in order to generate data that supports the establishment of a scientific bridge between the two reference products. Furthermore, both EU-approved Neulasta (RMP) and US-licensed Neulasta were used in the non-clinical and clinical (Phase III) studies and the US-licensed Neulasta was used in the Phase I PK/PD study.

The clinical comparability exercise begins with pharmacokinetic (PK) and pharmacodynamic (PD) comparative studies followed by comparative clinical efficacy trial versus the chosen reference medicinal product. Additionally, the pivotal Phase III 3-arm Efficacy and Safety Study conducted in breast cancer patients on docetaxel, doxorubicin, and cyclophosphamide (TAC) chemotherapy, comparing Pelgraz to both US-licensed and EU-approved Neulasta.

In general, the Applicant´s development programme to demonstrate the similarity between Pelgraz and Neulasta with respect to the pharmacokinetic (PK) is considered adequate and was performed according to the guidance on biosimilars.

This study Pelgraz-02 was a phase I, comparative, randomized, single-dose, assessor-blinded, 2-way crossover PK/PD study of subcutaneously administered Pelgraz (Test) and Neulasta (reference) (USA) in healthy volunteer subjects under fasting conditions separated by a washout period of 8 weeks.

The 6 mg SC dose was selected because it is the only dose approved and used as current standard of care for the comparator product Neulasta and therapeutic comparability was based on the clinical efficacy endpoint DSN in the first cycle of chemotherapy in a comparative way.

The selection of $AUC_{0\text{-tlast}}$ as primary PK endpoint is in line with the Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor (EMEA/CHMP/BMWP/31329/2005). C_{max} is also evaluated as primary endpoint, which is in line with the Guidance mentioned above, and to the Biosimilar Guideline (EMEA/CHMP/BMWP/42832/2005 Rev1) in which, $AUC_{0\text{-inf}}$ and C_{max} should be evaluated as co-primary endpoint for subcutaneously administered products. In addition, AUC_{inf} should also be considered as primary PK endpoint according to the PKWP

document in the assessment of biosimilarity since AUC_{inf} reflects CL/F for subcutaneously administered products. In conclusion, the PK endpoints are considered acceptable since the AUC_{0-inf}, AUC_{0-t} and C_{max} has been assessed.

Prior to performing the PK/PD statistical analysis, a treatment-blinded review of the pegfilgrastim, ANC and $CD34^+$ data was performed by evaluating the data and comparing the period 1 versus period 2 data for each subject. The blinded review of the pegfilgrastim concentration-time data for all subjects revealed that the concentration-time profiles for subject GC01 and GC02 were not consistent between periods. This is not considered acceptable. However, as the two subjects under investigation were finally included in the dataset, this issue is considered irrelevant.

For the adjusted data, the results demonstrate that the 90% confidence interval of the test/reference ratio for the primary PK endpoints of the study, AUC_{0-t} and C_{max}, are within 80–125% and the 100% was included within the 90 % CI. Moreover, the secondary and tertiary PK endpoints further support the PK conclusions of this study as the 90% confidence intervals for the AUC_{inf}, Cl and V_d parameters are contained within 80-125% and the 90% confidence intervals for the untransformed parameters t_{max}, K_{el} and t_{half} are also contained within the limits of 80-125%.

Following the clinical phase of the study and prior to conducting the pharmacokinetic and statistical analysis, it was noted that the applied dose of Pelgraz appeared to be on average higher by 5% than the dose of Neulasta used as the reference product and an statistical analysis in which adjustment for drug content was performed prior to performing database lock and unblinding of data and statistical analysis of the data. Based on the information submitted, it appears that the decision to adjust was not made after unblinding of the study. A specific memo detailing the need of drug content-adjustment of data was issued by the sponsor to the PK/stats group prior to unblinding the study data. This memo was issued on March 28th, 2014, prior to database lock on March 31st, 2014 and prior to unblinding of the study on 1 April 01st, 2014. Adjustment was based on the measured pegfilgrastim protein concentration and purity data as listed on the Certificate of Analysis for each product to account for the difference in pegfilgrastim content between the batches of Pelgraz and US-licensed Neulasta used in this study in order to meet the predefined acceptance range.

Results based on the unadjusted data demonstrate that the 90% confidence interval of the test/reference ratio for the primary, secondary and tertiary PK endpoints of the study are within 80–125% with the exception of AUC_{0-t} which had an upper limit of 125.5% and for Cl which had a lower limit of 79.7%. In our opinion AUC_{inf} is more relevant for biosimilars that AUC_{0-t} since it reflects CL/F, and the results for the AUC_{0-inf} demonstrate that the 90% confidence interval of the test/reference ratio is within 80–125%.

The AUC_{inf} could not be determined in 3 subjects for reference product and in 6 subjects for the test product and it appears that the subjects were excluded based on the SOP criteria. When these subjects data were considered the results change slightly and AUC 90% CI for the ratio test / reference falls slightly outside of the predefined acceptance range 80-125% if the data is not adjusted, but it is expected that it will be within the limits if adjusted.

In addition, the Applicant has conducted an additional comparative PK and PD study (study 154-14), an Assessor Blind, Randomized, Single-Dose, Crossover, Comparative Pharmacokinetic and Pharmacodynamic Study of Two Dose Levels Of INTP5 with Two Dose Levels of Neulasta (EU-Licensed Product) in Healthy volunteers Under Fasting Condition. In this study, the comparison of the key PK parameters (AUC_{0-t}, AUC_{0-inf}, and $C_{max)}$ of the test product, INTP5, relative to the reference product, EU-Neulasta, were within the 80.00% to 125.00% reference interval. Therefore, it confirmed that the applied product exhibits a similar exposure.

The Pharmacodynamics of Pelgraz were assessed in a single phase 1 PK/PD study (APO-Peg-02), which included healthy volunteers and compared the PK/PD characteristics of Pelgraz and US-licensed Neulasta (Amgen Inc.). The possibility to use a non-EU authorised comparator in part of the comparability exercise is reflected in the CHMP guideline for biosimilars ("Guideline on similar biological medicinal products CHMP/437/04 Rev 1"). The pivotal PK/PD study was a single-dose, randomized, assessor-blinded, two-way crossover, active-controlled, study in healthy volunteers. The primary PD endpoints were the AUECt and Emax parameters of the absolute neutrophil count (ANC), while secondary endpoints were: Tmax (for ANC endpoint), AUECt and Emax (for CD34+ endpoint). The 95% CI of the primary PD endpoint parameters (ANC-AUECt and ANC-Emax) were within the pre-defined acceptance margins of 80-125%, as well as those for ANC-Tmax and CD34+ cells-AUECt and Emax (secondary endpoints). Results were consistent between the PD population (patients who completed both periods) and the ITT population (patients who received at least one dose of the investigational product).

Further a new PK/PD study was submitted. In this newly submitted PK/PD study (154-14), the 95% CIs of the GMRs derived from the analysis on the ln-transformed baseline non-adjusted ANC PD parameters AUEC0-t and Emax of the test product, INTP5, relative to the reference product, Neulasta, were also within the 80.00% to 125.00% interval. Therefore, from a PK/PD perspective, results from study 154-14 demonstrated comparability between INTP5 and EU-Neulasta.

Overall, the findings conducted in healthy volunteers show a low immunogenic potential of Pelgraz, with no apparent differences between Pelgraz and Neulasta (US-licensed). Data presented indicate that there is no evidence to support a significant impact of ADA development on the pharmacokinetics or pharmacodynamics of Pelgraz or Neulasta (see clinical Efficacy and safety sections).

2.4.5. Conclusions on clinical pharmacology

From a PK/PD perspective, results from available studies demonstrated comparability between Pelgraz AS and Neulasta.

Overall the findings from the study conducted in healthy volunteers show a low immunogenic potential of Pelgraz, with no apparent differences between Pelgraz and Neulasta (US-licensed). Data presented from healthy volunteers indicate that there is no evidence of a significant impact of ADA development on the pharmacokinetics or pharmacodynamics of Pelgraz or Neulasta.

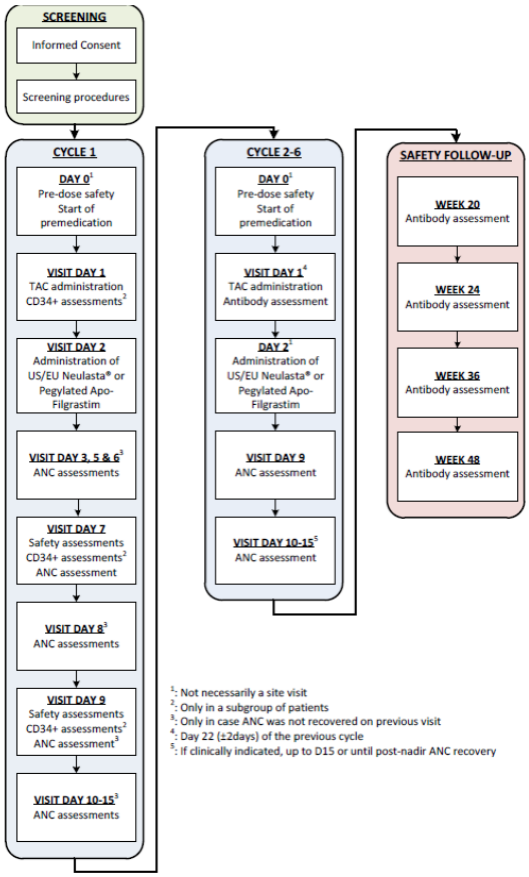
2.5. Clinical efficacy

2.5.1. Dose response study(ies)

No specific dose-response studies were submitted with the initial application. The Applicant selected the dose based on the approved one for US- and EU-Neulasta a fixed SC dose of 6 mg, once per cycle.

2.5.2. Main study(ies)

Study Pelgraz-03 was a phase III, multicentre, randomized, active controlled, assessor blinded, safety and efficacy equivalence trial in patients undergoing adjuvant TAC therapy after surgical resection of breast cancer. Subjects were randomized (2:1:1) to either Pelgraz, Neulasta US or Neulasta EU.



Source: APO-Peg-03, Figure 1

Figure 17 Apo-Peg-03 Overall Study Design

Methods

Study Participants

Main Inclusion Criteria:

- Female, ≥18 of age, suitable and intended to undergo adjuvant TAC (docetaxel, doxorubicin, cyclophosphamide) chemotherapy.
- Body weight within 40 and 120 kg.
- Subjects within 60 days of complete surgical resection of the primary breast tumor; either lumpectomy or mastectomy with sentinel lymph node biopsy or axillary dissection, with clear margins for both invasive and ductal carcinoma in situ (DCIS).
- Stage IIA, IIB or IIIA breast cancer.
- ECOG performance status ≤ 2.
- ANC ≥1.5 x 109/L; platelet count ≥100 x 109/L
- Adequate renal [serum creatinine <1.5 x upper limit of normal (ULN)] and hepatic function (bilirubin < ULN, transaminases and alkaline phosphatase (AP) <1.5 x ULN)

- Normal cardiac function evidenced by a left ventricle ejection fraction (LVEF) ≥55%.
- No evidence of metastatic disease.
- Baseline bilateral mammography (or other scan to exclude cancer on the contralateral breast).

Main Exclusion Criteria:

- Bilateral breast cancer (concomitant or prior) except in situ lesion of the contralateral breast.
- Prior chemotherapy (either adjuvant or neoadjuvant) for this breast cancer.
- History of myocardial infarction, heart failure, uncontrolled angina, severe uncontrolled arrhythmias, pericardial disease, or electrocardiographic evidence of acute ischemic changes.
- Immunotherapy, hormonal therapy (e.g. tamoxifen or aromatase inhibitors), Herceptin (trastuzumab) concurrently or within 30 days of screening.
- Concurrent radiation therapy.
- Investigational therapy concurrently or within 30 days of screening.
- Peripheral neuropathy above Grade 1.
- Major organ allograft or condition requiring chronic immunosuppression.
- Serious uncontrolled intercurrent medical or psychiatric illness.
- Active hepatitis B or hepatitis C with abnormal liver function tests (LFTs) or known to be HIV positive.
- History of other malignancy within the last 5 years.
- Pregnancy or breastfeeding.

Treatments

Table 30 Investigational medicinal products

Treatment T (considered as investigational test product)	Name: APO-Peg (Apotex) Dose: 6 mg/0.6 mL in pre-filled syringe Mode of Administration: Subcutaneous
Treatment R_{US} (considered as investigational reference product)	Name: Neulasta® inj. (Amgen Manufacturing Limited, USA) Dose: 6 mg/0.6 mL in pre-filled syringe Mode of Administration: Subcutaneous
Treatment R_{EU} (considered as investigational reference product)	Name: Neulasta® inj. (Amgen B.V.; EU) Dose: 6 mg/0.6 mL in pre-filled syringe Mode of Administration: Subcutaneous

Non-investigational medicinal products:

The only allowed chemotherapy regimen for this study was TAC, consisting of:

- docetaxel 75 mg/m^2 i.v. Day 1
- doxorubicin 50 mg/m^2 i.v. Day 1

- cyclophosphamide 500 mg/m^2 i.v. Day 1.

Premedication for Chemotherapy*:*

Antiemetic treatment with dexamethasone and ondansetron were required to improve the cytostatic tolerability. Dexamethasone premedication (six doses of 8 mg by mouth, twice daily) started on the day before chemotherapy and ended in the evening of the day after chemotherapy in order to prevent docetaxel-related hypersensitivity and fluid retention. Three doses of dexamethasone were prescribed to be taken before administration of chemotherapy. Ondansetron (8 mg) premedication was given to prevent chemotherapy related nausea and vomiting. It could be given either orally or intravenously, and it was given before and approximately 12 hours after chemotherapy.

Prior and Concomitant Treatments

Prior therapy (i.e. any therapy given 4 weeks prior to the screening visit) was recorded in the eCRFs. In the event that additional concomitant therapy became necessary during the study treatment period (i.e. any medication starting on or before Week 20), those also had to be documented. Any change in documented, permitted concomitant medication being taken at the beginning of the clinical study had to be dose, duration, and indication. If the administration of a non-permitted concomitant medication became necessary, participation in the study was discontinued prematurely in that subject.

Use of other concurrent haematopoietic growth factors was not allowed by the protocol. Use of other biotechnologically produced medications, medications which could have potentiated the release of neutrophils, such as lithium and medications known to lower platelet count were discouraged, but if considered necessary had to be discussed with the study medical monitor.

Primary prophylactic antibiotic therapy was not allowed, in line with NCCN, Practice Guidelines in Oncology, Myeloid growth factors, NCCN 2011 Myeloid Growth Factors. Secondary antibiotic prophylaxis was initiated upon development of episode of febrile neutropenia and was implemented in accordance with the recommendations of NCCN Clinical Practice Guidelines in Oncology, Prevention and Treatment of Cancer Related Infections, (NCCN 2011 Cancer Related Infections).

Objectives

Primary objective:

The primary objective of this study was to demonstrate an equivalent efficacy of Pelgraz as compared to US and EU licensed Neulasta in subjects suffering from early breast cancer and receiving TAC.

Secondary objectives:

• To assess the safety of Pelgraz as compared to that of Neulasta US and Neulasta EU when administered through 6 cycles of TAC anticancer chemotherapy.

• To assess the potential antigenicity of Pelgraz during chemotherapy and 30 weeks after the completion of chemotherapy.

Outcomes/endpoints

Primary efficacy endpoint:

Duration of severe neutropenia (DSN) in Cycle 1. Severe neutropenia was defined as ANC below 0.5 x 10^9/L.

Secondary efficacy endpoints:

• The frequency of Grade 3 and 4 severe neutropenia (ANC <1.0 x 10^9/L and <0.5 x 109/L, respectively) in Cycle 1.

• The depth and peak of ANC nadir in Cycle 1.

• The time to the post nadir ANC recovery (ANC ≥2.0 x 10^9/L) in Cycle 1.

• The rates of febrile neutropenia by cycle and across the cycles.

• The ANC-time profile in Cycle 1 (time from beginning chemotherapy to the occurrence of the ANC nadir).

• The frequency and type of (culture-confirmed) infections.

• The incidence of intravenous (i.v.) antibiotic therapy and hospitalization.

• The mobilization of CD34+ cells (in selected centres only) in Cycle 1.

• Incidence, severity and distribution of bone pain.

• Percentage of scheduled chemotherapy dose that was delivered.

• Proportion of subjects with chemotherapy doses reduced, omitted, or delayed.

• Number of days of delay of chemotherapy.

• Occurrence and/or resolution of chemotherapy-induced mucositis.

Sample size

The required sample size for efficacy evaluation was calculated based on equivalence design (two separate equivalence tests for comparison to Neulasta US and Neulasta EU) and based on the standard deviation (SD=1.4 days) of DSN in Cycle 1 reported by Green et al. 2003.

To test the equivalent efficacy of Pelgraz as compared to Neulasta US and Neulasta EU, sample sizes of 135 subjects in each reference prod*u*ct treatment arm and 270 in the investigational treatment arm were needed to achieve 90% power for the 95% CI of the difference in mean DSN to be within the equivalence range of [-0.5 day, +0.5 day]. Anticipating a 10% attrition/or protocol deviation rate in Cycle 1 (it is justifiable according to previous publications (Green et al. 2003), enrolment of 600 subjects (300 subjects for Pelgraz arm and 150 subjects for each Neulasta arm) was determined to be needed to achieve the required number of evaluable subjects to test the equivalence of Pelgraz and Neulasta US and Neulasta EU. In calculating the sample size, a difference of 0.05 day in mean DSN between products was assumed.

Beside efficacy consideration, requirements regarding safety were also taken into account when selecting the sample size for the study.

Randomisation

The randomization scheme was generated by using SAS Software version 9.3. Permuted block randomization was used and block size were as considered as blinded information.

Blinding (masking)

The investigator performing the assessments (the assessor), the study subjects as well as all other sponsor/clinical research organization (CRO) personnel monitoring and analyzing the study had to remain blinded. Unblinded team members were identified at each centre whose role in the study was limited to handling the IMP test and reference products (a pharmacist, an investigator and/or a study nurse not involved in other study procedures or assessments). Unblinded team members were responsible for the receipt, accountability, and administration of pegfilgrastim. Treatment assignment was not allowed to be disclosed to the investigator or any sponsor representative except the site monitor responsible for unblinded monitoring.

Statistical methods

Demonstration of equivalent efficacy of Pelgraz as compared to Neulasta US and Neulasta EU was performed. To test the equivalence of Pelgraz and each Neulasta product (US and EU) the 2-sided 95% confidence interval (CI) for the difference (Pelgraz minus Neulasta) of DSN in Cycle 1 was calculated. The two-sided 95% CIs were derived from a one-way analysis of variance (ANOVA) model accounting for the treatment effect. For declaring equivalence, the CI had to lie within the equivalence range of [-0.5 to +0.5 day]. The effects of baseline ANC and the interaction between country and treatment were examined, using analysis of covariance (ANCOVA). Secondary efficacy endpoints were calculated and summarized for all treatment arms. Log transformation was applied for the secondary endpoints to satisfy the normality assumption.

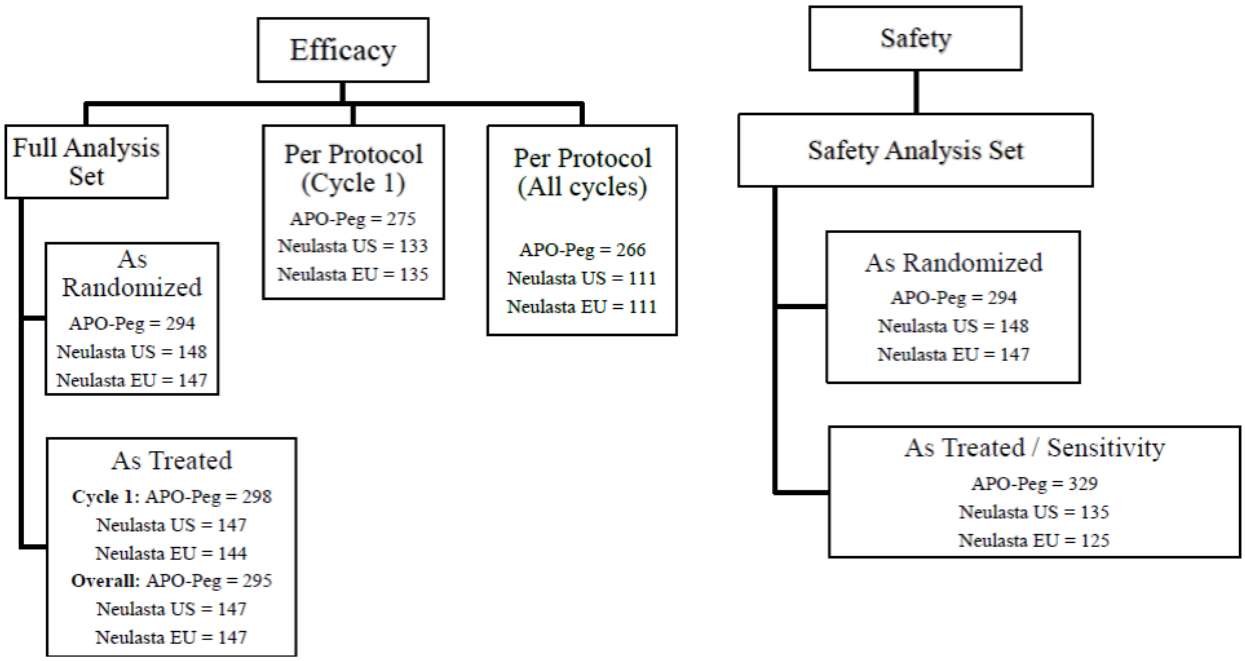
Safety endpoints were summarized using descriptive statistics.

