EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

25 July 2013
EMEA/CHMP/303037/2013
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

GRASTOFIL

International non-proprietary name: Filgrastim

Procedure No. EMEA/H/C/2150

Note

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

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List of abbreviations

Abbreviation	Meaning
ANC	Absolute Neutrophil Count
AUC	Area under curve
CI	Confidence interval
CPP	Critical Process Parameter
E. coli	Escherichia coli
ELISA	Enzyme-linked immunosorbent assay
FN	Febrile Neutropenia
GCSF	Granulocyte Colony-Stimulating Factor
HCP	Host Cell Protein
IEF	Isoelectric Focusing
i.v.	Intravenous
KPP	Key Process Parameter
MCB	Master Cell Bank
MU	Million Units
NCPP	Non-Critical Process Parameter
PBPC	Peripheral blood progenitor cells
PD	Pharmacodynamic
PK	Pharmacokinetic
Ph. Eur.	European Pharmacopoeia
PFS	Pre-Filled Syringe
rHu-met-GCSF	recombinant Human methionyl Granulocyte Colony-Stimulating Factor
RP-HPLC	Reversed Phase High-performance liquid chromatography
s.c.	Subcutaneous
SE-HPLC	Size Exclusion High-performance liquid chromatography
TSE	Transmissible spongiform encephalopathy
WCB	Working Cell Bank

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Apotex Europe B.V. submitted on 30 April 2012 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Grastofil, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication:

• Reduction in the duration of neutropenia and the incidence of febrile neutropenia in patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes) and for the reduction in the duration of neutropenia in patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia. The safety and efficacy of Filgrastim is similar in adults and children receiving cytotoxic chemotherapy.

• For the mobilisation of peripheral blood progenitor cells (PBPC).

• In patients, children or adults, with severe congenital, cyclic, or idiopathic neutropenia with an absolute neutrophil count (ANC) of 0.5 x 10^9/L, and a history of severe or recurrent infections, long term administration of Grastofil is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.

• For the treatment of persistent neutropenia (ANC less than or equal to 1.0 x10^9/L) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other options to manage neutropenia are inappropriate.

The legal basis for this application refers to:

Article 10(4) of Directive 2001/83/EC, as amended – 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.

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 on 18 October 2006. The Scientific Advice pertained to non-clinical and clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Manufacturer of the biological active substance

Intas Biopharmaceuticals Ltd.
Plot no: 423P/A
Sarkhej Bavla Highway
Village Moraiya; Taluka Sanand,
Ahmedabad – 382213 Gujarat
India

Manufacturer responsible for batch release

Apotex Nederland B.V.
Bio Science Park
Archimedesweg 2
NL-2333 CN Leiden
Netherlands

1.3. Steps taken for the assessment of the product

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

Rapporteur: Robert James Hemmings Co-Rapporteur: Sol Ruiz

- The application was received by the EMA on 30 April 2012.
- The procedure started on 23 May 2012.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 10 August 2012. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 10 August 2012.
- During the meeting on 17-20 September 2012, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 21 September 2012.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 12 March 2013.
- The summary report of the inspection carried out at Intas Biopharmaceuticals Limited, Ahmedabad, India between 19-22 February 2013 was issued on 8 May 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 30 April 2013.
- During the CHMP meeting on 27-30 May 2013, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 24 June 2013.
- During the meeting on 22-25 July 2013, 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 Grastofil on 25 July 2013.

2. Scientific discussion

2.1. Introduction

Problem statement

Cytotoxic chemotherapy suppresses the hematopoietic system causing profound and sometimes prolonged neutropenia. Chemotherapy-induced neutropenia is the major dose-limiting toxicity of systemic cancer chemotherapy. It may result in hospitalisation for treatment of fever or cause potentially fatal infection. Such complications of chemotherapy treatment often result in dose reduction or treatment delay which may compromise clinical outcome. Risk factors for cytotoxic chemotherapy-induced neutropenia are: advanced age, poor performance status, poor nutritional status and low baseline and first cycle nadir blood cell count along with high chemotherapy dose intensity. Some chemotherapy regimens are more myelosuppressive than others. High cyclophosphamide dose, etoposide and high anthracycline doses have been identified as significant predictors for severe neutropenia.

Prophylactic antibacterial, antifungal, and antiviral agents have been administered to prevent the development of infection as a complication of neutropenia. Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are used to reduce the duration and degree of neutropenia. G-CSF increases the proliferation and differentiation of neutrophils from progenitor cells, induces maturation and enhances the survival and function of mature neutrophils.

According to the European Organisation for Research and Treatment of Cancer (EORTC) guideline, primary prophylactic G-CSF treatment is recommended in case the overall risk of febrile neutropenia (FN) for a patient is ≥20%. When using chemotherapy regimens associated with a FN risk of 10-20%, particular attention should be given to the assessment of patient characteristics that may increase the overall risk of FN (Aapro et al., EJC, 2006; 42: 2433-53). Evidence from multiple randomised trials supports the benefit of primary prophylaxis in reducing the frequency of hospitalisation for antibiotic therapy, documented infection, and rates of neutropenic fever in adults. The impact on survival is less clear (Kuderer et al., J. Clin Oncol 2007; 25:3158).

Recombinant hG-CSF (filgrastim) has been introduced in clinical use since 1991 under the trade name Neupogen. Recombinant hG-CSF is produced in E. coli. Its amino acid sequence is identical to that of natural human G-CSF, except for the addition of an N-terminal methionine necessary for the expression in E. coli and it is not glycosylated.

About the product

The natural human granulocyte colony stimulating factor (G-CSF) is a glycoprotein composed of a single polypeptide chain of 174 amino acids and is glycosylated at a threonine residue. It:

- regulates the proliferation and differentiation of progenitor cells within the bone marrow and the release of mature neutrophils into the peripheral blood
- is a positive regulator of granulopoiesis, acting at different stages of myeloid cell development
- enhances the effector functions of normal mature neutrophils, including chemotaxis, phagocytosis and oxidative metabolism

exerting its effects via a high-affinity G-CSF-specific receptor mechanism, which accounts for its selective action compared to many other cytokines.

Grastofil, is a formulation of non-glycosylated recombinant granulocyte colony stimulating factor (G-CSF or filgrastim) developed as a biosimilar medicinal product to the reference product Neupogen. Filgrastim in Grastofil is also referred to as “Apo-filgrastim”, which was the company development code for the product.

Grastofil is presented in single use prefilled syringes in two strengths, 300µg/0.5ml and 480µg/0.5ml. It is administered via the intravenous (i.v. infusion) or subcutaneous (s.c. injection) route of administration.

Grastofil is indicated for the reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes) and for the reduction in the duration of neutropenia in adult patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia.

Grastofil is indicated for the mobilisation of peripheral blood progenitor cells (PBPCs) in adults.

In adult patients with severe congenital, cyclic, or idiopathic neutropenia with an absolute neutrophil count (ANC) of ≤ 0.5 x 10^9/L, and a history of severe or recurrent infections, long term administration of Grastofil is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.

Grastofil is indicated for the treatment of persistent neutropenia (ANC less than or equal to 1.0 x 10^9/L) in adults with advanced HIV infection, in order to reduce the risk of bacterial infections when other options to manage neutropenia are inappropriate.

Grastofil therapy should only be given in collaboration with an oncology centre which has experience in granulocyte-colony stimulating factor (G-CSF) treatment and haematology and has the necessary diagnostic facilities. The mobilisation and apheresis procedures should be performed in collaboration with an oncology-haematology centre with acceptable experience in this field and where the monitoring of haematopoietic progenitor cells can be correctly performed.

The recommended dose of filgrastim is 0.5 MU/kg/day (5 µg/kg/day). The first dose of Grastofil should not be administered less than 24 hours following cytotoxic chemotherapy. In patients with myeloablative therapy, the recommended starting dose of filgrastim is 1.0 MU/kg/day (10 µg/kg/day). In patients undergoing myelosuppressive or myeloablative therapy followed by autologous PBPC transplantation the recommended dose of filgrastim for PBPC mobilisation when used alone is 1.0 MU/kg/day (10 µg/kg/day) for 5 - 7 consecutive days, whereas in normal donors, the recommended dosage is 1.0 MU/kg/day (10 µg/kg/day) for 4 - 5 consecutive days. In congenital neutropenia, the recommended starting dose is 1.2 MU/kg/day (12 µg/kg/day) as a single dose or in divided doses. The recommended starting dose for idiopathic or cyclic neutropenia is 0.5 MU/kg/day (5 µg/kg/day) as a single dose or in divided doses. The recommended starting dose of filgrastim is 0.1 MU/kg/day (1 µg/kg/day) given daily with titration up to a maximum of 0.4 MU/kg/day (4 µg/kg/day) until a normal neutrophil count is reached and can be maintained (ANC > 2.0 x 10^9/L).