The following analysis populations were planned for the study:

- **Safety Analysis Set** (SAS): The SAS included all enrolled subjects who received at least one dose of the active treatment.
- **Full Analysis set** (FAS): The FAS comprised all enrolled subjects who received at least one dose of the active treatment and who provided any follow-up data for the primary target variables.
- **Per Protocol Analysis Set** (PP): Subjects having protocol deviations affecting the integrity of the data and the endpoint of the efficacy analysis/safety and well-being of the subjects, with premature termination of the treatment due to reasons that were definitely not related to study medication, were excluded from the PP analyses. Handling of drop-outs and missing values were performed as for the full analysis dataset.

In the Statistical Analysis Plan (SAP) the primary analysis set was the FAS, in line with the intent-to-treat principle. The **FAS** was analysed with subjects allocated **As Randomized** (i.e., regardless of any mixed dosing) and **As Treated** (subjects were allocated to the treatment they received in each cycle). The FAS-As Randomized and the SAS were identical in this study.

The prespecified primary analysis set was the FAS-As Randomized.



Results

Participant flow

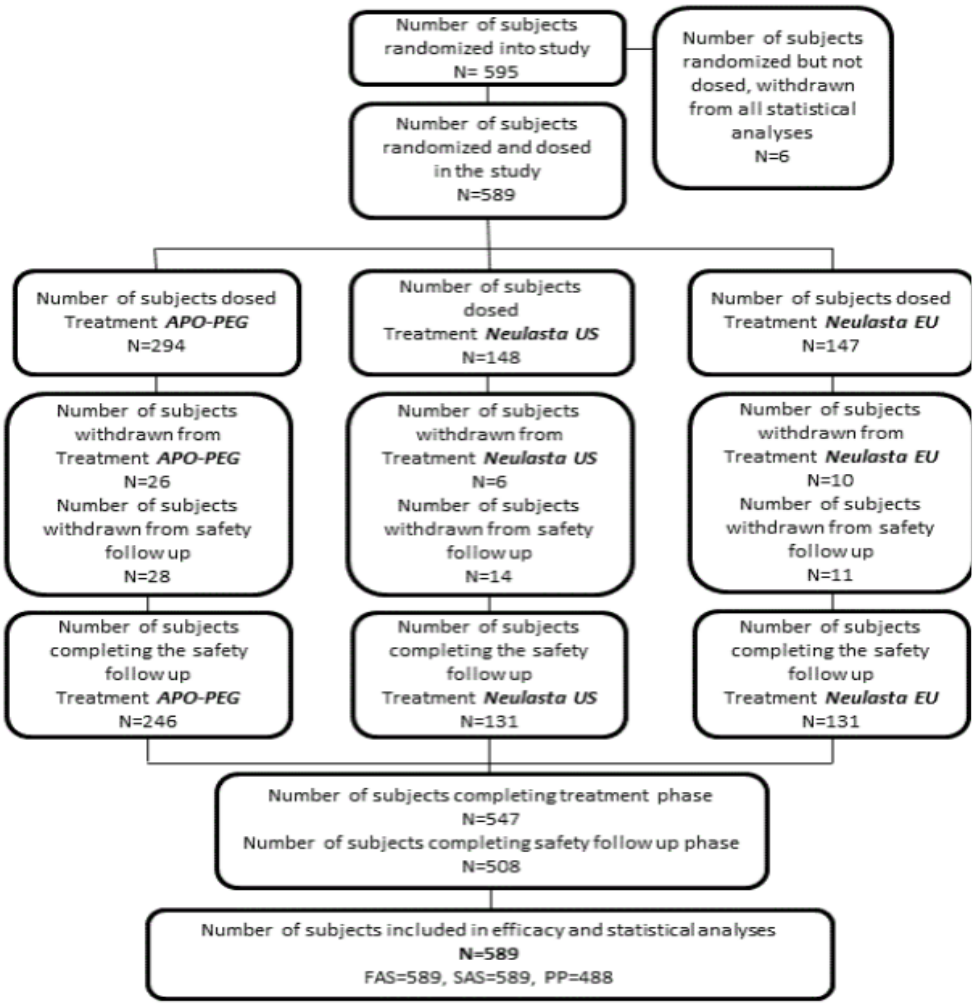


Figure 18 Disposition of Subjects As-Randomized

Recruitment

In total, 58 study centres screened subjects (out of 81 initiated centres) in 12 countries (Hungary, Slovakia, Czech Republic, Poland, Romania, Bulgaria, Serbia, Bosnia and Herzegovina, Ukraine, Georgia, Armenia and Russia).

The study took place from Mar 28, 2012 until May 30, 2014 (end of the safety follow-up phase).

Conduct of the study

Amendments:

There were three protocol amendments and two country-specific amendments to the initial clinical study protocol. The main amendments and changes are mentioned below.

Amendment 1 (13.1.2012) consisted of the following main changes:

- The number of subjects was changed from 105 to 150 for each Neulasta arm, 210 subjects to 300 in the Pelgraz arm. Therefore, the total number was increased from 420 to 600 subjects. The number of study centres was modified from up to 74 to 120.
- The exact time of anti-infective treatment was clarified; at least 72 hours should have elapsed between the last dose of anti-infective treatment and TAC administration.

- The equivalence range was changed from [-0.8 day, +0.8 day] to [-0.5 day, +0.5 day] within the statistical methods section, defining a new sample size as reflected in the first bullet. To test the equivalent efficacy of Pelgraz as compared to Neulasta US and Neulasta EU based on the revised equivalence range, sample sizes of 135 subjects in each reference arm and 270 in the investigational treatment arm were needed to achieve 90% power for the 95% CI of the difference in mean DSN to be within the equivalence range of [-0.5 day, +0.5 day].

Amendment 2 (25.6.2012) included administrative changes, typing omissions and addition of the most recent version of Helsinki Declaration.

Amendment 3 (21.11.2012) consisted of the following main changes:

- Blood sampling for blood chemistry assessment was allowed to be done on Day 0 or Day 1.
- CDISC standards concerning AE severity assessment and clarification of AEs outcome were implemented.

Protocol deviations:

- Exclusion of Subjects from the PP (Cycle 1) Analysis Set: Based on the assessment and classification of the protocol deviations, 46 (7.8%) subjects were excluded from PP Analysis Set for Cycle 1, resulting in 543 subjects in this analysis set.

Table 31 Subjects Excluded from PP (Cycle 1) Analysis Set

	Number (%) subjects affected			
PD Classification Cycle 1	APO-Peg N=294	Neulasta US N=148	Neulasta EU N=147	Total N=589
Exclusion/Inclusion Criteria Failed	3 (1.0%)	1 (0.7%)	4 (2.7%)	8 (1.4%)
The Intended Medication Not Received Based on Randomization arm (i.e. mixed dosing)	1 (0.3%)	5 (3.4%)	5 (3.4%)	11 (1.9%)
Intake of Prohibited Medication	4 (1.4%)	1 (0.7%)	2 (1.4%)	7 (1.2%)
TAC dose adjustment per body weight (the dose difference was greater than 5%)	4 (1.4%)	8 (5.4%)	1 (0.7%)	13 (2.2%)
PI Unblinding	2 (0.7%)	0 (0%)	0 (0%)	2 (0.3%)
ANC profile could not be characterized				
ANC result missing from Day 9 to Day 15	3 (1.0%)	0 (0%)	0 (0%)	3 (0.5%)
Discontinued on Cycle 1 Day 3	2 (0.7%)	0 (0%)	0 (0%)	2 (0.3%)

- Additional Subjects Excluded from the PP (All Cycles) Analysis Set Due to Deviations in Cycles 2-6: An additional 55 (9.3%) subjects were excluded from the PP (All Cycles) Analysis Set due to deviations in Cycle 2-6, resulting in 488 subjects in this analysis set.

Table 32 Subjects Excluded from PP (All Cycles) Analysis Set due to Deviations in Cycle 2-6

	Number (%) subjects affected			
PD Classification Cycle 2-6	**APO-Peg N=294**	**Neulasta US N=148**	**Neulasta EU N=147**	**Total N=589**
The Intended Medication Not Received Based on Randomization arm (i.e. mixed dosing)	3 (1.0%)	19 (12.8%)	20 (13.6%)	42 (7.1%)
Intake of Prohibited Medication	1 (0.3%)	0 (0%)	0 (0%)	1 (0.2%)
Discontinued treatment due to reasons that were definitely not related to study medication	3 (1.0%)	1 (0.7%)	3 (2.0%)	7 (1.2%)
TAC Withdrawal due to AE	0 (0%)	1 (0.7%)	1 (0.7%)	2 (0.3%)
TAC dose adjustment per body weight (the dose difference was greater than 5%)	1 (0.3%)	1 (0.7%)	0 (0%)	2 (0.3%)
IMP administered 8 days after TAC	1 (0.3%)	0 (0%)	0 (0%)	1 (0.2%)

Baseline data

Table 33 APO-Peg-03: Demographic Data-FAS-As Randomized

Demographic data	**APO-Peg Treatment (N=294)**	**Neulasta US Treatment (N=148)**	**Neulasta EU Treatment (N=147)**	**Total (N=589)**
Age (years)				
Mean (SD)	51.9 (10.00)	51.4 (10.37)	51.5 (10.22)	51.7 (10.13)
Median	52	52	53	52
Minimum – Maximum	24 – 75	27 – 80	22 – 77	22 - 80
Race, n (%) White	294 (100.0)	148 (100.0)	147 (100.0)	589 (100.0)
Body weight (kg)				
Mean (SD)	73.88 (14.41)	72.01 (14.06)	72.61 (12.87)	73.09 (13.95)
Median	73.0	70.0	70.0	72.0
Minimum – Maximum	40.0 - 120.0	40.0 - 118.0	48.0 - 119.0	40.0 - 120.0
Body height (cm)				
Mean (SD)	162.5 (6.77)	162.7 (6.58)	162.6 (6.42)	162.6 (6.63)
Median	163.0	163.0	163.0	163.0
Minimum – Maximum	140.0 - 180.0	142.0 - 180.0	148.0 - 183.0	140.0 - 183.0

Source: APO-Peg-03, Table 14.1.1 and Table 14.1.43

Table 34 APO-Peg-03: Breast Cancer History (FAS-As Randomized)

		Number (%) subjects affected			
Tumour parameter		APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
Tumour	T1	72 (24.5)	47 (31.8)	49 (33.3)	168 (28.5)
	T2	210 (71.4)	95 (64.2)	85 (57.8)	390 (66.2)
	T3	12 (4.1)	6 (4.1)	13 (8.8)	31 (5.3)
Node	N0	83 (28.2)	26 (17.6)	28 (19.0)	137 (23.3)
	N1	129 (43.9)	75 (50.7)	76 (51.7)	280 (47.5)
	N2	81 (27.6)	47 (31.8)	42 (28.6)	170 (28.9)
	N3	1 (0.3)	0 (0.0)	1 (0.7)	2 (0.3)
Metastasis	M0	294 (100.0)	148 (100.0)	147 (100.0)	589 (100.0)

Tumour parameter		APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
Staging	IIa	129 (43.9)	59 (39.9)	58 (39.5)	246 (41.8)
	IIb	79 (26.9)	40 (27.0)	44 (29.9)	163 (27.7)
	IIIa	86 (29.3)	49 (33.1)	45 (30.6)	180 (30.6)

T1, T2, T3 means the size and/or extent of the primary tumour stage (increasing order from 1 to 3). N0 means there is no regional lymph node involvement. N1, N2, N3 means the degree of regional lymph node involvement (number and location of lymph nodes – increasing order from 1 to 3). M0 means there is no distant metastasis.

Source: APO-Peg-03, Table 14.1.7

Numbers analysed

Table 35 APO-Peg-03: Treatment Period Completion Summary – All Randomized and Treated Subjects

		Number (%) subjects affected			
Analysis Dataset	Parameter	APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
Full Analysis Set (and Safety Analysis Set)- As Randomized	All subjects	294 (100.0%)	148 (100.0%)	147 (100.0%)	589 (100.0%)
	Discontinued treatment period	26 (8.8%)	6 (4.1%)	10 (6.8%)	42 (7.1%)
	Completed treatment period	268 (91.2%)	142 (95.9%)	137 (93.2%)	547 (92.9%)
	All subjects in safety follow-up	274 (100.0%)	145 (100.0%)	142 (100.0%)	561 (100.0%)
	Discontinued safety follow-up	28 (9.5%)	14 (9.5%)	11 (7.5%)	53 (9.0%)
	Completed safety follow-up	246 (83.7%)	131 (88.5%)	131 (89.1%)	508 (86.2%)

	Completed treatment and follow-up	245 (83.3%)	130 (87.8%)	131 (89.1%)	506 (85.9%)
Full Analysis Set-As Treated (Overall)	All subjects	295 (100.0%)	147 (100.0%)	147 (100.0%)	589 (100.0%)
	Discontinued treatment period	27 (9.2%)	5 (3.4%)	10 (6.8%)	42 (7.1%)
	Completed treatment period	268 (90.8%)	142 (96.6%)	137 (93.2%)	547 (92.9%)
Per Protocol (Cycle 1) Analysis Set	All subjects	275 (100.0%)	133 (100.0%)	135 (100.0%)	543 (100.0%)
	Discontinued treatment period	21 (7.6%)	4 (3.0%)	9 (6.7%)	34 (6.3%)
	Completed treatment period	254 (92.4%)	129 (97.0%)	126 (93.3%)	509 (93.7%)
Per Protocol (All Cycles) Analysis Set	All subjects	266 (100.0%)	111 (100.0%)	111 (100.0%)	488 (100.0%)
	Discontinued treatment period	18 (6.8%)	1 (0.9%)	5 (4.5%)	24 (4.9%)
	Completed treatment period	248 (93.2%)	110 (99.1%)	106 (95.5%)	464 (95.1%)
Safety Analysis Set Sensitivity	All subjects	329 (100.0%)	135 (100.0%)	125 (100.0%)	589 (100.0%)
	Discontinued treatment period	28 (8.5%)	4 (3.0%)	10 (8.0%)	42 (7.1%)
	Completed treatment period	301 (91.5%)	131 (97.0%)	115 (92.0%)	547 (92.9%)
	All subjects in safety follow-up	307	134	120	561
	Discontinued safety follow-up	32 (9.7%)	11 (8.1%)	10 (8.0%)	53 (9.0%)
	Completed safety follow-up	275 (83.6%)	123 (91.1%)	110 (88.0%)	508 (86.2%)
	Completed overall	274 (83.3%)	122 (90.4%)	110 (88.0%)	506 (85.9%)

Source: APO-Peg-03, Table 14.1.11, Table 14.3.1.13, Table 14.3.1.16

Outcomes and estimation

Primary endpoint

Table 36 APO-Peg-03, Summary of Duration of Severe Neutropenia in Cycle 1 (Full Analysis Set – As Randomized and As Treated)

Statistics for duration (days)	APO-Peg	Neulasta US	Neulasta EU	Total
FAS-As Randomized				
N	294	148	147	589
Mean (SD)	1.6 (1.48)	1.4 (1.17)	1.6 (1.34)	1.6 (1.37)
Median	2.0	1.0	2.0	1.0
Minimum - Maximum	0 - 10	0 - 5	0 - 10	0 – 10
FAS-As Treated				
N	298	147	144	589
Mean (SD)	1.6 (1.48)	1.4 (1.17)	1.6 (1.34)	1.6 (1.37)
Median	1.5	1.0	2.0	1.0
Minimum - Maximum	0 - 10	0 - 5	0 - 10	0 – 10

Source: APO-Peg-03, Table 14.2.1 and Table 14.2.2

Table 37 APO-Peg-03, Summary of Efficacy Results for the Duration of Severe Neutropenia in Cycle 1, Estimated with ANOVA accounting for the Treatment Effect following treatment with APO-Peg, US-Licensed and EU-Approved Neulasta (Full Analysis Set – As Randomized and as Treated).

Statistics for duration (days)	APO-Peg	Neulasta US	Neulasta EU	APO-Peg - Neulasta US	APO-Peg - Neulasta EU	Neulasta EU - Neulasta US
FAS-As Randomized						
LS Mean	1.63	1.39	1.61	0.24	0.02	0.21
95% CI	1.47 to 1.79	1.17 to 1.61	1.38 to 1.83	-0.03 to 0.51	-0.25 to 0.30	-0.10 to 0.53
FAS-As Treated						
LS Mean	1.62	1.39	1.63	0.23	-0.01	0.24
95% CI	1.46 to 1.77	1.17 to 1.61	1.41 to 1.86	-0.04 to 0.50	-0.29 to 0.26	-0.07 to 0.56

Source: APO-Peg-03, Table 14.2.9 and Table 14.2.10

Secondary endpoints

Grade 3 and 4 Severe Neutropenia

Table 38 Frequency of Grade 3 and Grade 4 Neutropenia – FAS-As Randomized

Cycle		Number (%) subjects affected			
		APO-Peg	Neulasta US	Neulasta EU	Total
1	Grade 3	28 of 294 (9.5)	20 of 148 (13.5)	13 of 147 (8.8)	61 of 589 (10.4)
	Grade 4	227 of 294 (77.2)	111 of 148 (75.0)	117 of 147 (79.6)	455 of 589 (77.2)
2	Grade 3	18 of 288 (6.3)	6 of 146 (4.1)	8 of 144 (5.6)	32 of 578 (5.5)
	Grade 4	26 of 288 (9.0)	11 of 146 (7.5)	13 of 144 (9.0)	50 of 578 (8.7)
3	Grade 3	36 of 285 (12.6)	16 of 146 (11.0)	15 of 144 (10.4)	67 of 575 (11.7)
	Grade 4	14 of 285 (4.9)	11 of 146 (7.5)	13 of 144 (9.0)	38 of 575 (6.6)
4	Grade 3	19 of 284 (6.7)	14 of 144 (9.7)	17 of 144 (11.8)	50 of 572 (8.7)
	Grade 4	25 of 284 (8.8)	9 of 144 (6.3)	15 of 144 (10.4)	49 of 572 (8.6)
5	Grade 3	23 of 276 (8.3)	16 of 143 (11.2)	19 of 142 (13.4)	58 of 561 (10.3)
	Grade 4	24 of 276 (8.7)	7 of 143 (4.9)	13 of 142 (9.2)	44 of 561 (7.8)
6	Grade 3	22 of 269 (8.2)	14 of 142 (9.9)	10 of 138 (7.2)	46 of 549 (8.4)
	Grade 4	25 of 269 (9.3)	8 of 142 (5.6)	17 of 138 (12.3)	50 of 549 (9.1)
Overall	Grade 3	24 of 294 (8.2)	18 of 148 (12.2)	14 of 147 (9.5)	56 of 589 (9.5)
	Grade 4	233 of 294 (79.3)	116 of 148 (78.4)	117 of 147 (79.6)	466 of 589 (79.1)

Subjects are summarized by the highest grade of neutropenia experienced in each cycle and overall

Source: Table 14.2.31

A similar distribution in the incidence of both Grade 3 and Grade 4 neutropenia was also evident between the treatment arms in the **FAS-As Treated** Analysis Set in Cycle 1: Grade 3 neutropenia occurred in 9.4%, 13.6% and 9.0% of subjects in the Pelgraz, Neulasta US and Neulasta EU arms, respectively, and Grade 4 neutropenia occurred in 76.8%, 74.8% and 80.6% subjects.

Results for **the PP Analysis** Set in Cycle 1 were: Grade 3 neutropenia occurred in 9.8%, 14.4% and 9.9% of subjects in the Pelgraz, Neulasta US and Neulasta EU arms, respectively, and Grade 4 neutropenia occurred in 77.8%, 73.9% and 79.3% subjects, respectively.

ANC Characteristics in Cycle 1

In Cycle 1, blood samples were collected for complete blood counts with differentials on Day 0, 1, 3, 5, 6, 7, and every day until post-nadir ANC recovery to ≥ 2.0 x 10^9/L or up to Day 15, if recovery did not occur earlier.

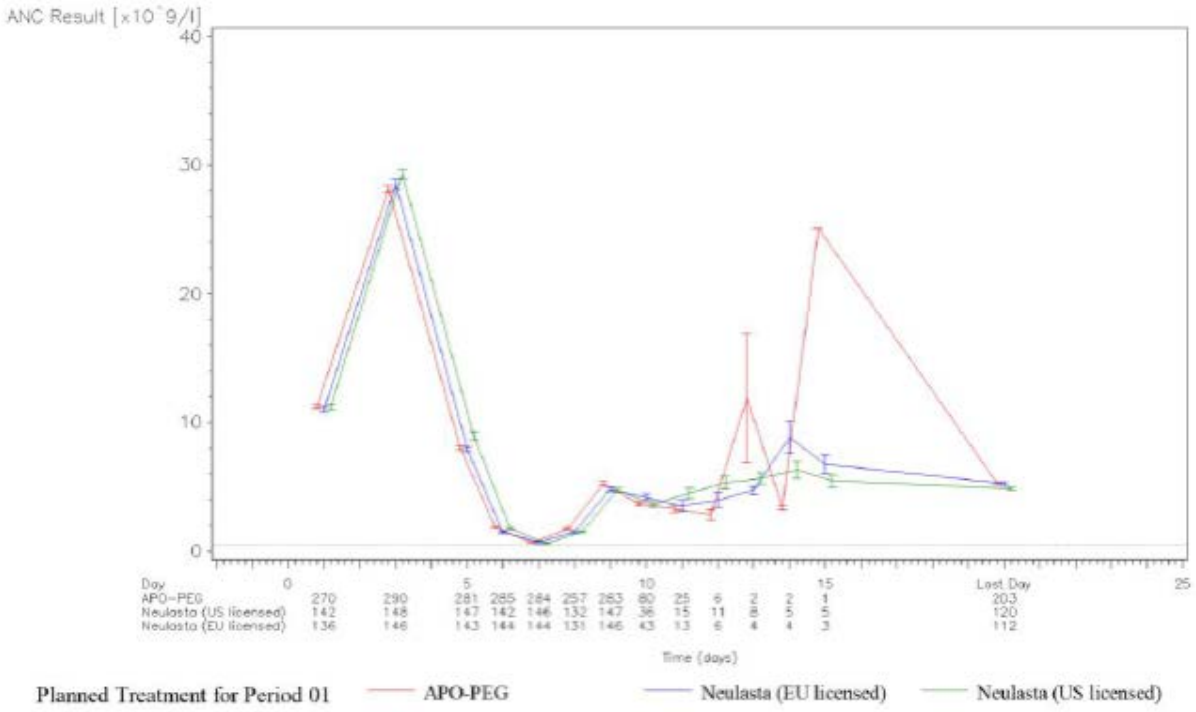
The results for FAS-As Treated were similar.

Results for the PP Analysis Set were consistent with those for the FAS-As Randomized Analysis Set.

Table 39 ANC Characteristics in Cycle 1 – FAS-As Randomized

Characteristics	Statistics	APO-Peg N=294	Neulasta US N=148	Neulasta EU N=147	Total N=589
Day on which the peak ANC value was reached	N	294	148	147	589
	Mean (SD)	3.4 (2.30)	3.1 (0.57)	3.1 (1.41)	3.2 (1.80)
	Median	3.0	3.0	3.0	3.0
	Minimum - Maximum	1 - 24	3 – 9	1 - 18	1 - 24
Peak ANC value (x10^9/L)	N	294	148	147	589
	Mean (SD)	28.4 (9.54)	29.9 (10.17)	28.7 (9.28)	28.9 (9.64)
	Median	27.7	29.4	27.9	28.0
	Minimum - Maximum	3.9 - 60.5	11.3 - 88.0	5.8 - 55.9	3.9 - 88.0
Day on which the depth of ANC nadir was reached	N	294	148	147	589
	Mean (SD)	7.1 (1.80)	7.1 (0.61)	7.3 (2.08)	7.1 (1.67)
	Median	7.0	7.0	7.0	7.0
	Minimum - Maximum	1 - 21	6 – 11	6 - 21	1 - 21
Depth of ANC nadir (x10^9/L)	N	294	148	147	589
	Mean (SD)	0.6 (1.12)	0.4 (0.61)	0.4 (0.74)	0.5 (0.92)
	Median	0.2	0.2	0.1	0.2
	Minimum - Maximum	0.0 - 9.8	0.0 - 3.4	0.0 - 6.0	0.0 - 9.8
Day on which recovery of ANC was reached	N	267	136	137	540
	Mean (SD)	9.4 (2.00)	9.5 (2.05)	9.2 (0.98)	9.4 (1.81)
	Median	9.0	9.0	9.0	9.0
	Minimum - Maximum	6 - 25	8 – 22	8 - 13	6 - 25

Source: Table 14.2.34



The last day of Cycle 1 is denoted by Day 20
The dotted line indicates the upper limit of severe neutropenia: 0.5x10^9/l.
Only the results of scheduled visits are presented on this plot

Source: Figure 14.2.45

Figure 19 Mean (SE) ANC Values Over Time in Cycle 1 (untransformed scale) FAS – As Randomised

Rates of Febrile Neutropenia by Cycle and Across Cycles

Table 40 Rates of Febrile Neutropenia by Cycle and Across the Cycles – FAS-As Randomized

	Number (%) of Subjects			
Cycle	**APO-Peg treatment**	**Neulasta US**	**Neulasta EU**	**Total**
1	15 of 294 (5.1)	6 of 148 (4.1)	5 of 147 (3.4)	26 of 589 (4.4)
2	0 of 288 (0.0)	1 of 146 (0.7)	0 of 144 (0.0)	1 of 578 (0.2)
3	0 of 285 (0.0)	0 of 146 (0.0)	1 of 144 (0.7)	1 of 575 (0.2)
4	0 of 284 (0.0)	0 of 144 (0.0)	0 of 144 (0.0)	0 of 572 (0.0)
5	2 of 276 (0.7)	0 of 143 (0.0)	0 of 142 (0.0)	2 of 561 (0.4)
6	0 of 270 (0.0)	0 of 143 (0.0)	0 of 139 (0.0)	0 of 552 (0.0)
Overall	17 of 294 (5.8)	7 of 148 (4.7)	5 of 147 (3.4)*	29 of 589 (4.9)*

*1 subject in Neulasta EU treatment arm experienced an episode of febrile neutropenia in Cycle 3 as well as Cycle 1

Source: Table 14.2.40

The results for PP Analysis Set were similar.

Maximum duration of febrile neutropenia was 7 days but for the majority of subjects was less than 5 days. Febrile neutropenia did not necessitate delay in TAC administration, or reduction in TAC dose.

CD34+ cell count

Table 41 APO-Peg-03: Summary of CD34+ results following treatment with APO-Peg, US-Neulasta, and EU-Neulasta (FAS-As Randomized)

Visit	Statistics for CD34+ (cells/µl)	APO-Peg (N= 59)	Neulasta US (N= 39)	Neulasta EU (N= 32)	Total (N=130)
Cycle 1 Day 1 (baseline)	N	42	26	17	85
	Mean (SD)	2.82 (6.57)	2.10 (3.96)	1.96 (1.86)	2.43 (5.15)
	Median	1.05	1.00	1.60	1.00
	Minimum – Maximum	0.0 - 42.8	0.0 - 20.7	0.0 - 6.7	0.0 - 42.8
Cycle 1 Day 7	N	40	26	16	82
	Mean (SD)	2.39 (2.14)	3.54 (6.12)	3.31 (2.61)	2.93 (3.91)
	Median	1.90	2.00	2.70	2.00
	Minimum – Maximum	0.0 - 10.6	0.0 - 31.3	0.0 - 8.6	0.0 - 31.3
	Mean change from baseline	-0.48	1.44	1.34	0.48
Cycle 1 Day 9	N	40	26	16	82
	Mean (SD)	45.64 (46.13)	57.33 (68.06)	59.79 (47.04)	52.11 (53.90)
	Median	30.50	34.50	47.85	35.00
	Minimum – Maximum	2.0 - 219.5	7.0 - 333.9	6.0 - 156.4	2.0 - 333.9
	Mean change from baseline	42.75	55.24	57.83	49.65

Source: APO-Peg-03, Table 14.2.73d

Additionally, for the comparison of CD34+ results between Pelgraz and US-Neulasta, the 95% CI was -40.79 to 13.22 and for the comparison of Pelgraz and EU-Neulasta the 95% CI was -43.95 to 18.28.

Frequency and Type of Culture-Confirmed Infections

A total of 13 samples were submitted for microbiological culture, six were confirmed by culture.

Table 42 APO-Peg-03: Frequency and Type of Culture-confirmed Infection FAS-As Randomized

	Statistics	APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total
Overall	Culture-confirmed infection	3 (1.0)	1 (0.7)	2 (1.4)	6 (1.0)
	Febrile neutropenia	1 (0.3) *(C1: HU01304*)*	1 (0.7) *(C1: HU01003*)*	0 (0.0%)	2 (0.3)
	Klebsiella infection	0 (0.0%)	0 (0.0%)	1 (0.7) (C1: *RO05710)*	1 (0.2)
	Oral candidiasis	1 (0.3) (C4: *RO05701)*	0 (0.0%)	1 (0.7) *(C1:RO05710)*	2 (0.3)
	Oropharyngeal pain	1 (0.3) (C1: *BA03901)*	0 (0.0%)	0 (0.0%)	1 (0.2)
	Fistula	0 (0.0%)	0 (0.0%)	1 (0.7) *(C3:RO05709)*	1 (0.2)

Note: None of these subjects received mixed dosing
*No isolated microorganism
Source: APO-Peg-03, Table 14.2.51 and Listing 16.2.6.8

Intravenous Antibiotic Therapy and Hospitalization

The similar results were also obtained in PP Analysis Set and FAS-As Treated population.

The slightly higher incidence of hospitalizations in the Pelgraz arm was due to the slightly higher rate of febrile neutropenia/neutropenia in the Pelgraz arm: 8 cases (2.7%) requiring hospitalization compared with 3 (2.0%) and 3 (2.0%) cases, respectively in the US-Neulasta and EU-Neulasta arms. Also there were 5 subjects (1.7%) in the Pelgraz arm with SAEs requiring hospitalization that were unrelated to IMP, compared with 1 subject (0.7%) with IMP-related toxic skin eruption in the US-Neulasta arm and 1 subject (0.7%) with cholecystitis (unrelated to IMP) in the EU-Neulasta arm.

Table 43 Number (%) of Subjects with i.v. Antibiotic Therapy and Hospitalization FAS-As Randomized

Parameter	APO-Peg N=294	Neulasta US N=148	Neulasta EU N=147	Total N=589
Subject with i.v. antibiotic therapy	8 (2.7)	5 (3.4)	4 (2.7)	17 (2.9)
Subject requires hospitalization	13 (4.4)	4 (2.7)	4 (2.7)	21 (3.6)

Table 44 Number (%) of Subjects with i.v. Antibiotic Therapy and Hospitalization PP (All Cycles) Analysis Set

Parameter	APO-Peg N=266	Neulasta US N=111	Neulasta EU N=111	Total N=488
Subject with i.v. antibiotic therapy	6 (2.3)	2 (1.8)	3 (2.7)	11 (2.3)
Subject requires hospitalization	9 (3.4)	2 (1.8)	3 (2.7)	14 (2.9)

Incidence, Severity and Distribution of Bone Pain

Table 45 APO-Peg-03: Incidence, Severity and Distribution of Bone Pain – FAS-As Randomized

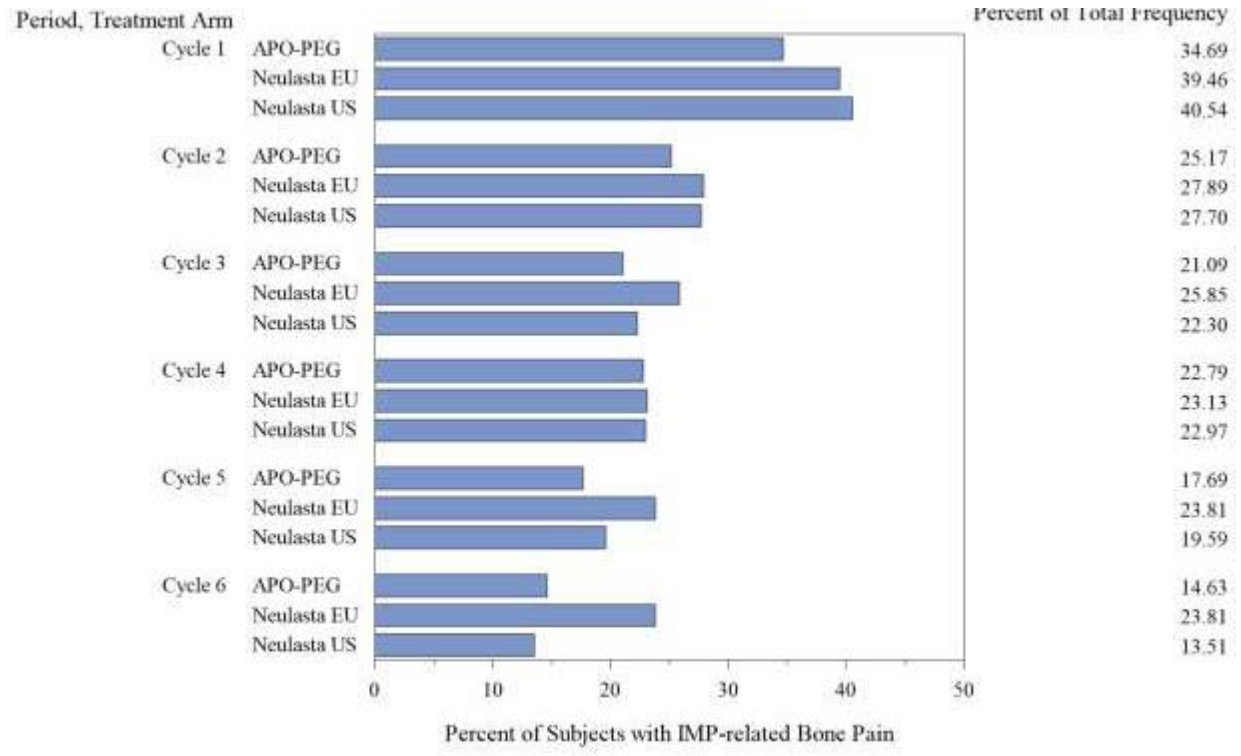
		APO-Peg N=294	Neulasta US N=148	Neulasta EU N=147	Total N=589
Any severity		139 (47.3)	73 (49.3)	76 (51.7)	288 (48.9)
Maximum intensity	Mild	42 (14.3)	24 (16.2)	25 (17.0)	91 (15.4)
	Moderate	52 (17.1)	26 (17.6)	31 (21.1)	109 (18.5)
	Severe	45 (15.3)	23 (15.5)	20 (13.6)	88 (14.9)
Location	Bone pain - Arm(s)	44 (15.0)	16 (10.8)	19 (12.9)	79 (13.4)
	Bone pain - Head	8 (2.7)	1 (0.7)	6 (4.1)	15 (2.5)
	Bone pain – Neck	9 (3.1)	8 (5.4)	6 (4.1)	23 (3.9)
	Bone pain - Pelvis	51 (17.3)	24 (16.2)	29 (19.7)	104 (17.7)
	Bone pain - Vertebral column	71 (24.1)	26 (17.6)	34 (23.1)	131 (22.2)
	Bone pain - Leg(s)	78 (26.5)	49 (33.1)	43 (29.3)	170 (28.9)
	Bone pain - Other	74 (25.2)	42 (28.4)	37 (25.2)	153 (26.0)
Subjects without Bone Pain		155 (52.7)	75 (50.7)	71 (48.3)	301 (51.1)

Source: APO-Peg-03, Table 14.2.57

Table 46 Patients Receiving concomitant treatment for Bone Pain in Treatment Period - SAS

Severity of bone pain	Number (%) Subjects			
	APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
Any grade	77 (26.19%)	35 (23.65%)	43 (29.25%)	155 (26.3%)
Mild	25 (8.5)	17 (11.5)	15 (10.2)	57 (9.7)
Moderate	51 (17.4)	19 (12.8)	26 (17.7)	96 (16.3)
Severe	32 (10.9)	12 (8.1)	10 (6.8)	54 (9.2)

Subjects are summarized once in each severity of bone pain



Bone pain events possibly, probably or definitely related to IMP are included in the figure

Figure 20 Pelgraz-03 Percent of Subjects with Bone Pain- FAS-As Randomized

Occurrence and/or Resolution of Chemotherapy-Induced Mucositis

Oral mucositis is a common complication of cytoreductive cancer chemotherapy and radiotherapy. The reporting incidence was 39 (6.6%) cases of oral mucositis during the course of the study and all cases were resolved, usually without treatment. The frequencies across the three therapeutic arms were similar with 21 (7.1%) subjects in the Pelgraz, 12 (8.1%) subjects in US-Neulasta and 6 (4.1%) subjects in EU-Neulasta arms, respectively.

Frequencies of Deviations from Originally Planned Chemotherapy (including doses delayed, reduced and omitted)

The dose of TAC therapy was calculated based on the subject BSA. More than a 5% difference to the baseline body weight meant the dose had to be recalculated. Dose reduction by 25% was possible if the subject had Grade 3/4 non-haematopoietic toxicities, two Grade 3/4 infectious episodes, or Grade 4 thrombocytopenia. Treatment could be terminated if non-haematologic Grade 4 toxic effects developed or persisted, Grade 3 toxic effects occurred despite a dose reduction, or a clinically significant cardiac event developed.

Deviations occurring in Cycle 1 could result in subject exclusion from both PP Analysis Sets, for the primary endpoint and for the whole study (ie. deviation of >5% in administered chemotherapy dose). Deviations, occurring in Cycles 2-6 resulted in the exclusion of the subject from the PP Analysis Set for later cycles.

Table 47 Frequencies of Deviations from Originally Planned chemotherapy – FAS-As Randomized

Cycle	Deviation from Planned Chemotherapy	Number (%) Subjects			
		APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
1	Not proper dose	6 (2.0)	8 (5.4)	2 (1.4)	16 (2.7)
	Reduced	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Omitted	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Delayed administration	156 (53.1)	79 (53.4)	82 (55.8)	317 (53.8)
2	Not proper dose	6 (2.0)	7 (4.7)	2 (1.4)	15 (2.5)
	Reduced	4 (1.4)	0 (0.0)	1 (0.7)	5 (0.8)
	Omitted	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Delayed administration	146 (49.7)	77 (52.0)	76 (51.7)	299 (50.8)
3	Not proper dose	8 (2.7)	7 (4.7)	3 (2.0)	18 (3.1)
	Reduced	4 (1.4)	1 (0.7)	3 (2.0)	8 (1.4)
	Omitted	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Delayed administration	144 (49.0)	75 (50.7)	76 (51.7)	295 (50.1)
4	Not proper dose	8 (2.7)	8 (5.4)	2 (1.4)	18 (3.1)
	Reduced	4 (1.4)	1 (0.7)	3 (2.0)	8 (1.4)
	Omitted	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Delayed administration	148 (50.3)	72 (48.6)	76 (51.7)	296 (50.3)
5	Not proper dose	7 (2.4)	10 (6.8)	3 (2.0)	20 (3.4)
	Reduced	5 (1.7)	1 (0.7)	3 (2.0)	9 (1.5)
	Omitted	0 (0.0)	1 (0.7)	1 (0.7)	2 (0.3)
	Delayed administration	138 (46.9)	77 (52.0)	76 (51.7)	291 (49.4)
6	Not proper dose	6 (2.0)	9 (6.1)	3 (2.0)	18 (3.1)
	Reduced	5 (1.7)	1 (0.7)	2 (1.4)	8 (1.4)
	Omitted	0 (0.0)	1 (0.7)	3 (2.0)	4 (0.7)
	Delayed administration	134 (45.6)	78 (52.7)	73 (49.7)	285 (48.4)

Delayed administration refers to start of the infusion rather than a late visit

Table 48 Summary of Days of Delay Outside Permitted Window for Chemotherapy – FAS-As Randomized

Cycle	Statistics	APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
2	N	11	1	4	16
	Mean (SD)	5.27 (3.29)	3.00 (not estimable)	4.75 (1.50)	5.00 (2.83)
	Median	4.0	3.0	4.0	4.0
	Minimum - Maximum	3 - 14	3 - 3	4 - 7	3 - 14
3	N	3	5	2	10
	Mean (SD)	5.00 (1.73)	12.40 (5.81)	7.00 (0.00)	9.10 (5.32)
	Median	4.0	13.0	7.0	7.0
	Minimum - Maximum	4 - 7	7 - 21	7 - 7	4 - 21
4	N	5	4	4	13
	Mean (SD)	6.20 (1.30)	8.75 (2.36)	4.75 (1.71)	6.54 (2.33)
	Median	7.0	8.0	4.5	7.0
	Minimum - Maximum	4 - 7	7 - 12	3 - 7	3 - 12
5	N	7	3	4	14
	Mean (SD)	5.43 (1.72)	9.33 (4.04)	7.00 (1.63)	6.71 (2.64)
	Median	5.0	7.0	7.0	7.0
	Minimum - Maximum	3 - 8	7 - 14	5 - 9	3 - 14
6	N	3	4	5	12
	Mean (SD)	6.33 (1.15)	8.25 (3.20)	5.80 (1.30)	6.75 (2.22)
	Median	7.0	7.0	6.0	7.0
	Minimum - Maximum	5 - 7	6 - 13	4 - 7	4 - 13

Source: Table 14.2.66a

Subgroup analyses

Age

Table 49 APO-Peg-03: Summary of Duration of Severe Neutropenia in Cycle 1 for Age Subgroups and Overall (FAS-As Randomized)

Statistics	APO-Peg	Neulasta US	Neulasta EU	Total
Subjects – 18-64 years at baseline				
N	262	133	136	531
Mean (SD)	1.7 (1.52)	1.4 (1.19)	1.6 (1.34)	1.6 (1.40)
Subjects ≥65years at baseline				
N	32	15	11	58
Mean (SD)	1.3 (1.11)	1.1 (0.99)	1.1 (1.22)	1.2 (1.08)
Subjects – Overall				
N	294	148	147	589
Mean (SD)	1.6 (1.48)	1.4 (1.17)	1.6 (1.34)	1.6 (1.37)

Source: APO-Peg-03, Table 14.2.86, Table 14.2.87, and Table 14.2.1

Table 50 Duration of Severe Neutropenia in Cycle 1: Least-Square Means (95% Confidence Intervals)of Treatment Arms and Differences between LSMeans, Estimated within ANOVA Accounting for Treatment, Age Category, and Interaction Between Treatment and Age Category

Age Category	Statistics	APO-PEG treatment	Neulasta (US licensed) treatment	Neulasta (EU licensed) treatment	APO-PEG treatment - Neulasta (US licensed) treatment	APO-PEG treatment - Neulasta (EU licensed) treatment	Neulasta (EU licensed) - Neulasta (US licensed) treatment
18-64 years	LSMean	1.68	1.42	1.65	0.25	0.03	0.23
	95% CI (Lower bound - Upper bound)	1.51 to 1.84	1.19 to 1.65	1.42 to 1.88	-0.03 to 0.54	-0.26 to 0.31	-0.10 to 0.55
65+ years	LSMean	1.25	1.13	1.09	0.12	0.16	-0.04
	95% CI (Lower bound - Upper bound)	0.77 to 1.73	0.44 to 1.83	0.28 to 1.90	-0.73 to 0.96	-0.78 to 1.10	-1.11 to 1.03

Table 51 Duration of Severe Neutropenia in Cycle 1: ANOVA P-Values for Treatment, Age Category, and Interaction Between Treatment and Age Category (Study APO-Peg-03: Full Analysis Set – As Randomized)

Effect	P-Value
Treatment	0.708
Age Category	0.043
Treatment x Age Interaction	0.893

Country

As an exploratory analysis the efficacy was assessed using descriptive statistics to compare results from the countries with clinical trial sites in Pelgraz-03. As the study was conducted in Eastern and Central Europe, the study population provides a good representation of the North American and European population.