2.2. Quality aspects

2.2.1. Introduction

Grastofil has been developed as a “similar biological medicinal product” according to Article 10 (4) and Annex 1, Part II, Chapter 4 of Directive 2001/83/EC, as amended. The chosen reference medicinal product is Neupogen, which is manufactured and marketed by Amgen Ltd.

Grastofil is presented as a solution for injection or infusion in prefilled glass syringes containing 30MU / 0.5ml or 48MU / 0.5ml filgrastim.

The reference comparator products are Neupogen 30 MU (300 mcg/0.5 ml) solution for injection in pre-filled syringe and Neupogen 48 MU (480 mcg/0.5 ml) solution for injection in pre-filled syringe, Amgen Europe B.V. sourced from the EU market. The same reference product, Neupogen, was used for the entire comparability exercise to demonstrate comparable quality, safety and efficacy of the test product.

2.2.2. Active Substance

Filgrastim is a recombinant Human Methionyl Granulocyte Colony-Stimulating Factor (rHu-met-GCSF), produced at Intas Biopharmaceuticals Ltd. (IBPL) from *E. coli* host cells transformed with the codon-optimized GCSF DNA.

The protein obtained by this technology is non-glycosylated and consists of 175 amino acids of molecular weight 18800.8 Da.

Filgrastim contains 5 cysteine residues; these 5 cysteine residues form 2 disulfide bridges, leaving 1 free cysteine residue.

The mature and unmodified form of G-CSF has a predominant alpha helical secondary structure.

Manufacture

The Cell Substrate was generated by transfer of codon-optimized GCSF DNA to *E. coli* BL21 DE3 host cells using an expression vector. The vector development involved multiple steps of genetic engineering and manipulation.

A two tier cell bank system is followed at Intas Biopharmaceuticals Limited (IBPL), which consists of the Master Cell Bank (MCB) and a Working Cell Bank (WCB).

The MCBs and WCBs are characterized to ensure identity, purity, viability and stability of the cell bank for its intended use.

Appropriate data have been provided to demonstrate genetic stability of the host cell construct.

E.coli are expanded in fermentors using human and animal-free growth media. Filgrastim is concentrated in E.coli inclusion bodies (IB) which are isolated by cell disruption and centrifugation and then solubilised to allow protein re-folding.

Down-stream processing involves several filtration and chromatographic purification steps to separate filgrastim from other contaminating proteins and impurities. This is followed by further chromatography steps to yield the active substance solution.

In-Process Manufacturing Controls

The manufacturing process and control strategy has been adequately described. Classification and definitions of the Operating Parameters and Performance Parameters into critical (CPP) and key process parameters (KPP) were provided. In-process manufacturing controls (CPP or KPP) for each step of the manufacture process, together with acceptance criteria or expected ranges, were established on the basis of a risk assessment.

Process validation

CPPs or KPPs for each step of the commercial manufacturing process and Drug Substance release specifications were studied to qualify the manufacturing process performance during three consecutive

Drug Substance production batches. The results showed good reproducibility, often within narrower limits than the predefined ranges. The proven acceptable ranges for operating and performance parameters have been adequately supported with qualifying data and proposed process monitoring. Relevant information has been provided with respect process performance and consistency.

Manufacturing process development

The manufacturing process development has been an iterative process through nine sequential processes (Processes I to IX), not all of which have a direct relevance to Grastofil.

Non clinical studies were conducted with material from Process V, clinical trials used product from Process VII and the proposed commercial medicinal product will be obtained with Process IX. Comparability data were provided for material derived from process VII and process IX vs the reference medicinal product Neupogen, see discussion on comparability. Additionally, Process IX derived drug product has been used in the Phase I 3-arm bridging study (GCSF-SUIN-05Sb01-3FA) to support the claims of in vivo biosimilarity of Grastofil and Neupogen (see discussion on clinical pharmacology).

Specification

Characterisation

The structural and functional characteristics of the drug substance have been investigated using a variety of analytical tools, including N-terminal sequencing, SDS-PAGE, Isoelectric Focusing (IEF), peptide mapping, mass spectrometry, and determination of biological activity.