Subjects were screened in 58 centers from 12 countries and randomized in 56 centers from 11 countries, where 589 subjects were randomized and dosed. Although study procedures were standardized as per protocol, potential site or center effect was examined in an exploratory manner. As subject number per site was not balanced with respect to the treatment allocation and some sites had few subjects, summary of DSN in cycle 1 was presented by country.

Pooling was performed to combine data from countries Slovakia, Czech Republic and Poland as these countries had very low number of subjects.

The mean (SD) DSN between the three treatment arms are comparable in most countries; small variation of mean DSN was detected in countries with the largest number of subjects (Bulgaria, Georgia, and Hungary). The largest variation was observed for the pooled unit including Slovakia, Poland and Czech Republic [(Pelgraz treatment: 3.0 (1.41); US-Neulasta treatment: 0.7 (0.58); EU-Neulasta treatment: 1.7 (1.03)].

For FAS-As Randomized, the main effect of country was statistically significant (P<0.001). Differences between countries are not uncommon in studies in which endpoints are measured in local laboratories. It is noteworthy however, that differences between treatment arms within most countries of this study were consistently small, which is suggestive of the lack of interaction between country and treatment effect.

Immunogenicity

Based on the test results of the 5421 samples, from 589 subjects dosed, 147 samples from 47 subjects were reported as potential positive in the ADA screening assay and were subsequently analysed in the confirmatory assay. Of the 147 samples analysed, 54 samples from 18 subjects were confirmed positive in the ADA confirmatory assay and further characterized for binding specificity and neutralizing activity. Of the 54 samples, 8 samples were positive for neutralizing antibodies; 3 samples from 3 subjects were determined to be positive for neutralizing antibodies to Pelgraz; 4 samples from 2 subjects were determined to be positive for neutralizing antibodies to G-CSF and 1 sample from 1 subject was determined to be positive for neutralizing antibodies to both Pelgraz and G-CSF. The remaining 46 samples were negative for neutralizing antibodies.

There was no apparent impact of ADA or neutralizing antibodies observed in this study on the pharmacodynamic activity of Pelgraz or Neulasta. In Cycle 1, presence of pre-existing antibodies had no negative impact on the ANC, Depth ANC Nadir and Duration of Severe Neutropenia measurements taken in the confirmed positive subjects. In Cycles 1-6, there was no correlation between the presence of detected ADA and failure to recover neutrophil counts. In addition, there were no apparent differences in ANC recovery between the ADA or neutralizing antibody positive subjects in the Pelgraz, US-Neulasta or EU-Neulasta groups.

Ancillary analyses

Supportive analyses for the Primary Efficacy Analysis

PP (Cycle 1) Analysis Set

Table 52 APO-Peg-03: Summary of Duration of Severe Neutropenia in Cycle 1 – PP (Cycle 1) Analysis Set

Statistics for duration (days)	APO-Peg N=275	Neulasta US N=133	Neulasta EU N=135	Total N=543
N	275	133	135	543
Mean (SD)	1.6 (1.26)	1.4 (1.19)	1.6 (1.36)	1.6 (1.27)
Median	2.0	1.0	2.0	1.0
Minimum – Maximum	0 – 7	0 – 5	0 – 10	0 – 10

Source: APO-Peg-03 Table 14.2.3

Table 53 APO-Peg-03: Duration of Severe Neutropenia in Cycle 1 – Least-Square Means (and 95% Confidence Intervals) of Treatment Arms and Differences between LS Means, Determined from an ANCOVA Accounting for the Treatment Effect and Baseline ANC (FAS – As Randomized and As Treated)

Statistics	APO-Peg	Neulasta US	Neulasta EU	APO-Peg - Neulasta US	APO-Peg - Neulasta EU	Neulasta EU - Neulasta US
As Randomized						
LS Mean	1.63	1.39	1.62	0.23	0.01	0.22
95% CI	1.47 to 1.78	1.17 to 1.61	1.40 to 1.84	-0.04 to 0.50	-0.26 to 0.28	-0.09 to 0.54
As Treated						
LS Mean	1.62	1.39	1.64	0.23	-0.03	0.25
95% CI	1.46 to 1.77	1.17 to 1.61	1.42 to 1.87	-0.04 to 0.50	-0.30 to 0.25	-0.06 to 0.57

Source: APO-Peg-03, Table 14.2.13 and Table 14.2.14

Summary of main study(ies)

The following tables summarise the 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 benefit risk assessment (see later sections).

Table 54 Summary of efficacy for trial Pelgraz-03

Title: A phase III, randomized, active controlled, assessor-blinded study of safety and efficacy of Pelgraz versus US and EU licensed Neulasta in subjects with stage IIa, IIb or IIIa breast cancer receiving TAC anticancer chemotherapy in adjuvant setting		
Study identifier	Pelgraz-03	
Design	Phase III, randomized (2:1:1), active controlled (US-, EU- Neulasta), assessor blinded study.	
	Duration of main phase:	18 months
	Duration of Run-in phase:	not applicable
	Duration of Extension phase:	not applicable
Hypothesis	Equivalence	
Treatments groups	Pelgraz-03	One single-dose 6 mg/0.6 mL pre-filled syringe, s.c., on Day 2 of each cycle, for up to 6 cycles. (n= 294)
	EU- Neulasta	One single-dose 6 mg/0.6 mL pre-filled syringe, s.c., on Day 2 of each cycle, for up to 6 cycles. (n= 147)

	EUS- Neulasta		One single-dose 6 mg/0.6 mL pre-filled syringe, s.c., on Day 2 of each cycle, for up to 6 cycles. (n= 148)
Endpoints and definitions	Primary endpoint	DSN (days)	Duration of severe neutropenia (DSN) in Cycle 1. Severe neutropenia was defined as ANC below 0.5 x 10^9/L.
	Secondary endpoint	Frequency of Grade 3 and 4 severe neutropenia	Frequency of Grade 3 and 4 severe neutropenia (ANC <1.0 x 109/L and <0.5 x 109/L, respectively) in Cycle 1.
	Secondary endpoint	Peak ANC/ Depth of ANC nadir in Cycle 1	The peak of ANC and depth of ANC nadir in Cycle 1
	Secondary endpoint	The time to the post nadir ANC recovery) in Cycle 1	The time to the post nadir ANC recovery (ANC ≥2.0 x 109/L) in Cycle 1
	Secondary endpoint	Rates of febrile neutropenia	Febrile neutropenia: a single temperature ≥38.3°C measured orally or ≥38.0°C for over 1 hour; neutropenia: ANC <0.5 x 109/L or <1 x 109/L and a predicted decline to ≤0.5 x 109/L over the next 48 hours, or AE of febrile neutropenia reported.
	Secondary endpoint	ANC-time profile in Cycle 1	ANC-time profile in Cycle 1 (time from beginning chemotherapy to the occurrence of the ANC nadir).
Database lock	N/A		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat (FAS-as randomized			
Descriptive statistics and estimate variability	Treatment group	Pelgraz	US-Neulasta	EU-Neulasta
	Number of subject	294	148	147

DSN (Cycle 1)

Statistics for duration (days)	APO-Peg	Neulasta US	Neulasta EU
FAS-As Randomized			
N	294	148	147
Mean (SD)	1.6 (1.48)	1.4 (1.17)	1.6 (1.34)
Median	2.0	1.0	2.0
Minimum - Maximum	0 - 10	0 - 5	0 - 10

Grade 3 and 4 Neutropenia (Cycle 1): n (%)	G3: 28 (9.5%) G4: 227 (77.2)	G3: 20 (13.5%) G4: 111 (75%)	G3: 13 (8.8%) G4: 117 (79.6%)
Peak ANC in Cycle 1 : mean $x10^9$/L (SD)	28.4 (9.54)	29.9 (10.17)	28.7 (9.28)
Depth of ANC nadir in Cycle 1: mean $x10^9$/L(SD)	0.6 (1.12)	0.4 (0.61)	0.4 (0.74)
Rate FN-cycle 1: n (%)	15 (5.1)	6 (4.1)	5 (3.4)

Effect estimate per comparison

Primary endpoint: DSN

Statistics for duration (days)	APO-Peg	Neulasta US	Neulasta EU	APO-Peg - Neulasta US	APO-Peg - Neulasta EU	Neulasta EU - Neulasta US
FAS-As Randomized						
LS Mean	1.63	1.39	1.61	0.24	0.02	0.21
95% CI	1.47 to 1.79	1.17 to 1.61	1.38 to 1.83	-0.03 to 0.51	-0.25 to 0.30	-0.10 to 0.53
FAS-As Treated						
LS Mean	1.62	1.39	1.63	0.23	-0.01	0.24
95% CI	1.46 to 1.77	1.17 to 1.61	1.41 to 1.86	-0.04 to 0.50	-0.29 to 0.26	-0.07 to 0.56

Source: APO-Peg-03, Table 14.2.9 and Table 14.2.10

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

Not applicable

Clinical studies in special populations

No studies in special populations have been conducted. In study Pelgraz-03, subgroup analysis of the primary efficacy endpoint, Duration of Severe Neutropenia (DSN) in Cycle 1, by age was conducted.

In the study, there were 55 subjects (9.3%) with age at baseline between 65-74 years, and only 3subjects (0.5%) with age above 74 years at baseline. The maximum age at baseline was 80 years. There were no subjects with age at baseline exceeding or equal to 85 years.

Table 55 APO-Peg-03: Summary of Duration of Severe Neutropenia in Cycle 1 for Age Subgroups and Overall (FAS-As Randomized)

Statistics	APO-Peg	Neulasta US	Neulasta EU	Total
Subjects – 18-64 years at baseline				
N	262	133	136	531
Mean (SD)	1.7 (1.52)	1.4 (1.19)	1.6 (1.34)	1.6 (1.40)
Subjects ≥65years at baseline				
N	32	15	11	58
Mean (SD)	1.3 (1.11)	1.1 (0.99)	1.1 (1.22)	1.2 (1.08)
Subjects – Overall				
N	294	148	147	589
Mean (SD)	1.6 (1.48)	1.4 (1.17)	1.6 (1.34)	1.6 (1.37)

Source: APO-Peg-03, Table 14.2.86, Table 14.2.87, and Table 14.2.1

Supportive study(ies)

No supportive efficacy/safety studies have been performed.

2.5.3. Discussion on clinical efficacy

The efficacy of this biosimilar application is based on study Pelgraz-03, which included patients undergoing adjuvant TAC therapy after surgical resection of breast cancer.

Design and conduct of clinical studies

No dedicated dose-finding studies or multiple-dose pharmacodynamic studies have been conducted with Pegylated Apo-Filgrastim. The dose and dosing regimen used in the phase 3 study (a fixed dose of 6 mg, once per cycle) was selected based on the approved ones for US- and EU-Neulasta. This is considered acceptable, since mandatory dose-finding studies are not required for a biosimilar application.

Main Study (Pelgraz-03) was a phase III, multicentre, randomized (2:1:1), active controlled, assessor-blinded, safety and efficacy equivalence trial. The study included two active comparators, US-license Neulasta and EU-authorised Neulasta. The study was powered to demonstrate the equivalence of Pelgraz vs. each active comparator, as well as directly comparing both active comparators. The focus of this assessment is the demonstration of equivalence of Pelgraz vs. EU-Neulasta.

Patients were included if they were to undergo adjuvant TAC (docetaxel, doxorubicin, cyclophosphamide) chemotherapy for stage IIA, IIB or IIIA breast cancer. This population is considered homogenous in terms of tumour type, planned chemotherapy and disease stage (local-regional, non-metastatic breast cancer). In comparison with the original MAA for Neulasta-EU, some

small differences can be observed, since the population in the pivotal trials allowed the inclusion of patient with more advanced disease (stage II/III/IV), and who could have received prior chemotherapy (either adjuvant therapy and/or no more than 1 regimen of chemotherapy for metastatic disease).

Several populations were pre-defined for analyses: FAS (all randomised subject who received at least one dose of the active treatment; this population was the initially intended to be the main population for analysis), the PP (per-protocol, patients without major protocol violations) and the SAS (safety population). During the conduct of the study, 53 subjects (9.0%) were not treated as per the randomization scheme (referred to as 'mixed dosing' subjects). As a result, the FAS was re-defined as two separate subsets: FAS-as randomized (original FAS) and FAS-as treated (according to the treatment actually received). All pre-planned analyses were conducted with both FAS populations subsets, as additional sensitivity analyses. The main focus of this assessment are the results from the FAS-as randomized population, which closely follow the ITT principle and is the population for the primary analysis. Results from the FAS-as treated, defined post-hoc, are considered informative and are included as supportive.

The selected primary endpoint was the duration of severe neutropenia (DSN) in Cycle 1, defined as ANC below 0.5 x 10^9/L. This is a pharmacodynamic endpoint, closely related to its mechanism of action. DSN was also the primary endpoint used in the pivotal studies of the original MAA for Neulasta-EU and it was considered appropriate for peg-filgrastim by the CPMP[7].
The secondary endpoints included further characterisation of ANC in cycle 1 (depth and peak of ANC nadir, time to the post nadir ANC recovery, and ANC-time profile); grade 3-4 neutropenia (cycle 1); rate of FN (per cycle and across cycles); mobilization of CD34+ cells (in a subset of patients, in cycle 1); frequency and type of (culture-confirmed) infections; incidence of IV antibiotic therapy and hospitalization; incidence, severity and distribution of bone pain; occurrence and/or resolution of chemotherapy-induced mucositis and modifications to the planned chemotherapy (% of scheduled chemotherapy dose delivered, proportion of subjects with chemotherapy doses reduced, omitted, or delayed; number of days of delay of chemotherapy). Only descriptive analyses were initially planned for the secondary endpoints.

Efficacy data and additional analyses

A total of 595 patients were included in the study, of which 589 were treated (294, and 148, 147 randomised to Pelgraz, US-Neulasta and EU-Neulasta, respectively) and comprise the main population for analysis (FAS-as randomised).

Demographic characteristics were fairly balanced, all patients were women and Caucasian. Elderly patients were underrepresented, since only 58 patients over the age of 65 were included in the study, only 3 of them were very elderly patients (> 75 years old) and the maximum age was 80 years of age.

Regarding to baseline disease characteristics, all patients were chemotherapy naïve. A slightly higher proportion of patients in the Pelgraz group had stage IIa disease (43.9%, vs. 39.9% and 39.5% for the US- and EU-Neulasta, respectively), while stage IIIa was more frequent in the US-Neulasta group (33.1%, vs. Pelgraz and EU-neulasta, 29.3% and 30.6%, respectively).

In general, the study population can be considered representative of a population with mostly local (non-metastatic) breast cancer.

[7] Neulasta EPAR. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000420/WC500025941.pdf

The study met its primary objective, by demonstrating an equivalent efficacy of Pelgraz as compared to EU-approved Neulasta in terms of DSN. However, it failed to demonstrate equivalence of Pelgraz vs. US-Neulasta and the equivalence of both active comparator (US vs. EU Neulasta), since these results fell outside of the pre-defined 95%CIs. Considering that US- Neulasta was used in the PD pivotal study and that in the phase 3 study the comparability between both reference products (US-EU Neulasta) fell outside of the pre-defined 95%CI (i.e., ±0.5 days) for the primary endpoint, the clinical bridging was not considered convincing . The assessment of the new PK/PD study (154-14), provided additional clinical bridging by demonstrating the similarity between Pelgraz and EU-Neulasta.

Regarding the secondary endpoints, only descriptive statistics were initially provided. In general, a few differences were observed between treatment groups in terms of FN rates, CD34+ cells mobilization, bone pain and ANC characteristics in cycle 1. Most of these differences appear to be small, mostly numerical and in general, they tend to favour EU-neulasta. Likewise, a similar trend can be observed in some of the related endpoints in the safety section. As part of the responses to the CHMP´s D120 LoQ, the Applicant provided *post hoc* statistical analyses for these secondary endpoints, which showed that the differences were not statistically significant. Although these results need to be taken with caution, they do not suggest that the differences are due to lack of efficacy.

Regarding the subgroup analyses according to age, the study failed to demonstrate equivalence of Pelgraz vs. either active comparator in the subgroup of patients >65 years old. The Applicant clarified that the study was not formally powered for this subgroup comparison. This limitation is acknowledged.

Regarding the potential impact of immunogenicity on efficacy and PD and considering the low incidence of ADA and neutralizing antibodies in study Pelgraz-03, a clinically relevant impact on efficacy seems unlikely.

The Applicant is requesting the only indication currently approved for EU-Neulasta (“Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy [with the exception of chronic myeloid leukaemia and myelodysplastic syndromes”]). This indication was supported by pivotal studies conducted in patients with breast cancer, as it was considered a good model due to the high risk of neutropenia related to cytotoxic chemotherapy. It can be agreed that mode of action of pegfilgrastim is the same across patient populations receiving cytotoxic therapy for other solid cancers, and the effect of Pelgraz would be expected to be comparable.

2.5.4. Conclusions on the clinical efficacy

Biosimilarity in efficacy of Pelgraz as compared to EU-approved Neulasta in terms of DSN has been demonstrated.

2.6. Clinical safety

The data from the clinical studies, Pelgraz-02 (conducted in a healthy subject population) and Pelgraz-03 (conducted in a breast cancer subject population) form the basis of the safety assessment of Pegylated Apo-Filgrastim.

Table 56 Studies Constituting the Pegylated Apo-Filgrastim Safety Database

Study Number	Location and Date	Phase	Indication and Population	Study Design	Test Product; Reference Product; Dose/ Regimen; Route of Administration	Number and Disposition of Subjects	Primary Safety Parameter(s)
APO-Peg-02	1 site; Toronto, Ontario 4/15/2013-7/22/2013	I	Healthy volunteer PK/PD	Single center, comparative, randomized, single-dose, assessor-blinded, 2-way crossover PK and PD study	6 mg Pegylated Apo-Filgrastim (6 mg/0.6 mL); 6 mg Neulasta (US-licensed) (6 mg/0.6 mL); Single dose (1 x 6 mg/0.6 mL); subcutaneous administration	66 (49 Male, 17 Female) 65 completed period 1, 56 completed period 2	Adverse events, Laboratories, Vital Signs, Immunogenicity
APO-Peg-03	56 centers in 11 countries (randomized and dosed subjects); Hungary, Slovakia, Czech Republic, Poland, Romania, Bulgaria, Serbia, Bosnia and Herzegovina, Ukraine, Georgia, and Russia 3/28/2012-5/30/2014	III	Subjects with stage IIa, IIb or IIIa breast cancer receiving TAC anticancer chemotherapy in adjuvant setting	Multicenter, randomized, active controlled, assessor-blinded study of safety and efficacy	6 mg Pegylated Apo-Filgrastim (6 mg/0.6 mL); 6 mg Neulasta (EU-approved) (6 mg/0.6 mL) and 6 mg Neulasta (US-licensed) (6 mg/0.6 mL); Single 6 mg/0.6 mL dose administered subcutaneously once per chemotherapy cycle for 6 cycles	595 randomized (Female) 589 randomized and dosed 547 completed treatment period 506 completed treatment and safety follow-up periods	Adverse events, Injection site reactions, Vital signs, Presence of antibodies, Abnormal clinical laboratory results

Patient exposure

In both Pelgraz-02 and Pelgraz-03, Pelgraz and Neulasta were administered subcutaneously with fixed doses of 6 mg per administration.

Table 57 Summary of Exposure to Study Drug

	Cumulative Dose	Pegylated Apo-Filgrastim, 6 mg	US-Neulasta, 6 mg	EU-Neulasta, 6 mg
Number of Healthy Volunteer Subjects (APO-Peg-02) – Period 1	6 mg	33	33	-
Number of Healthy Volunteer Subjects (APO-Peg-02) – Period 2	6 mg	27	30	-
Number of Cancer Subjects* (APO-Peg-03) – Cycle 1	6 mg	294	148	147
Number of Cancer Subjects (APO-Peg-03) – Cycle 2	12 mg	288	146	144
Number of Cancer Subjects (APO-Peg-03) – Cycle 3	18 mg	285	146	144
Number of Cancer Subjects (APO-Peg-03) – Cycle 4	24 mg	284	144	144
Number of Cancer Subjects (APO-Peg-03) – Cycle 5	30 mg	276	143	142
Number of Cancer Subjects (APO-Peg-03) – Cycle 6	36 mg	270	143	139

*Number of Cancer Subjects for drug exposure is the same for both Full Analysis Set (FAS) – As Randomized and Safety Analysis Set (SAS)
Source: APO-Peg-02, Listing 16.2.1.1; APO-Peg-03, Table 14.1.34

Study Pelgraz-02: Sixty- six (66) subjects were dosed in this study.

A total of 57 subjects (27 for Pelgraz and 30 for US-Neulasta) received a total of 12 mg of pegfilgrastim exposure (cumulative dose); of which fifty-six (56) subjects (84.85%) completed both phases of the study.

Healthy subjects in Pelgraz-02 were all adults (mean age 40.05 years, range 20-55 years). The majority were male (49 subjects, 74.24%), and most were White (39 subjects, 59.09%) with the remainder of subjects relatively equally distributed amongst the other racial groups (Black/African American: 10.61%, Asian: 9.09%, Multi-Racial: 10.61% and Aboriginal: 10.61%). The Pelgraz and US-Neulasta subjects were well balanced between groups for demographic variables.

Study Pelgraz-03: was a 3-arm, active controlled, study in which study drug was administered on day 2 following chemotherapy, for up to 6 cycles of chemotherapy treatment with maximal total cumulative pegfilgrastim exposures up to 36 mg. A total of 595 subjects were randomized, 589 were administered study drug, and 547 subjects completed the treatment phase of the study. Similar total numbers of subjects were exposed to Pelgraz, 294 subjects, as were exposed to the combined total of US-Neulasta (148 subjects) and EU-Neulasta (147 subjects) (295 subjects in the combined Neulasta arms).

In APO- Peg-03, subjects were well balanced across the three treatment arms (Pelgraz, US- Neulasta and EU-Neulasta) for these demographic variables. All subjects enrolled in Pelgraz-03 were females and all were White. The age range of subjects in Pelgraz-03 was 22-80 years, with 58 out of 589 subjects (9.8%) in Pelgraz-03 being geriatric subjects (≥ 65 years). Within each treatment arm in Pelgraz-03, the median body weight was similar; 73.0, 70.0, and 70.0 kg for Pelgraz, US-Neulasta, and EU-Neulasta, respectively.

Adverse events

Healthy Subjects (Pelgraz-02)

Of 66 (100%) randomized subjects, the number of subjects included in the safety analysis set was 60 (90.91%) for Pelgraz and 63 (95.45%) for US-Neulasta. A similar total number of adverse events were documented for the two drugs, 369 events with Pelgraz and 386 with US- Neulasta. One subject (1 out of 63, 1.6%) taking US- Neulasta (Subject GC60) experienced a serious adverse event of hypersensitivity.

Table 58 APO-Peg-02: Adverse Events by Treatment Group

	Pegylated Apo-Filgrastim Treatment	US-Neulasta Treatment
Number of Subjects who received study medication	60	63
Total Number of Subjects with Adverse Events	60	63
Total Number of Adverse Events	369	386
Total Number of Mild Adverse Events	331	329
Total Number of Moderate Adverse Events	38	57
Total Number of Severe Adverse Events	0	0
Total Number of Serious Adverse Events	0	1
Number of Possibly Related Adverse Events	139	154
Number of Probably Related Adverse Events	153	163

Source: APO-Peg-02, Table 14 and 15, Appendix 16.2.7.1 and 16.2.7.2.

Common AEs

The most common AEs, defined as those occurring in at least 5% of exposures to either Pelgraz or US-Neulasta, are shown below organized by system organ class.

Table 59 Most Common Adverse Events from APO-Peg-02 with ≥5% Incidence

		Period 1			Period 2			Post-Trial
System Organ Class	AE Preferred Term	Pegylated Apo-Filgrastim (N= 33)	US-Neulasta (N= 33)	Total (N= 66)	Pegylated Apo-Filgrastim (N= 27)	US-Neulasta (N= 30)	Total (N= 57)	Total (N= 57)
Any AE, n/N (%)		33 (100.0)	33 (100.0)	66 (100.0)	27 (100.0)	30 (100.0)	57 (100.0)	1 (1.75)
Cardiac Disorders	Palpitations	3 (9.09)	2 (6.06)	5 (7.58)		1 (3.33)	1 (1.75)	
Gastrointestinal Disorders	Nausea	3 (9.09)	4 (12.12)	7 (10.61)	1 (3.70)	6 (20.00)	7 (12.28)	
General Disorders And Administration Site Conditions	Asthenia	3 (9.09)	1 (3.03)	4 (6.06)				
	Chest Discomfort	2 (6.06)	2 (6.06)	4 (6.06)	1 (3.70)		1 (1.75)	
	Chest Pain	2 (6.06)	2 (6.06)	4 (6.06)	3 (11.11)	1 (3.33)	4 (7.02)	
	Feeling Hot	3 (9.09)	4 (12.12)	7 (10.61)	3 (11.11)	1 (3.33)	4 (7.02)	
	Non-Cardiac Chest Pain	3 (9.09)	2 (6.06)	5 (7.58)		1 (3.33)	1 (1.75)	
Investigations	Blood Pressure Diastolic Increased	2 (6.06)	1 (3.03)	3 (4.55)	2 (7.41)	2 (6.67)	4 (7.02)	
	Blood Pressure Increased	3 (9.09)	1 (3.03)	4 (6.06)	2 (7.41)	1 (3.33)	3 (5.26)	
	Blood Pressure Systolic Increased	1 (3.03)	2 (6.06)	3 (4.55)	3 (11.11)	6 (20.00)	9 (15.79)	
	Body Temperature Increased	3 (9.09)	1 (3.03)	4 (6.06)	1 (3.70)		1 (1.75)	
	Heart Rate Increased	2 (6.06)	3 (9.09)	5 (7.58)	1 (3.70)		1 (1.75)	
	Neutrophil Count Decreased	5 (15.15)		5 (7.58)		2 (6.67)	2 (3.51)	
	White Blood Cell Count Decreased	19 (57.58)	16 (48.48)	35 (53.03)	14 (51.85)	14 (46.67)	28 (49.12)	
	White Blood Cell Count Increased	33 (100.0)	33 (100.0)	66 (100.0)	27 (100.0)	30 (100.0)	57 (100.0)	
Musculoskeletal And Connective Tissue Disorders	Bone Pain	29 (87.88)	25 (75.76)	54 (81.82)	18 (66.67)	22 (73.33)	40 (70.18)	
	Musculo-skeletal Stiffness	1 (3.03)	4 (12.12)	5 (7.58)				
	Myalgia	3 (9.09)	4 (12.12)	7 (10.61)	1 (3.70)	1 (3.33)	2 (3.51)	
	Pain In Extremity	2 (6.06)	2 (6.06)	4 (6.06)				
Nervous System Disorders	Dizziness	3 (9.09)	5 (15.15)	8 (12.12)	1 (3.70)	3 (10.00)	4 (7.02)	
	Headache	19 (57.58)	19 (57.58)	38 (57.58)	10 (37.04)	17 (56.67)	27 (47.37)	
Respiratory, Thoracic And Mediastinal Disorders	Cough	3 (9.09)	4 (12.12)	7 (10.61)	1 (3.70)		1 (1.75)	
	Oropharyngeal Pain	2 (6.06)	3 (9.09)	5 (7.58)	1 (3.70)	4 (13.33)	5 (8.77)	
Skin And Subcutaneous Tissue Disorders	Hyperhidrosis	2 (6.06)	2 (6.06)	4 (6.06)		1 (3.33)	1 (1.75)	

Source: APO-Peg-02, Table 14.3.1.7 and Appendix 16.2.7.1

White blood cell count increases were noted in 100% of subjects receiving test or reference drug product, regardless of study treatment period. While white blood cell count increase is a known and expected pharmacodynamic effect of pegfilgrastim, values outside of normal ranges were reported as AEs in Pelgraz-02. The white cell count AEs were mild in severity without any apparent difference between the Pelgraz and Neulasta treatment groups.

White cell count decrease was also judged as mild in severity in 55.0% (33 out of 60 subjects) in the Pelgraz treatment group versus 47.6% (30 out of 63 subjects) in the Neulasta treatment group. One subject (GC58) (1.52%) in the Pelgraz group withdrew from the study due to white cell count increased adverse event.

The incidence of bone pain was not unexpected as this is a known and well reported AE for Neulasta treatment (SmPC Neulasta; USPI Neulasta). The related bone pain AEs were mild to moderate in severity without any apparent difference in severity between the Pelgraz and Neulasta treatment groups. Bone pain was judged as mild in severity in 35 % (21 out of 60 subjects) in the Pelgraz treatment group versus 23.8 % (15 out of 63 subjects) in the Neulasta treatment group. Bone pain was judged as moderate in severity in 43.3% (26 out of 60 subjects) in the Pelgraz treatment group versus 50.8% (32 out of 63 subjects) in the Neulasta treatment group. No subject withdrew from the study due to a bone pain adverse event and the pain was controllable with non-narcotic analgesics.

All reported headaches were either mild or moderate in severity; none resulted in discontinuation from the study.

Breast Cancer Subjects (Pelgraz-03)

Table 60 Overview of TEAEs in Treatment Period - SAS

Overview	APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
Subjects (%) reporting at least one AE	265 (90.1)	138 (93.2)	136 (92.5)	539 (91.5)
Number of AEs	3887	2386	2410	8683
Subjects (%) with SAEs	14 (4.8)	5 (3.4)	6 (4.1)	25 (4.2)
Number of SAEs	19	6	7	32
Death	0 (0.0)	1 (0.7)[a]	0 (0.0)	1 (0.2)
Subjects (%) with AEs leading to early withdrawal	7 (2.4)	3 (2.0)	2 (1.4)	12 (2.0)
Subjects (%) with IMP-related AEs	155 (52.7)	83 (56.1)	81 (55.1)	319 (54.2)
Subjects (%) with IMP-related SAEs	0	0	0	0
Subjects (%) with IMP-related bone pains	139 (47.3)	73 (49.3)	76 (51.7)	288 (48.9)
Subjects (%) with ISR	17 (5.8)	7 (4.7)	9 (6.1)	33 (5.6)

[a] This subject received mixed dosing and therefore appears in the APO-Peg arm in Table 54
A total of 6 subjects with AEs had their reason for discontinuation recorded as switched to safety follow-up (2 APO-Peg; 2 Neulasta US and 2 Neulasta EU in Table 7).
Source: Table 14.3.1.1 Table 14.3.6.10. Listing 16.2.7.6

-Safety Follow Up Phase:

Table 61 APO-Peg-03: Adverse Events by Treatment Groups (Safety Follow Up Phase)

Overview	Statistics[a]	APO-Peg (N-SFU=274)	US-Neulasta (N-SFU=145)	EU-Neulasta (N-SFU=142)	Total (N-SFU=561)
Subjects not reporting any Adverse Events	n/N (%)	262 (95.6)	134 (92.4)	138 (97.2)	534 (95.2)
Subjects reporting at least one Adverse Event	n/N (%)	12 (4.4)	11 (7.6)	4 (2.8)	27 (4.8)
Death	n/N (%)	1 (0.4)	1 (0.7)	0 (0.0)	2 (0.4)
SAEs	n/N (%)	2 (0.7)	4 (2.8)	0 (0.0)	6 (1.1)
Subjects with AEs leading to early withdrawal	n/N (%)	2 (0.7)	1 (0.7)	0 (0.0)	3 (0.5)
Life-threatening AEs	n/N (%)	1 (0.4)	2 (1.4)	0 (0.0)	3 (0.5)
Total number of subjects with unlikely related AEs	Number of Patients with AEs, n (%)	3 (1.1)	0 (0.0)	1 (0.7)	4 (0.7)
Total number of subjects with possibly related AEs	Number of Patients with AEs, n (%)	2 (0.7)	0 (0.0)	0 (0.0)	2 (0.4)
Total number of subjects with probably related AEs	Number of Patients with AEs, n (%)	1 (0.4)	0 (0.0)	0 (0.0)	1 (0.2)
Total number of subjects with definitely related AEs	Number of Patients with AEs, n (%)	1 (0.4)	3 (2.1)	0 (0.0)	4 (0.7)
Total number of subjects with unrelated AEs	Number of Patients with AEs, n (%)	8 (2.9)	9 (6.2)	3 (2.1)	20 (3.6)
Total number patients with mild AEs	Number of Patients with AEs, n (%)	9 (3.3)	4 (2.8)	3 (2.1)	16 (2.9)
Total number patients with moderate AEs	Number of Patients with AEs, n (%)	3 (1.1)	5 (3.4)	2 (1.4)	10 (1.8)
Total number patients with severe AEs	Number of Patients with AEs, n (%)	2 (0.7)	3 (2.1)	0 (0.0)	5 (0.9)

[a] n=Number of subjects
Source: APO-Peg-03, Table 14.3.1.3; Table 14.3.1.7; 14.3.1.11; Table 14.3.1.15;, Listing 16.2.7.6

Most common AEs

The most common AEs, defined as those occurring in ≥ 5% of subjects, are shown below organized by system organ class for the treatment phase. In the safety follow up phase, there were no AEs that occurred with ≥ 5% incidence.

Table 62 APO-Peg-03: Frequency Table of Most Common Adverse Evets (≥5% of subjects0 in Treatment Period-SAS

SOC	PT	APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
Any AE n/N (%)		265 (90.1)	138 (93.2)	136 (92.5)	539 (91.5)
Blood And Lymphatic System Disorders	All PTs	164 (55.8)	94 (63.5)	88 (59.9)	346 (58.7)
	Anaemia	14 (4.8)	8 (5.4)	8 (5.4)	30 (5.1)
	Febrile Neutropenia	15 (5.1)	7 (4.7)	4 (2.7)	26 (4.4)
	Leukocytosis	20 (6.8)	18 (12.2)	14 (9.5)	52 (8.8)
	Leukopenia	62 (21.1)	41 (27.7)	41 (27.9)	144 (24.4)
	Neutropenia	149 (50.7)	85 (57.4)	77 (52.4)	311 (52.8)
	Neutrophilia	13 (4.4)	14 (9.5)	11 (7.5)	38 (6.5)
	Thrombocytopenia	12 (4.1)	5 (3.4)	16 (10.9)	33 (5.6)
Ear And Labyrinth Disorders	All PTs	17 (5.8)	12 (8.1)	14 (9.5)	43 (7.3)
	Vertigo	17 (5.8)	9 (6.1)	14 (9.5)	40 (6.8)
Gastrointestinal Disorders	All PTs	168 (57.1)	82 (55.4)	86 (58.5)	336 (57.0)
	Abdominal Pain	19 (6.5)	9 (6.1)	10 (6.8)	38 (6.5)
	Abdominal Pain Upper	18 (6.1)	13 (8.8)	18 (12.2)	49 (8.3)
	Diarrhoea	51 (17.3)	32 (21.6)	37 (25.2)	120 (20.4)
	Dyspepsia	10 (3.4)	7 (4.7)	11 (7.5)	28 (4.8)
	Nausea	138 (46.9)	67 (45.3)	72 (49.0)	277 (47.0)
	Stomatitis	20 (6.8)	10 (6.8)	5 (3.4)	35 (5.9)
	Vomiting	43 (14.6)	18 (12.2)	28 (19.0)	89 (15.1)
General Disorders And Administration Site Conditions	All PTs	138 (46.9)	72 (48.6)	77 (52.4)	287 (48.7)
	Asthenia	72 (24.5)	44 (29.7)	37 (25.2)	153 (26.0)
	Fatigue	43 (14.6)	18 (12.2)	32 (21.8)	93 (15.8)
	Malaise	9 (3.1)	8 (5.4)	5 (3.4)	22 (3.7)
	Oedema Peripheral	15 (5.1)	10 (6.8)	9 (6.1)	34 (5.8)
	Pyrexia	21 (7.1)	10 (6.8)	21 (14.3)	52 (8.8)
Metabolism And Nutrition Disorders	All PTs	28 (9.5)	16 (10.8)	26 (17.7)	70 (11.9)
	Decreased Appetite	12 (4.1)	9 (6.1)	17 (11.6)	38 (6.5)
Musculoskeletal And Connective Tissue	All PTs	156 (53.1)	79 (53.4)	88 (59.9)	323 (54.8)
	Arthralgia	13 (4.4)	8 (5.4)	10 (6.8)	31 (5.3)
Disorders	Bone Pain	139 (47.3)	73 (49.3)	78 (53.1)	290 (49.2)
	Myalgia	28 (9.5)	19 (12.8)	15 (10.2)	62 (10.5)
Nervous System Disorders	All PTs	109 (37.1)	64 (43.2)	62 (42.2)	235 (39.9)
	Dizziness	58 (19.7)	27 (18.2)	28 (19.0)	113 (19.2)
	Headache	66 (22.4)	38 (25.7)	32 (21.8)	136 (23.1)
	Hypoaesthesia	9 (3.1)	9 (6.1)	6 (4.1)	24 (4.1)
Respiratory, Thoracic And Mediastinal Disorders	All PTs	31 (10.5)	15 (10.1)	20 (13.6)	66 (11.2)
	Oropharyngeal Pain	14 (4.8)	8 (5.4)	6 (4.1)	28 (4.8)
Skin And Subcutaneous Tissue Disorders	All PTs	88 (29.9)	46 (31.1)	49 (33.3)	183 (31.1)
	Alopecia	75 (25.5)	37 (25.0)	39 (26.5)	151 (25.6)

Source: APO-Peg-03, Table 14.3.1.17

Although the *safety follow up phase* did not have AEs that occurred with ≥ 5% incidence, the most common AEs that occurred in more than one subject were:

bone pain was reported in 4 subjects (0.7%), 1 subject (0.4%) in Pelgraz and 3 subjects (2.1%) in US-Neulasta arms, respectively. Oedema peripheral was reported in 3 subjects (0.5%), 2 subjects (0.7%) in Pelgraz and 1 subject (0.7%) in US-Neulasta arms, respectively. Asthenia was reported in 2 subjects (0.4%), 1 subject (0.4%) in Pelgraz and 1 subject (0.7%) in EU-Neulasta arms, respectively. Disease progression, paraesthesia and oropharyngeal pain were each reported in 2 subjects (0.4%), 1 subject (0.4%) in Pelgraz and 1 subject (0.7%) in the US-Neulasta arms. All other events in the follow-up period were single occurrences.

Adverse Events of Interest

-Cancer Subjects (Pelgraz-03)

Bone Pain

Table 63 Summary of Bone Pain AEs in Treatment Period-SAS

			APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
All bone pain AEs		Number (%) patients	139 (47.3)	73 (49.3)	78 (53.1)	290 (49.2)
		Number of AEs	1095	534	545	2174
Relationship to IMP	Not related	Number (%) patients	2 (0.7)	1 (0.7)	3 (2.0)	6 (1.0)
		Number of AEs	2	2	3	7
	Unlikely	Number (%) patients	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.2)
		Number of AEs	0	0	1	1
	Definitely	Number (%) patients	139 (47.3)	73 (49.3)	76 (51.7)	288 (48.9)
		Number of AEs	1093	532	541	2166
Severity	Mild	Number (%) patients	99 (33.7)	55 (37.2)	51 (34.7)	205 (34.8)
		Number of AEs	464	262	254	980
	Moderate	Number (%) patients	87 (29.6)	41 (27.7)	44 (29.9)	172 (29.2)
		Number of AEs	387	179	204	770
	Severe	Number (%) patients	46 (15.6)	23 (15.5)	20 (13.6)	89 (15.1)
		Number of AEs	244	93	87	424
SAEs of bone pain		Number (%) patients	0	0	0	0
		Number of AEs	0	0	0	0
Withdrawals due to AEs of bone pain			0	0	0	0

Source: Table 14.3.1.5, Table 14.3.1.9, Table 14.3.1.13, Listing 16.2.7.5, Listing 16.2.7.6

The proportions of subjects reporting bone pain of any intensity who received concomitant treatment for bone pain were similar across treatment arms: 26.2% (77 out of 294), 23.6%(35 out of 148) and 29.3% (43 out of 147) for Pelgraz, US-Neulasta and EU-Neulasta arms,respectively (Fisher's Exact test, p=0.547). These incidences were lower than the 75-79%reported in literature (Vogel et al., 2005;Kubista et al., 2003), further supporting that the occurrence of bone pain and the intensity of bone pain reported were not masked by potential over-prescribing of pain medication. In Pelgraz-03 study, the rates of bone pain events of any intensity that were treated with concomitant medication were also similar across the three treatment arms: 28.1% (308 out of 1095), 29.8% (159 out of 534) and 27.5% (150 out of 545) forPelgraz, US-Neulasta and EU-Neulasta, respectively.

Table 64 Injection Site Reactions

		Statistics	APO-PEG Treatment (N=294)	Neulasta (US licensed) Treatment (N=148)	Neulasta (EU licensed) Treatment (N=147)	Total (N=589)
Any injection site reaction		n (%)	17 (5.8)	7 (4.7)	9 (6.1)	33 (5.6)
Location	LEFT ARM	n (%)	9 (3.1)	3 (2.0)	5 (3.4)	17 (2.9)
	RIGHT ARM	n (%)	9 (3.1)	4 (2.7)	5 (3.4)	18 (3.1)
	LEFT THIGH	n (%)	0 (0.0)	1 (0.7)	0 (0.0)	1 (0.2)
	RIGHT THIGH	n (%)	2 (0.7)	0 (0.0)	1 (0.7)	3 (0.5)
	LEFT BUTTOCK	n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	RIGHT BUTTOCK	n (%)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.2)
	LEFT PART OF ABDOMEN	n (%)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.2)
	RIGHT PART OF ABDOMEN	n (%)	1 (0.3)	1 (0.7)	1 (0.7)	3 (0.5)
Intensity	MILD	n (%)	16 (5.4)	7 (4.7)	9 (6.1)	32 (5.4)
	MODERATE	n (%)	2 (0.7)	1 (0.7)	0 (0.0)	3 (0.5)
	SEVERE	n (%)	0 (0.0)	1 (0.7)	0 (0.0)	1 (0.2)
	NK	n (%)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.2)
Symptom/Sign	PAIN	n (%)	12 (4.1)	5 (3.4)	6 (4.1)	23 (3.9)
	TENDERNESS	n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	WARMTH	n (%)	7 (2.4)	5 (3.4)	4 (2.7)	16 (2.7)
	ERYTHEMA	n (%)	4 (1.4)	1 (0.7)	0 (0.0)	5 (0.8)
	SWELLING	n (%)	4 (1.4)	1 (0.7)	0 (0.0)	5 (0.8)
	OTHER	n (%)	1 (0.3)	0 (0.0)	1 (0.7)	2 (0.3)

Splenomegaly

Splenomegaly is a known significant risk with G-CSFs such as filgrastim and pegfilgrastim and considered potentially lethal from splenic rupture. No such adverse event was reported during Pelgraz-03, nor was splenomegaly detected by physical examination.

The most common AE indicative of splenic rupture was abdominal pain upper. It was reported in 18 (6.1%) subjects in the Pelgraz arm and 13 (8.8%) and 18 (12.2%) in the US- Neulasta and EU-Neulasta arms, respectively. Out of the 127 reported abdominal pain upper AEs, none of them were associated with splenomegaly or splenic rupture.

Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) also is a reported significant, potentially lethal risk with filgrastim or pegfilgrastim medicinal products such as Neulasta. In Pelgraz-03, no respiratory events consistent with such toxicity were reported.

Allergic Reactions

Data for allergic reactions indicate that possible allergic reactions to Pelgraz are similar to those in Neulasta. Numerical differences in the overall incidence of these AEs were due to non-SAEs, mainly mild HS AE. The profile of AEs by preferred terms is within that known for these medicinal products, with no unexpected AEs and thus, no issues of particular concerns.

Sickle Cell Disorder

No subject with known sickle cell disorder was enrolled in the Pelgraz-03.

Febrile neutropenia

The overall incidence of febrile neutropenia was higher in Pelgraz treatment arm: 5.1% vs 4.7% vs 2.7%, in US and EU Neulasta, respectively. A higher proportion of subjects had severe febrile neutropenia in the AI-Peg arm compared with Neulasta Us and Neulasta EU arms: 3.7%, 1.4% and 2.7% as was incidence of SAEs of febrile neutropenia: 3.1%, 2%, 1.4%. Most of the cases occurred in Cycle 1, consistent with literature. There were no withdrawals due to this AEs.

Table 65 Summary of Febrile Neutropenia AEs in Treatment Period-SAS

			APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
All febrile neutropenia AEs		Number (%) patients	15 (5.1)	7 (4.7)	4 (2.7)	26 (4.4)
		Number of AEs	15	7	5	27
Relationship to IMP	Not related	Number (%) patients	14 (4.8)	6 (4.1)	4 (2.7)	24 (4.1)
		Number of AEs	14	6	5	25
	Unlikely	Number (%) patients	1 (0.3)	1 (0.7)	0 (0.0)	2 (0.3)
		Number of AEs	1	1	0	2
Severity	Mild	Number (%) patients	1 (0.3)	1 (0.7)	0 (0.0)	2 (0.3)
		Number of AEs	1	1	0	2
	Moderate	Number (%) patients	3 (1.0)	4 (2.7)	0 (0.0)	7 (1.2)
		Number of AEs	3	4	0	7
	Severe	Number (%) patients	11 (3.7)	2 (1.4)	4 (2.7)	17 (2.9)
		Number of AEs	11	2	5	18
SAEs of febrile neutropenia		Number (%) patients	9 (3.1)	3 (2.0)	2 (1.4)	14 (2.4)
		Number of AEs	9	3	2	14
Withdrawals due to AEs of febrile neutropenia			0	0	0	0

Source: Table 14.3.1.5, Table 14.3.1.9, Table 14.3.1.13, Listing 16.2.7.5, Listing 16.2.7.6

Serious adverse event / deaths / other significant events

Deaths

There were no deaths in Pelgraz-02.

In the treatment period of Pelgraz-03, 1 out of 589 subjects (0.2%) died prior to dosing in cycle 4, due to progression of metastatic breast cancer. The subject was randomized to US-Neulasta arm, but received Pelgraz from Cycle 1 to 3. The event was reported by the Investigator as not related to the study drug. In the safety follow-up period of Pelgraz-03, 2 out of 561 subjects (0.4%) died, 1 subject (RU06606) (0.4%) from the Pelgraz arm and 1 subject (BA03803) (0.7%) from the US-Neulasta arm due to disease progression.

Other Serious Adverse Events

-Healthy Subjects (Pelgraz-02)

1 in 66 subjects (1.5%) experienced an SAE that was reported in Period 1 where one subject taking US-Neulasta experienced a hypersensitivity reaction, which required hospital admission but resolved without sequelae. The SAE was moderate in severity and considered probably related to Neulasta.

-Cancer Subjects (Pelgraz-03)

In the treatment period, the incidence of SAEs was similar across the treatment arms. SAEs were reported for 4.8%, 3.4% and 4.1% of subjects in the Pelgraz, US-Neulasta and EU-Neulasta treatment arms, respectively. None of the SAEs were considered related (either possibly, probably or definitely) to the study drug. One of the SAEs had a fatal outcome due to disease progression in the US-Neulasta arm (the same event was also reported as life threatening).

The most common serious adverse event was febrile neutropenia. Fourteen (2.4%) cases were reported altogether, 9 (3.1%) in Pelgraz arm, 3 (2.0%) in US-Neulasta arm and 2 (1.4%) in the EU-Neulasta arm, respectively. In the Pelgraz arm, the following additional SAEs occurred: thrombocytopenia, duodenal ulcer, vertebral fracture, cecum perforation, acute sinusitis, anaemia, pancytopenia and pulmonary embolism. Thrombocytopenia was assessed as definitely TAC related, its severity was moderate and it resolved without sequelae. All other SAEs can be associated with TAC chemotherapy or with the primary disease. In the US-Neulasta arm the following additional SAEs occurred: pneumonia and toxicoderma, and in the EU-Neulasta arm the following additional SAEs occurred: pneumonia, cholecystitis and acute pancreatitis. None of the SAEs were considered to be related to the study medication.

In safety follow up period 6 SAEs were reported for 2 (0.7%) subjects in the Pelgraz arm and 4 (2.7%) in the US-Neulasta arm and 0% in EU Neulasta. Two of these SAEs were fatal. None of these events were related to the study drug.

Laboratory findings

-Healthy Subjects (Pelgraz-02)

Abnormal laboratory events were common during the study, occurring in 61 subjects (92.42%) having abnormal laboratory results [44 subjects (66.67%) with abnormal laboratory results at Screening, 43 subjects (65.15%) during Period 1, and 44 subjects (66.67%) during Period 2]. Laboratory abnormality results were not linked to a specific diagnosis.

-Cancer Patients (Pelgraz-03)

Table 66 APO-Peg-03: Frequencies of All Clinically Significant Laboratory Results

Parameter	APO-Peg (N=294)	US-Neulasta (N=148)	EU-Neulasta (N=147)	Total (N=589)
Overall - Screening	3 (1.0)	3 (2.0)	1 (0.7)	7 (1.2)
Overall - Treatment	146 (49.7)	77 (52.0)	77 (52.4)	300 (50.9)

Source: APO-Peg-03, Table 14.3.4.3

Table 67 Frequencies of Shifts for Neutrophils and Haemoglobin from Baseline to Week 20

	APO-Peg (N=294)				Neulasta US (N=148)				Neulasta EU (N=147)			
	Week 20 Classification				Week 20 Classification				Week 20 Classification			
	Missing	Low	Normal	High	Missing	Low	Normal	High	Missing	Low	Normal	High
Neutrophils												
Baseline												
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Low	1 (0.3%)	0 (0.0%)	1 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.7%)	1 (0.7%)	0 (0.0%)
Norm	9 (3.1%)	9 (3.1%)	53 (18.0%)	2 (0.7%)	6 (4.1%)	9 (6.1%)	19 (12.8%)	0 (0.0%)	7 (4.8%)	7 (4.8%)	27 (18.4%)	0 (0.0%)
High	28 (9.5%)	36 (12.2%)	149 (50.7%)	6 (2.0%)	6 (4.1%)	20 (13.5%)	83 (56.1%)	5 (3.4%)	5 (3.4%)	16 (10.9%)	80 (54.4%)	3 (2.0%)
Haemoglobin												
Baseline												
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Low	4 (1.4%)	25 (8.5%)	5 (1.7%)	0 (0.0%)	1 (0.7%)	9 (6.1%)	3 (2.0%)	0 (0.0%)	2 (1.4%)	5 (3.4%)	5 (3.4%)	0 (0.0%)
Norm	33 (11.2%)	67 (22.8%)	157 (53.4%)	0 (0.0%)	10 (6.8%)	33 (22.3%)	91 (61.5%)	0 (0.0%)	10 (6.8%)	54 (36.7%)	68 (46.3%)	0 (0.0%)
High	0 (0.0%)	0 (0.0%)	3 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.7%)	0 (0.0%)	0 (0.0%)	1 (0.7%)	2 (1.4%)	0 (0.0%)

Source: Table 14.3.5.2.1.

Table 68 Frequencies of All Clinically Significant Haematology Laboratory Results by Parameter

Parameter	APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
Haemoglobin - Screening	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.2)
Haemoglobin - Treatment	14 (4.8)	5 (3.4)	6 (4.1)	25 (4.2)
Erythrocytes - Screening	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Erythrocytes - Treatment	6 (2.0)	3 (2.0)	4 (2.7)	13 (2.2)
Haematocrit - Screening	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Haematocrit - Treatment	6 (2.0)	4 (2.7)	4 (2.7)	14 (2.4)
Leukocytes - Screening	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Leukocytes - Treatment	66 (22.4)	43 (29.1)	47 (32.0)	156 (26.5)

Parameter	APO-Peg (N=294)	Neulasta US (N=148)	Neulasta EU (N=147)	Total (N=589)
Neutrophils - Screening	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.2)
Neutrophils - Treatment	134 (45.6)	72 (48.6)	72 (49.0)	278 (47.2)
Lymphocytes - Screening	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lymphocytes - Treatment	7 (2.4)	7 (4.7)	4 (2.7)	18 (3.1)
Platelets - Screening	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Platelets - Treatment	14 (4.8)	6 (4.1)	14 (9.5)	34 (5.8)

Source: Table 14.3.4.3

Safety in special populations

Table 69 Safety Profile by MedDRA Term and Subject Age (SAS-As Randomized population)

		APO-Peg N=294			US-Neulasta N=148			EU-Neulasta N=147			Total N=589			
MedDRA Terms		Age <65 N=262	Age 65-74 N=31	Age 75-84 N=1	Age <65 N=133	Age 65-74 N=14	Age 75-84 N=1	Age <65 N=136	Age 65-74 N=10	Age 75-84 N=1	Age <65 N=531	Age 65-74 N=55	Age 75-84 N=3	P-value *
Total AEs	Number of Patients with AEs, n (%)	234 (89.3%)	30 (96.8%)	1 (100.0%)	126 (94.7%)	12 (85.7%)	0 (0.0%)	125 (91.9%)	10 (100.0%)	1 (100.0%)	485 (91.3%)	52 (94.5%)	2 (66.7%)	0.935
	Number of AEs	3623	382	2	2146	291	0	2245	196	16	8014	869	18	
Serious AEs – Total	Number of Patients with AEs, n (%)	13 (5.0%)	3 (9.7%)	0 (0.0%)	6 (4.5%)	2 (14.3%)	0 (0.0%)	6 (4.4%)	0 (0.0%)	0 (0.0%)	25 (4.7%)	5 (9.1%)	0 (0.0%)	0.269
	Number of AEs	17	4	0	8	2	0	7	0	0	32	6	0	
- Fatal	Number of Patients with AEs, n (%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	2 (1.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.6%)	0 (0.0%)	0 (0.0%)	0.575
	Number of AEs	1	0	0	2	0	0	0	0	0	3	0	0	
- Hospitalization/ prolong existing hospitalization	Number of Patients with AEs, n (%)	11 (4.2%)	3 (9.7%)	0 (0.0%)	4 (3.0%)	1 (7.1%)	0 (0.0%)	4 (2.9%)	0 (0.0%)	0 (0.0%)	19 (3.6%)	4 (7.3%)	0 (0.0%)	0.284
	Number of AEs	14	4	0	5	1	0	5	0	0	24	5	0	
- Life-threatening	Number of Patients with AEs, n (%)	3 (1.1%)	2 (6.5%)	0 (0.0%)	3 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (1.1%)	2 (3.6%)	0 (0.0%)	0.194
	Number of AEs	4	2	0	3	0	0	0	0	0	7	2	0	
- Disability/ incapacity	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
- Other (medically significant)	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
AE leading to drop-out	Number of Patients with AEs, n (%)	8 (3.1%)	1 (3.2%)	0 (0.0%)	3 (2.3%)	1 (7.1%)	0 (0.0%)	2 (1.5%)	0 (0.0%)	0 (0.0%)	13 (2.4%)	2 (3.6%)	0 (0.0%)	0.716
	Number of AEs	8	1	0	3	2	0	2	0	0	13	3	0	
Psychiatric disorders	Number of Patients with AEs, n (%)	6 (2.3%)	1 (3.2%)	0 (0.0%)	7 (5.3%)	1 (7.1%)	0 (0.0%)	11 (8.1%)	0 (0.0%)	0 (0.0%)	24 (4.5%)	2 (3.6%)	0 (0.0%)	0.665
	Number of AEs	9	1	0	10	1	0	14	0	0	33	2	0	
Nervous system disorders	Number of Patients with AEs, n (%)	99 (37.8%)	15 (48.4%)	0 (0.0%)	61 (45.9%)	4 (28.6%)	0 (0.0%)	56 (41.2%)	6 (60.0%)	0 (0.0%)	216 (40.7%)	25 (45.5%)	0 (0.0%)	0.992
	Number of AEs	321	53	0	229	43	0	204	18	0	754	114	0	
Accidents and injuries	Number of Patients with AEs, n (%)	3 (1.1%)	0 (0.0%)	0 (0.0%)	3 (2.3%)	1 (7.1%)	0 (0.0%)	3 (2.2%)	0 (0.0%)	0 (0.0%)	9 (1.7%)	1 (1.8%)	0 (0.0%)	0.972
	Number of AEs	3	0	0	3	4	0	4	0	0	10	4	0	
Cardiac disorders	Number of Patients with AEs, n (%)	13 (5.0%)	2 (6.5%)	0 (0.0%)	9 (6.8%)	1 (7.1%)	0 (0.0%)	7 (5.1%)	1 (10.0%)	0 (0.0%)	29 (5.5%)	4 (7.3%)	0 (0.0%)	0.745
	Number of AEs	18	3	0	18	7	0	7	1	0	43	11	0	
Vascular disorders	Number of Patients with AEs, n (%)	24 (9.2%)	4 (12.9%)	0 (0.0%)	14 (10.5%)	2 (14.3%)	0 (0.0%)	14 (10.3%)	0 (0.0%)	0 (0.0%)	52 (9.8%)	6 (10.9%)	0 (0.0%)	0.998
	Number of AEs	41	5	0	20	3	0	16	0	0	77	8	0	
Cerebrovascular disorders	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
Infections and infestations	Number of Patients with AEs, n (%)	21 (8.0%)	1 (3.2%)	0 (0.0%)	15 (11.3%)	1 (7.1%)	0 (0.0%)	15 (11.0%)	2 (20.0%)	0 (0.0%)	51 (9.6%)	4 (7.3%)	0 (0.0%)	0.454
	Number of AEs	38	1	0	18	1	0	22	4	0	78	6	0	

For pegfilgrastim, while the pharmacokinetics are reported to have a high variability particularly in cancer patients (CPM Neulasta), various intrinsic factors such as age and gender are not reported to have an impact on the pharmacokinetics of pegfilgrastim, and hence its overall disposition and efficacy. Additionally, based on the available information in the literature, co- morbidities such as renal impairment and hepatic impairment are also not expected to have an effect on the disposition of pegfilgrastim as the clearance of pegfilgrastim is primarily mediated via neutrophils.

Additionally, considering the homogenous subject populations in study Pelgraz- 02 and Pelgraz-03, it would be difficult to clearly ascertain the impact of various intrinsic factors on the overall efficacy and safety profile of Pelgraz in comparison to Neulasta. An exploratory gender analysis was conducted for informational purposes only in Pelgraz-02, which included both male (74.24%) and female (25.76%) subjects. The results from this analysis reaffirmed the lack of expected gender effects on the disposition of pegfilgrastim.

		APO-Peg N=294			US-Neulasta N=148			EU-Neulasta N=147			Total N=589			
MedDRA Terms		Age <65 N=262	Age 65-74 N=31	Age 75-84 N=1	Age <65 N=133	Age 65-74 N=14	Age 75-84 N=1	Age <65 N=136	Age 65-74 N=10	Age 75-84 N=1	Age <65 N=531	Age 65-74 N=55	Age 75-84 N=3	P-value *
Anticholinergic syndrome	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
Quality of life decreased	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	Number of Patients with AEs, n (%)	47 (17.9%)	11 (35.5%)	0 (0.0%)	25 (18.8%)	4 (28.6%)	0 (0.0%)	26 (19.1%)	2 (20.0%)	0 (0.0%)	98 (18.5%)	17 (30.9%)	0 (0.0%)	0.099
	Number of AEs	92	31	0	61	15	0	66	6	0	219	52	0	
- Postural hypotension	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
- Falls	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
- Black outs	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
- Syncope	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	1 (1.8%)	0 (0.0%)	0.080
	Number of AEs	0	0	0	2	1	0	0	0	0	2	1	0	
- Dizziness	Number of Patients with AEs, n (%)	47 (17.9%)	11 (35.5%)	0 (0.0%)	24 (18.0%)	4 (28.6%)	0 (0.0%)	26 (19.1%)	2 (20.0%)	0 (0.0%)	97 (18.3%)	17 (30.9%)	0 (0.0%)	0.092
	Number of AEs	92	31	0	59	14	0	66	6	0	217	51	0	
- Ataxia	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	
- Fractures	Number of Patients with AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NE
	Number of AEs	0	0	0	0	0	0	0	0	0	0	0	0	

		APO-Peg N=294			US-Neulasta N=148			EU-Neulasta N=147			Total N=589			
MedDRA Terms		Age <65 N=262	Age 65-74 N=31	Age 75-84 N=1	Age <65 N=133	Age 65-74 N=14	Age 75-84 N=1	Age <65 N=136	Age 65-74 N=10	Age 75-84 N=1	Age <65 N=531	Age 65-74 N=55	Age 75-84 N=3	P-value *
Other AEs appearing more frequently in older patients**:														
- Bone Pain	Number of Patients with AEs, n (%)	123 (46.9%)	16 (51.6%)	0 (0.0%)	66 (49.6%)	7 (50.0%)	0 (0.0%)	69 (50.7%)	6 (60.0%)	1 (100.0%)	258 (48.6%)	29 (52.7%)	1 (33.3%)	0.763
	Number of AEs	1038	67	0	473	62	0	508	27	6	2019	156	6	
- Anemia	Number of Patients with AEs, n (%)	11 (4.2%)	3 (9.7%)	0 (0.0%)	6 (4.5%)	2 (14.3%)	0 (0.0%)	6 (4.4%)	2 (20.0%)	0 (0.0%)	23 (4.3%)	7 (12.7%)	0 (0.0%)	0.023
	Number of AEs	22	6	0	9	2	0	8	2	0	39	10	0	
- Leukocytosis	Number of Patients with AEs, n (%)	18 (6.9%)	3 (9.7%)	0 (0.0%)	16 (12.0%)	3 (21.4%)	0 (0.0%)	14 (10.3%)	0 (0.0%)	0 (0.0%)	48 (9.0%)	6 (10.9%)	0 (0.0%)	0.856
	Number of AEs	42	15	0	36	4	0	38	0	0	116	19	0	
- Eye Disorders	Number of Patients with AEs, n (%)	11 (4.2%)	1 (3.2%)	0 (0.0%)	6 (4.5%)	3 (21.4%)	0 (0.0%)	9 (6.6%)	2 (20.0%)	0 (0.0%)	26 (4.9%)	6 (10.9%)	0 (0.0%)	0.128
	Number of AEs	16	3	0	10	9	0	18	2	0	44	14	0	

MedDRA Terms		Age <65 N=262	Age 65-74 N=31	Age 75-84 N=1	Age <65 N=133	Age 65-74 N=14	Age 75-84 N=1	Age <65 N=136	Age 65-74 N=10	Age 75-84 N=1	Age <65 N=531	Age 65-74 N=55	Age 75-84 N=3	P-value *
- Gastrointestinal Disorders	Number of Patients with AEs, n (%)	151 (57.6%)	19 (61.3%)	1 (100.0%)	76 (57.1%)	7 (50.0%)	0 (0.0%)	79 (58.1%)	6 (60.0%)	1 (100.0%)	306 (57.6%)	32 (58.2%)	2 (66.7%)	0.838
	Number of AEs	755	84	1	518	63	0	512	52	6	1785	199	7	
- Vomiting	Number of Patients with AEs, n (%)	36 (13.7%)	8 (25.8%)	0 (0.0%)	17 (12.8%)	1 (7.1%)	0 (0.0%)	28 (20.6%)	2 (20.0%)	0 (0.0%)	81 (15.3%)	11 (20.0%)	0 (0.0%)	0.603
	Number of AEs	60	11	0	30	2	0	49	2	0	139	15	0	
- General Disorders And Administration Site Conditions	Number of Patients with AEs, n (%)	125 (47.7%)	16 (51.6%)	0 (0.0%)	66 (49.6%)	9 (64.3%)	0 (0.0%)	69 (50.7%)	8 (80.0%)	0 (0.0%)	260 (49.0%)	33 (60.0%)	0 (0.0%)	0.495
	Number of AEs	403	37	0	257	24	0	261	26	0	921	87	0	
- Asthenia	Number of Patients with AEs, n (%)	63 (24.0%)	10 (32.3%)	0 (0.0%)	39 (29.3%)	5 (35.7%)	0 (0.0%)	34 (25.0%)	4 (40.0%)	0 (0.0%)	136 (25.6%)	19 (34.5%)	0 (0.0%)	0.390
	Number of AEs	178	19	0	103	14	0	95	11	0	376	44	0	
- Fatigue	Number of Patients with AEs, n (%)	39 (14.9%)	6 (19.4%)	0 (0.0%)	15 (11.3%)	3 (21.4%)	0 (0.0%)	28 (20.6%)	4 (40.0%)	0 (0.0%)	82 (15.4%)	13 (23.6%)	0 (0.0%)	0.270
	Number of AEs	72	10	0	38	6	0	61	7	0	171	23	0	
- Investigations	Number of Patients with AEs, n (%)	21 (8.0%)	3 (9.7%)	1 (100.0%)	10 (7.5%)	1 (7.1%)	0 (0.0%)	13 (9.6%)	1 (10.0%)	1 (100.0%)	44 (8.3%)	5 (9.1%)	2 (66.7%)	0.090
	Number of AEs	58	6	1	21	1	0	31	3	1	110	10	2	
- Metabolism And Nutrition Disorders	Number of Patients with AEs, n (%)	25 (9.5%)	6 (19.4%)	0 (0.0%)	14 (10.5%)	4 (28.6%)	0 (0.0%)	26 (19.1%)	3 (30.0%)	0 (0.0%)	65 (12.2%)	13 (23.6%)	0 (0.0%)	0.062
	Number of AEs	50	14	0	29	12	0	48	9	0	127	35	0	
- Myalgia	Number of Patients with AEs, n (%)	24 (9.2%)	5 (16.1%)	0 (0.0%)	18 (13.5%)	1 (7.1%)	0 (0.0%)	13 (9.6%)	1 (10.0%)	1 (100.0%)	55 (10.4%)	7 (12.7%)	1 (33.3%)	0.304
	Number of AEs	39	9	0	38	1	0	33	1	2	110	11	2	
- Respiratory, Thoracic And Mediastinal Disorders	Number of Patients with AEs, n (%)	27 (10.3%)	4 (12.9%)	0 (0.0%)	15 (11.3%)	2 (14.3%)	0 (0.0%)	17 (12.5%)	3 (30.0%)	0 (0.0%)	59 (11.1%)	9 (16.4%)	0 (0.0%)	0.432
	Number of AEs	52	6	0	33	5	0	24	9	0	109	20	0	

NE – Not evaluable
*Two-sided P-value (alpha level = 0.05) of the Cochran-Armitage test for age-related incidence trend
**AEs occurring in at least 10% of elderly subjects (age ≥65) and occurring more frequently than in subjects under 65 years of age

Immunological events

-Healthy Subjects (Pelgraz-02)

A total of 10 samples from 6 subjects were confirmed positive in the ADA confirmatory assay and underwent further characterization for binding specificity and neutralizing activity. In Period 1, three subjects had detectable treatment emergent ADA after receiving Pelgraz in Period 1, representing an incidence of 9% (3 out of 33). Three subjects had detectable treatment emergent ADA after receiving US-Neulasta in Period 1, representing an incidence of 9% (3 out of 33). In Period 2 of this study, no additional subjects developed ADA and no ADA positive subjects had increased ADA titers. Two subjects became ADA negative; therefore, 4 subjects were positive for ADA at the end of Period 2.

ADA has the potential to affect clinical safety by mediating hypersensitivity or other immune reactions or by affecting the activity of an endogenous counterpart. Therefore, the adverse event (AE) profiles of subjects with ADA were evaluated and compared to those of ADA negative subjects. Assessment of their adverse event profiles reveals no clinically significant differences in type of event, or severity, when compared to subjects that were ADA negative. In addition, adverse event profiles of the 6 subjects with treatment emergent ADAs were not in keeping with clinical outcomes suggestive of immune mediated reactions. Only one subject experienced an SAE (hypersensitivity reaction) however all samples collected for this subject were ADA negative.

In the healthy subject study population, ADA impact on AEs, as it relates to white blood cell (WBC) count, and more specifically ADA impact on neutrophilia, was evaluated. There were no differences between the frequency of WBC count AEs and neutrophilia in the ADA positive and negative populations. In addition, the frequency of these events was similar in subjects who developed ADA after exposure to Pelgraz and subjects who developed ADA after exposure to US-Neulasta.

-Cancer Patients (Pelgraz-03)

In the study, 18 of 589 (3%) subjects assessed for immunogenicity were confirmed to be positive for ADA at one or more time point and were further assessed to characterize their ADA responses and their clinical impact. Pre-existing antibodies to pegfilgrastim were detected in a low percentage (2.2%) of the subjects (13/581) prior to their initial treatment in the study. Incidence of treatment-emergent induced ADA was low and highly similar between the three treatment groups: 1.0% (3/294) in the Pelgraz population, 0.7% (1/148) in the US-Neulasta population and 0.7% (1/147) in the EU-Neulasta population. In addition, ADA titers were low across all 3 treatment groups, and there was no pattern of increasing titers over time in the post-exposure period.

Reactivity was primarily directed to the PEG moiety, although a few subjects had pre-existing or induced reactivity to the filgrastim moiety or to rhuGCSF.

Neither Pelgraz nor Neulasta exposure resulted in the induction of neutralizing antibodies to Pegylated Apo-Filgrastim. Although pre-existing antibodies to pegfilgrastim were detected in a small number of subjects prior to treatment, none of these subjects remained positive following treatment and moreover, no subjects developed neutralizing antibodies to Pelgraz after exposure to any of the 3 products.

Neutralizing antibodies to GCSF were detected in 3 subjects. Two of these subjects were positive at the screen visit, of which one was negative at all post-dosing timepoints and one was only positive for anti-GCSF neutralizing antibodies at one other time point (W20) although other time points were positive

for ADA (Apo-Filgrastim and GCSF). The third subject (treated with Pelgraz) was positive for anti-GCSF neutralizing antibodies at two post-treatment time points. The GCSF neutralizing antibodies appeared to be transient and all 3 subjects were negative for neutralizing antibodies at their last time points tested.

An assessment of AE profiles of subjects that were positive to ADA or GCSF neutralizing antibodies, showed that no anaphylaxis or other immunologically related AEs were reported. Overall the AEs reported are consistent with the subjects' disease and treatment and were similar to those reported in the ADA negative subjects. There was no apparent relationship between the duration, timing, or specificity of ADA results and the AEs reported. In addition, there was no apparent differences in patterns of AEs reported for ADA confirmed positive subjects in the Pelgraz, US-Neulasta and EU-Neulasta groups. With respect to the subjects who had GCSF neutralizing antibodies, one subject completed the study and had no reported AEs and the other 2 subjects were withdrawn from the study due to AE/SAE that were apparently unrelated to the presence of neutralizing antibodies.

Safety related to drug-drug interactions and other interactions

No formal drug-drug interaction studies were performed with Pegylated Apo-Filgrastim. Similarly, no formal drug interaction studies between Neulasta and other drugs have been performed.

Discontinuation due to adverse events

A relatively small number of subjects did not complete studies Pelgraz-02 (10 subjects, 15.15%) and Pelgraz-03 (42 subjects, 7.1%). Reasons for subject withdrawal are shown below.

Table 70 Subject Withdrawals by Study

	APO-Peg-02 Healthy Subjects	**APO-Peg-03** Breast Cancer Subjects (Safety Analysis Set-Treatment Phase)	**APO-Peg-03** Breast Cancer Subjects (Safety Analysis Set-Safety Follow Up Phase)
Number of subjects/patients randomized	66	589	561
Completed	56 (84.8%)	547 (92.9%)	508 (90.6%)
Discontinued	10 (15.2%)	42 (7.1%)	53 (9.4%)
Reason for discontinuation			
Adverse event	3 (4.6%)	6 (1.0%)	9 (1.6%)
Consent withdrawn*	1 (1.5%)	18 (3.1%)	30 (5.3%)
Non-compliance with study drug	4 (6.1%)	1 (0.2%)	0 (0.0%)
Withdrawn due to PK/PD reasons**	2 (3.0%)	0 (0.0%)	0 (0.0%)
Protocol violation	0 (0.0%)	3 (0.5%)	0 (0.0%)
Switched to Safety Follow-up**	0 (0.0%)	14 (2.4%)	0 (0.0%)
Physician Decision	0 (0.0%)	0 (0.0%)	1 (0.2%)
Lost to Follow Up	0 (0.0%)	0 (0.0%)	13 (2.3%)

* Subjects withdrew informed consent because they were unable to continue due to personal reasons (i.e. moving to another country, wanted to stop chemotherapy, or without giving a reason).
** Subjects were withdrawn prior to the completion of the clinical phase and hence prior to PK/PD analysis.
*** Subjects who did not attend or were terminated before the last TAC cycle but came for safety follow-up visits were switched to safety follow-up regardless of which cycle they finished the chemotherapy treatment. Six of these discontinuations were in subjects with AEs but the reason for discontinuation was recorded as switched to safety follow-up (2 APO-Peg; 2 US-Neulasta and 2 EU-Neulasta).

Source: APO-Peg-02, Table 14.1.3; APO-Peg-03, Table 14.1.18

-Healthy Subjects (Pelgraz-02)

Adverse events in these subjects leading to discontinuation included wrist fracture in 1 subject (1.5%), hypersensitivity in 1 subject (1.5%) (both occurred in Period 1) and those subjects had been administered US-Neulasta. One subject (1.5%) experienced increased white cell count, neutrophil count and lymphocyte count that occurred in Period 1. He had been administered Pegylated Apo-Filgrastim.

-Cancer Subjects (Pelgraz-03) – Treatment Phase

Altogether 42 (7.1%) subjects were discontinued from the study during the treatment phase, 26 (8.8%) in the Pelgraz arm, 6 (4.1%) in the US-Neulasta arm and 10 (6.8%) in EU-Neulasta arm. A summary of the reasons for the discontinuation of these subjects is provided below.

The most common reason for study discontinuation was subject's withdrawal of consent: thirteen (4.4%) subjects withdrew their consent in the Pelgraz treatment arm, 1 (0.7%) subject in the US-Neulasta treatment arm and 4 (2.7 %) subjects in the EU-Neulasta treatment arm.

During the Treatment Period in the Pelgraz arm, 7 (2.4%) subjects were withdrawn due to the following AEs: paresthesia, cecum perforation, disease progression (2 subjects), pancytopenia, pulmonary embolism, and ejection fraction decreased G3. Out of these AEs cecum perforation, pancytopenia and pulmonary embolism were assessed as serious. None of these events were considered related to IMP. In the US-Neulasta arm, 3 (2.0%) subjects were withdrawn due to the

following AEs: left subclavian vein thrombosis, toxicoderma and redness, metastatic breast cancer. Of these events, the toxicoderma and metastatic breast cancer were assessed as serious, and the redness was considered possibly IMP-related. In the EU-Neulasta arm 2 (1.4%) subjects were withdrawn due to the following AEs: toxicoderma and sensory neuropathy. Neither was assessed as serious but the toxicoderma was assessed as probably related to IMP.

– Safety Follow up Phase

Altogether 53 (9.4%) subjects were discontinued from the study during the safety follow up phase, 28 (10.2%) in the Pelgraz treatment arm, 14 (9.7%) in the US-Neulasta treatment arm and 11 (7.7%) in EU-Neulasta treatment arm. The most common reason for discontinuation in the safety follow up phase was subject's withdrawal of consent: eighteen (6.6%) subjects withdrew their consent in the Pelgraz treatment arm, 8 (5.5%) subject in the US-Neulasta treatment arm and 4 (2.8 %) subjects in the EU-Neulasta treatment arm.

During the safety follow-up AEs leading to early withdrawal were reported for 3 (0.5%) subjects: 2 subjects (0.7%) withdrew in the Pelgraz arm and one (0.7%) subject in the US-Neulasta arm. Thirteen subjects (2.3%) were lost to follow-up, 6 (2.2%) subjects in the Pelgraz treatment arm, 3 (2.1%) subject in the US-Neulasta treatment arm and 4 (2.8%) subjects in the EU- Neulasta treatment arm.

Post marketing experience

There is no post-marketing experience with Pelgraz.

2.6.1. Discussion on clinical safety

The data from the clinical studies, Pelgraz-02 (conducted in a healthy subject population) and Pelgraz-03 (conducted in a breast cancer subject population) form the basis of the safety assessment of Pegylated Apo-Filgrastim. Safety data from these two studies have not been integrated given the differences between studies.

Patient exposure

Study Pelgraz-02 included healthy volunteers, with groups balanced with respect to demographic characteristics.

Study Pelgraz-03 included a highly selected (homogeneous) subset of white women with early Stage (IIA, IIB and IIIA) breast cancer and receiving TAC (docetaxel, doxorubicin, cyclophosphamide) anticancer chemotherapy in adjuvant setting, with a fairly balanced distribution of demographic and patients characteristics across treatment arms.

The studied populations are considered adequate for the comparability exercise.

Adverse events

-**Study Pelgraz-02**: 100% of subjects experienced at least 1 AEs on each study treatment. A similar total number of AEs were documented for the two drugs, 369 events with Pelgraz and 386 with US-Neulasta. All events were mild to moderate in severity and were similar between treatment groups, with slightly more moderate events for US-Neulasta (57/386 events, 14.7%) compared to Pelgraz

(38/369 events, 10.1%). There were no severe AEs. There was only one SAE reported by 1 subject (1.6%) taking US- Neulasta (Subject GC60), who experienced a serious adverse event of hypersensitivity. Most of the AEs were considered possibly or probably related to study drug.

A relatively small number of subjects did not complete Study Pelgraz-02 (10 subjects, 15.15%). Most subjects discontinued for reasons related to compliance in 4 subjects (6.1%). There were 3 subjects (4.6%) that withdrew due to adverse events.

Overall, in Study Pelgraz-02, the distribution of common AEs was relatively similar after exposure to Pelgraz and US-Neulasta, and relatively similar in the two study periods. The most common AE reported in 66 subjects was increased white blood cell count (100%), followed by bone pain (83.3% subjects), and headache (66.7% subjects). The frequencies of the most common AEs for each treatment arm and for each study period (Period 1 or Period 2) were overall similar.

No deaths occurred in Study 02. Only 1 SAE was reported: 1 HS reaction in the US Neulasta treated arm.

There were no notable differences between Pelgraz and Neulasta with respect to change in laboratory parameters from screening to 672 hours in either period 1 or period 2. The maximum AST and ALT values were greater for the Pelgraz arm, as was the change from screening to 672 hours in both periods 1 and 2. It is argued that subject GC42, who had individual values at approximately 3x upper limit of normal (ULN) during Period 1 while taking Pegylated Apo-Filgrastim, is likely contributing to the noted differences. An analysis excluding these patients has been provided to support this statement. Changes in AST/ALT were observed in different direction, were mostly mild and do not raise any particular concern.

It is noted that mean change from baseline in LDH at the end of period 2 was substantially higher for both treatment arms compared to the same analyses by the end of Period 1. In fact, mean change from baseline in period 1 was higher for Pelgraz vs Neulasta. These changes led to higher mean (minimum-maximum) values in the Pelgraz treatment arm. The magnitude of the mean change is not substantial. The LDH incremental changes that were observed in both Pelgraz-02 and Pelgraz-03 studies, which are well documented with the use of pegfilgrastim (Neulasta USPI), were transient laboratory findings that had no impact on the overall treatment course or safety of subjects receiving pegfilgrastim.

-**Study Pelgraz-03**: A total of 265 subjects (90.1%) in the Pelgraz arm, 138 subjects (93.2%) in the US-Neulasta arm, and 136 subjects (92.5%) in the EU-Neulasta arm reported adverse events. Most events were mild (61.2% Pelgraz-arm, 62% US Neulasta, 62.3% EU Neulasta) and to a lesser extent moderate (25.3%, 27.7%, 25.6% in APO-Pe-, US Neulasta and EU Neulasta, respectively) and severe (13.5%, 10.14%, 11.9% in APO-Pe-, US Neulasta and EU Neulasta, respectively) in all three groups. The proportion of subjects with SAEs was low: 14 (4.8%) subjects in Pelgraz, 5 (3.4%) subjects in US-Neulasta and 6 (4.1%) subjects in EU-Neulasta treatment arms.

Treatment was in general well tolerated, with the number of discontinuation being relatively low in all treatment arms: 26 (8.8%) in the Pelgraz arm, 6 (4.1%) in the US-Neulasta arm and 10 (6.8%) in EU-Neulasta arm. The rate of treatment discontinuations during the treatment phase and the safety FU were numerically higher in the Pelgraz arm (8.8% and 10.2%, respectively) compared to the rates reported in the EU Neulasta treated arm (6.8% and 7.7%, respectively). These differences are mainly driven by a higher rate of discontinuations due to withdrawal of consent in the Pelgraz treated arm, but these could not be attributed to lack of efficacy or particular safety issues. The overall incidence of discontinuations due to AEs was low with no major differences across study arms, both during

treatment phase (2.4%, 2% and 1.4% in Pelgraz, US Neulasta, and EU Neulasta, respectively) and the safety follow-up (0.7% in each treatment arm).

During the safety follow-up period, the overall incidence of AEs was low in all treatment arms: 4.4% Pelgraz arm, 7.6% US Neulasta, 2.8% EU Neulasta, mostly mild AEs. SAEs were reported in 2 (0.7%) subjects in the Pelgraz arm and 4 (2.8%) subjects in the US-Neulasta arm and 0 subjects in EU Neulasta. Two of the 6 SAEs had fatal outcome [1 (0.4%) in the Pelgraz arm, and 1 (0.7%) in the US-Neulasta arm]. None of these events were related to the study drug. No SAEs were reported in the EU Neulasta treated arm during this FU-period. Withdrawals due to AEs were rare and similar across study arms: 0.7% Pelgraz, 0.7% US Neulasta, 0.7% EU Neulasta.

Some numerical differences in the overall incidence of AEs, AEs leading to withdrawal, seriousness of AEs, SAEs, life threatening and fatal events, both during the treatment phase and the safety FU period are observed between EU-Neulasta and Pelgraz treatment arms. Differences are rather modest and of doubtful relevance. Most of the AEs driving these results are related to the underlying condition and/or the concomitant chemotherapy.

The occurrence of the three most common AEs (neutropenia, bone pain and nausea) was similar in all the three treatment arms. For neutropenia: 50.7%, 57.4% and 52.4% in Pelgraz, US-Neulasta and EU-Neulasta, respectively. For bone pain: 47.3%, 49.3% and 53.1% in Pelgraz, US-Neulasta and EU-Neulasta, respectively. For nausea: 46.9%, 45.3% and 49.0% in Pelgraz, US- Neulasta and EU-Neulasta, respectively. Other frequent AEs for which a difference >5% between Pelgraz arm and EU Neulasta was found include: leukopenia (21.1% vs 27.9%), thrombocytopenia (4.1% vs 10.9%), abdominal pain upper (6.1% vs 12.2%), fatigue (14.6% vs 21.8%), pyrexia (7.1% vs 14.3%), decrease appetite (4.1% vs 11.6%). The overall incidence of cytopenic disorders and GI disorders tended to be higher in the EU Neulasta treatment arm as compared to the Pelgraz-arm. These are known AEs of the concomitant QT, and no explanation is given for these differences. In the safety follow up phase, there were no AEs that occurred with ≥ 5% incidence.

Deaths and other SAEs: During the pivotal study, 3 deaths occurred, 1 during the treatment phase and 2 during the safety FU. In all cases these were not considered related to study treatment but the disease progression.

The total number of SAEs was 32. SAEs were reported for 4.8%, 3.4% and 4.1% of subjects in the Pelgraz, US-Neulasta and EU-Neulasta treatment arms, respectively. One of the SAEs had a fatal outcome due to disease progression in the US-Neulasta arm (the same event was also reported as life threatening). The most common serious adverse event was febrile neutropenia: 9 (3.1%) in Pelgraz arm, 3 (2.0%) in US-Neulasta arm and 2 (1.4%) in the EU-Neulasta arm, respectively. Other SAEs reported across the treatment groups appear more related to the underlying condition and/or to chemotherapy, i.e. thrombocytopenia, anemia, pulmonary embolism. In the safety follow up period 6 SAEs were reported for 2 (0.7%) subjects in the Pelgraz arm and 4 (2.7%) in the US-Neulasta arm, apparently not related to study drug toxicity.

AESI (only assessed in the Study Pelgraz-03):

-The overall incidence of *bone pain and related AEs*was slightly lower in the Pelgraz treated arm compared to the EU Neulasta (bone pain 47.3% vs 53.1%, respectively, all PTs 53.1% vs 59.9%, respectively). By contrary, the incidence of severe bone pain AEs was slightly higher in Pelgraz (15.6% vs 13.6%). The slightly higher incidence of severe bone pain in Pelgraz arm when compared with EU-Neulasta arm could be due to chance and are not considered relevant based on the totality of the safety data. In addition it was observed that none of the cases of bone pain were reported as an SAE, nor led to discontinuation from the study.

-ISR was reported in 17 (5.8%) subjects in Pelgraz arm, in 7 (4.7%) subjects in US-Neulasta arm and in 9 (6.1%) subjects in EU-Neulasta arm. Only one severe AEs was reported in US Neulasta. No discontinuations were reported.

-Splenomegaly and splenic rupture are known significant risks with G-CSFs such as filgrastim and pegfilgrastim. No such adverse event was reported during Pelgraz-03, nor was splenomegaly detected by physical examination. The most common AE indicative of splenic rupture was abdominal pain upper. It was reported in 18 (6.1%) subjects in the Pelgraz arm and 13 (8.8%) and 18 (12.2%) in the US-Neulasta and EU-Neulasta arms, respectively. All 26 events of moderate and severe upper abdominal pain were related to gastric pain, epigastric pain, stomach pain or stomach ache, asper the investigator's clinical judgment. None of the cases were judged to be related to splenomegaly or splenic rupture. In addition, none of the events of upper abdominal pain were considered as SAEs, nor led to withdrawal of study treatment, and all of the events resolved by the end of the study (18 out of 26 events were treated with concomitant medications while the remaining 8 events resolved without intervention. Further investigations were not conducted in any of the cases. While the presence of splenomegalia in some of these cases cannot be totally ruled out, the fact that all of them resolved (with or without treatment) and that none of them were considered SAEs is reassuring.

-No Acute respiratory distress syndrome (ARDS) events consistent with such a toxicity were reported. The most common AE indicative of ARDS was pneumonia, reported in 1 subject (0.3%) in the Pelgraz arm, 1 subject (0.7%) and 2 subjects (1.4%) in the US-Neulasta and EU-Neulasta arms, respectively. Two of these cases were considered serious, one each in the US-Neulasta and EU-Neulasta arms.

-Data for allergic reactions indicate that possible allergic reactions to Pelgraz are similar to those seen in EU-Neulasta.

-The overall incidence of febrile neutropenia, serious and severe febrile neutropenia were numerically higher in Pelgraz treatment arm: overall incidence 5.1% vs 4.7% vs 2.7%, in US and EU Neulasta, respectively, severe febrile neutropenia in the AI-Peg arm compared with Neulasta Us and Neulasta EU arms: 3.7%, 1.4% and 2.7% ; SAEs of febrile neutropenia: 3.1%, 2%, 1.4%. These were numerical differences of doubtful clinically relevance.

Laboratory changes:

The frequency of abnormal, clinically significant laboratory results was similar across treatment arms.

Overall out of range (abnormal), clinically significant laboratory parameters were recorded in 49.7% of subjects in the Pelgraz arm, 52.0% in the US-Neulasta arm and 52.4% in the EU- Neulasta arm. Most commonly the clinically significant abnormalities were in neutrophils (45.6%, 48.6% and 49.0%), leukocytes (22.4%, 29.1% and 32.0%) and platelets (4.8%, 4.1% and 9.5%) as would be expected in this patient population undergoing chemotherapy.

Overall, shifts to below normal range at W20 in neutrophils were similar between treatment arms. It is noted that the proportion of patients with shifts to below normal range in Hb values at W20 was higher in EU Neulasta (36.7%) compared to Pelgraz and US Neulasta treated arms (23% and 22.3%, respectively).

Vital signs and ECG: there are no relevant findings in any of the Studies.

Immunological events:

-*In Study Pelgraz-02:* a total of 6 subjects were confirmed ADA positive by the end of Period 1 (3 in each study treatment, representing an incidence of 9%). In Period 2 of this study, no additional subjects developed ADA and no ADA positive subjects had increased ADA titers. Two subjects became ADA negative; therefore, 4 subjects were positive for ADA at the end of Period 2. Subjects who were confirmed ADA positive had no clinical outcomes suggestive of immune mediated reactions, and hence ADA development was not correlated with any clinical safety concerns in this Study. Overall, no apparent difference was noted between treatment groups for the total number of AEs, severity, relationship to study drug, interventions, incidence rate and SOC of the most common AEs and immunogenicity results.

-*In Study Pelgraz-03:* 18 of 589 (3%) subjects assessed for immunogenicity were confirmed to be positive for ADA at one or more time point. Of them, 2.2% were ADA + at screening. Incidence of treatment-emergent induced ADA was low and highly similar between the three treatment groups: 1.0% (3/294) in the Pelgraz population, 0.7% (1/148) in the US-Neulasta population and 0.7% (1/147) in the EU-Neulasta population. The incidence of immunogenicity was low and highly similar across products. In addition, there were no apparent clinically meaningful effects associated with either pre-existing antibodies or induced ADA or NAb in these studies and no clinically meaningful differences between the immunogenic responses to Pelgraz and Neulasta. The Applicant concludes that an assessment of AE profiles of subjects that were positive to ADA or GCSF neutralizing antibodies, showed that no anaphylaxis or other immunologically related AEs were reported, which is reassuring. Overall the AEs reported are consistent with the subjects' disease and treatment and were similar to those reported in the ADA negative subjects.

Discontinuation due to adverse events

A relatively small number of subjects did not complete studies Pelgraz-02 (10 subjects, 15.15%) and Pelgraz-03 (42 subjects, 7.1%).

Overall, the rate of treatment discontinuations in the pivotal study was higher in the Pelgraz arm, 8.8% and 10.2% during the treatment phase and the safety follow up, respectively, compared to the rates reported in the EU Neulasta treated arm (6.8% and 7.7%, respectively). The Pelgraz arm had a higher incidence of premature discontinuation due to consent withdrawal (4.4%) and due to AEs (1.7%) in FAS-As Randomized; discontinuations due to other reasons were balanced among treatment arms. After conduction of an in depth analysis of these cases, it was concluded that these cannot be attributed to the lack of efficacy or to a particular safety concern related to Pelgraz. Differences in discontinuation rate among treatment arms were numerical but not statistically significant (Fisher's exact test, p=0.181). Based on these analyses, it can be concluded that these numerical differences were most likely due to chance.

Based on the comparative data presented, Pelgraz demonstrated a safety profile that was similar as compared to each of the commercially available US-licensed and EU-approved Neulasta.

Overall, adverse reactions reported in the studies were in line with the known safety profile of Neulasta, as described in its SmPC. The safety information of Pelgraz SmPC is fully aligned with the Neulasta SmPC. The list of safety concerns of the RMP of Pelgraz is also fully aligned with the one of Neulasta.

In addition, findings confirmed the low immunogenic potential of Pelgraz and support the biosimilarity of Pelgraz and Neulasta

2.6.2. Conclusions on the clinical safety

Pelgraz displayed a similar safety profile to Neulasta with no unexpected or significant safety findings. The safety profile of Pelgraz was consistent with the well-characterized mode-of-action of pegfilgrastim. There were no clinically relevant differences in the incidence, frequency, or duration of TEAEs between Pelgraz and Neulasta.

The available safety data support biosimilarity between Pelgraz and Neulasta.

2.7. Risk Management Plan

Safety concerns

Important identified risks	• Acute febrile neutrophilic dermatosis (Sweet's syndrome) • Serious Pulmonary Adverse Events including interstitial pneumonia and Acute Respiratory Distress Syndrome (ARDS) • Capillary leak syndrome • Cutaneous Vasculitis • Severe allergic reaction (anaphylactic reaction) • Sickle Cell Crisis in Patients with Sickle Cell Disease • Severe splenomegaly/splenic rupture • Musculoskeletal pain-related symptoms • Thrombocytopenia • Leukocytosis • Glomerulonephritis
Important potential risks	• Cytokine release syndrome • Immunogenicity (incidence and clinical implications of anti-G-CSF antibodies) • Drug Interaction with lithium • Malignant Cell Growth (myeloid malignancies such as acute myelogenous leukaemia [AML] and myelodysplastic syndrome [MDS]) • Off label use • Extramedullary hematopoiesis • Medication errors including overdose
Missing information	• Risks in children <18 years of age • Risks during pregnancy and lactation

Pharmacovigilance plan

There is no planned or ongoing additional study in the pharmacovigilance plan.

Routine pharmacovigilance activities are sufficient to address the safety concerns of this medicinal product.

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important Identified Risks		
Acute febrile neutrophilic dermatosis (Sweet's syndrome)	Routine risk minimisation measures: Section 4.8 of Pelgraz SmPC has information on this safety concern Section 4 of Pelgraz PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Serious Pulmonary Adverse Events including interstitial pneumonia and Acute Respiratory Distress Syndrome (ARDS)	Routine risk minimisation measures: Sections 4.4 and 4.8 of Pelgraz SmPC have information on this safety concern. Sections 2 and 4 of Pelgraz PIL have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Capillary leak syndrome	Routine risk minimisation measures: Sections 4.4 and 4.8 of Pelgraz SmPC have information on this safety concern Sections 2 and 4 of Pelgraz PIL have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: AE follow-up form for adverse reaction Additional pharmacovigilance activity: None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	haematology. Additional risk minimisation measures: None	
Cutaneous vasculitis	Routine risk minimisation measures: Section 4.8 of Pelgraz SmPC has information on this safety concern Section 4 of Pelgraz PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Severe allergic reaction (anaphylactic reaction)	Routine risk minimisation measures: Sections 4.3, 4.4 and 4.8 of Pelgraz SmPC have information on this safety concern. Sections 2 and 4 of Pelgraz PIL have information on this safety concern. Other routine risk minimisation measures include prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Sickle cell crisis in patients with sickle cell disease	Routine risk minimisation measures: Sections 4.4 and 4.8 of Pelgraz SmPC have information on this safety concern. Sections 2 and 4 of Pelgraz PIL have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	experienced in oncology and/or haematology. Additional risk minimisation measures: None	
Severe splenomegaly/splenic rupture	Routine risk minimisation measures: Sections 4.4 and 4.8 of Pelgraz SmPC have information on this safety concern. Sections 2 and 4 of Pelgraz PIL have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Musculoskeletal pain-related symptoms	Routine risk minimisation measures: Section 4.8 of Pelgraz SmPC has information on this safety concern Section 4 of Pelgraz PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Thrombocytopenia	Routine risk minimisation measures: Sections 4.4 and 4.8 of Pelgraz SmPC have information on this safety concern. Sections 2 and 4 of Pelgraz PIL have information on this safety concern. Other routine risk minimisation	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	
Leukocytosis	Routine risk minimisation measures: Sections 4.4 and 4.8 of Pelgraz SmPC have information on this safety concern Sections 2 and 4 of Pelgraz PIL have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Glomerulonephritis	Routine risk minimisation measures: Sections 4.4 and 4.8 of Pelgraz SmPC have information on this safety concern Sections 2 and 4 of Pelgraz PIL have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Important Potential Risks		
Cytokine release syndrome	Routine risk minimisation measures include the prescription only status of the product. Additional risk minimisation	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: AE follow-up form for adverse

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	measures: None	reaction Additional pharmacovigilance activity: None
Immunogenicity (incidence and clinical implications of anti-G-CSF antibodies)	Routine risk minimisation measures: Section 4.4 of Pelgraz SmPC has information on this safety concern. Section 2 of Pelgraz PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Drug interaction with lithium	Routine risk minimisation measures: Section 4.5 of Pelgraz SmPC has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: AE follow-up form for adverse reaction Additional pharmacovigilance activity: None
Malignant Cell Growth (myeloid malignancies such as acute myelogenous leukaemia [AML] and myelodysplastic syndrome [MDS])	Routine risk minimisation measures: Section 4.4 of Pelgraz SmPC has information on this safety concern Section 2 of Pelgraz PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	Additional risk minimisation measures: None	
Off label use	Routine risk minimization measures: Sections 4.1 and 4.4 of Pelgraz SmPC have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: AE follow-up form for adverse reaction Additional pharmacovigilance activity: None
Extramedullary haematopoiesis	Routine risk minimisation measures: Section 5.3 of Pelgraz SmPC has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Medication errors including overdose	Routine risk minimization measures: Sections 4.2, 4.5 and 4.9 of Pelgraz SmPC have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: AE follow-up form for adverse reaction Additional pharmacovigilance activity: None
Missing information		
Risks in children <18 years of age	Routine risk minimisation measures:	Routine pharmacovigilance activities beyond adverse

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	Sections 4.2 and 4.8 of Pelgraz SmPC have information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	reactions reporting and signal detection: None Additional pharmacovigilance activity: None
Risks during pregnancy and lactation	Routine risk minimisation measures: Sections 4.6 and 5.3 of Pelgraz SmPC have information on this safety concern. Section 2 of Pelgraz PIL has information on this safety concern. Other routine risk minimisation measures include the prescription only status of the product. Pelgraz therapy should be initiated and supervised by physicians experienced in oncology and/or haematology. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activity: None

Routine risk minimisation measures are considered sufficient to minimise the safety concerns of this medicinal product.

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.3 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

A justification for not performing a full user consultation with target patient groups on the package leaflet has been submitted by the applicant and has been found acceptable for a biosimilar of an authorised medicinal product.

2.9.2. Additional monitoring

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

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

3. Biosimilarity assessment

3.1. Comparability exercise and indications claimed

The indications claimed are as for the reference product (Neulasta):

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

A pre-submission meeting was held with EMA on January 15th, 2015 for Apotex to present an overview of the content of the proposed MAA for Pelgraz as a proposed biosimilar to Neulasta (pegfilgrastim) and receive feedback from the EMA on Apotex's readiness to proceed with the MAA filing for Pegylated Apo-Filgrastim.

As part of the global development program, Pelgraz has been developed as a proposed biosimilar to both the EU-approved and US-licensed Neulasta. Consistent with relevant guidances which were current at the time of product development, as follows:

- EMEA/CHMP/BMWP/42832/2005, February 2006
- EMEA/CHMP/BMWP/31329/2005,February 2006
- FDA Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product, February 2012
- FDA Guidance for Industry: Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to aReference Product, February 2012
- FDA Draft Guidance for Industry: Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product, May 2014

- Health Canada Guidance For Sponsors: Information and Submission Requirements for Subsequent Entry Biologics (SEBs), March 2010
- EMA - 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. 2015; 1-7.

EMA - Annex to Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Non-clinical and Clinical Issues. Guidance on Similar Medicinal Products Containing Recombinant Granulocyte-ColonyStimulating Factor. EMEA/CHMP/BMWP/31329/2005. 2006; 1-4.

3.2. Results supporting biosimilarity

Quality

Several biosimilarity studies conducted show comparability between the proposed biosimilar, Pegylated Apo-Filgrastim, and EU-approved and US-licensed Neulasta (the reference medicinal product), using a variety of test methods to confirm identity, structural features and biological activity. Results from the comparison of several batches derived from the commercial process of Pelgraz and several batches of Neulasta do not show significant differences that could have an impact on the comparability of the efficacy and safety profile. However, due to the limited number of batches used for the comparison concerns were raised regarding the representativeness of those batches to reflect the actual variability of both the biosimilar and the reference medicinal product and, therefore, the basis to establish biosimilarity from quality comparability studies was questionned. This was considered a critical point since some of the non-clinical and clinical studies have only been conducted with RMP derived from a non-EU source. Further, the Applicant provided results of new biosimilarity studies that resolve these concerns.

Non-clinical

The non-clinical overall data indicate that Pelgraz can be considered similar to the reference product Neulasta.

Clinical

The Applicant followed a stepwise approach to demonstrate similarity between Pelgraz and Neulasta, which consists of a phase 1 PK/PD study in healthy volunteers and a phase 3 study in breast cancer patients. Additional support derives from a new PK/PD study (154-14) was provided.

In the single dose PK/PD trial in healthy volunteers (Pelgraz-02) provided with the original submission, the PK/PD analyses revealed biosimilarity between the product and (US-labeled) Neulasta. Study 154-14 provided additional evidence on similarity between the product and EU-Neulasta.

In the phase 3 study, although the equivalence of Pelgraz vs. EU-Neulasta was demonstrated in terms of DSN (primary endpoint), the results of some of the secondary endpoints do not point in the same direction. Additional data provided by the Applicant do not suggest that these differences could be due to lack of efficacy.

Overall, comparable safety profiles of Pelgraz and US-Neulasta or EU-Neulasta were demonstrated in the Study conducted in early stage breast cancer subjects and of Pelgraz and US Neulasta in the

healthy volunteers Study.

3.3. Uncertainties and limitations about biosimilarity

There are no remaining uncertainties and limitations that have an impact on the conclusion of biosimilarity.

3.4. Discussion on biosimilarity

The clinical study submitted met its primary objective, by demonstrating an equivalent efficacy of Pelgraz as compared to EU-approved Neulasta in terms of DSN.

Descriptive statistics regarding the secondary endpoints, showed a few and small differences between treatment groups in terms of FN rates, CD34+ cells mobilization, bone pain and ANC characteristics in cycle 1, which do not suggest impact on efficacy. Regarding the potential impact of immunogenicity on efficacy and PD and considering the low incidence of ADA and neutralizing antibodies in study Pelgraz-03, a clinically relevant impact on efficacy or safety seems unlikely.

The claimed indication was supported by pivotal studies conducted in patients with breast cancer, as it was considered a good model due to the high risk of neutropenia related to cytotoxic chemotherapy. It can be agreed that mode of action of pegfilgrastim is the same across patient populations receiving cytotoxic therapy for other solid cancers, therefore the effect of Pelgraz in reduction of neutropenia is independent of the oncology condition under treatment.

3.5. Extrapolation of safety and efficacy

The Applicant is requesting the only indication currently approved for EU-Neulasta ("Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy [with the exception of chronic myeloid leukaemia and myelodysplastic syndromes"]). Therefore no extrapolation to other indications is discussed.

3.6. Additional considerations

Not applicable.

3.7. Conclusions on biosimilarity and benefit risk balance

Based on the review of the submitted data, Pelgraz 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 Pelgraz 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.