The biological activity was assessed using two orthogonal procedures: an in vitro cell proliferation assay and a receptor binding assay. A number of additional techniques have been used to assess higher-order structure, including Circular Dichroism (CD), FTIR (Fourier Transformed Infrared Spectroscopy), Analytical Ultracentrifugation and thermal stability by Differential Scanning Calorimetry (DSC). The data presented confirmed the expected primary, secondary and tertiary structure, with no major clipped species, and that the molecule is functional.

Impurities

The Filgrastim Concentrated Solution (2206) monograph in the current edition of the European Pharmacopoeia (Ph. Eur.) contains specified impurities for the Reversed Phase (RP-) and Size Exclusion (SE-) chromatography procedures (oxidised forms, dimer and aggregates). No additional product-related new impurities have been identified in the Grastofil Drug Substance.

Process-related impurities include host cell contaminants (host cell protein and residual DNA) which have been shown to be consistently cleared by the manufacturing process. Additives used during manufacture were shown to be adequately removed.

Control of Drug Substance

The proposed specification for the Drug Substance reflects the requirements of the Ph.Eur. monograph for filgrastim concentrated solution, as well as currently available batch release and stability data, and is accepted.

Routine testing is performed at release for Host Cell Protein (HCP) and residual DNA, bioburden, endotoxins. Identity is confirmed by peptide mapping and Isoelectric Focusing (IEF) against a reference solution, while the purity is tested by SDS-PAGE, RP-HPLC, SE-HPLC and IEF.

The in-vitro bioassay is based on the on the Ph.Eur monograph for filgrastim concentrated solution.

The Ph. Eur. filgrastim monograph methods have been modified by the manufacturer, in part, to match the materials and commercial kits available to the manufacturer in their own territory or to improve the sensitivity of the monograph methods. The rationale for the changes introduced has been provided and the analytical methods have been appropriately validated.

The applicant will review the drug substance specifications once data on a pre-determined number of batches is available.

Container closure system

The Drug Substance is filled into sterile glass bottles.

Stability

Stability studies of the Drug Substance were performed at long term (5 ± 3°C), and at short term with accelerated (25 ± 2°C), and stressed (40 ± 2°C) conditions according to ICH Q5C. Photostability studies indicate the drug substance is photolabile and should be protected from light.

The proposed shelf life of 24 months for the Drug Substance when stored at 5°C ± 3 °C in the proposed container is accepted.

2.2.3. Finished Medicinal Product

The Drug Product is a sterile, clear, and colourless liquid formulation (acetate buffered, pH 4.0, isotonic solution for injection) in a 1 mL glass (Type I), single-use, prefilled syringe for parenteral administration in two strengths:

- 30 MU (300 mcg/0.5 mL) dosage strength containing 300 mcg of Drug Substance
- 48 MU (480 mcg/0.5 mL) dosage strength containing 480 mcg of Drug Substance

Both strengths will be supplied in packs of one (1 x 1) or five (1 x 5) pre-filled syringes in a carton along with the prescribing information.

Subcutaneous (s.c.) injection is the primary route of administration, although the product may also be diluted for infusion administration (administered by short intravenous (i.v.) infusion or continuous i.v. infusion).

There are no validated markings on the syringe barrel that are compatible with paediatric posology.

The Drug Product contains Filgrastim as the active pharmaceutical ingredient and excipients, similar to those of the reference medicinal product Neupogen. The quantitative composition of the Drug Product is given in the Table below.

Table 1: Quantitative Composition of the Drug Product

Ingredient	Concentration (mg/mL)		Quantity per Syringe (mg/0.5mL)		Function
	30 MU / 0.5 mL strength	48 MU / 0.5 mL strength	30 MU / 0.5 mL strength	48 MU / 0.5 mL strength	
rHu G-CSF (Apo-Filgrastim Drug Substance)	0.60	0.96	0.30	0.48	Active Pharmaceutical Ingredient – Human Granulocyte Colony-Stimulating Factor
Glacial Acetic Acid					Buffering Agent
Sodium Hydroxide					Buffering Agent
D-Sorbitol					Tonicity Agent; Isotonicity Adjuster
Polysorbate 80					Stabilizer Nonionic surfactant
Water for Injection					Vehicle / Solvent

The Drug Product is a liquid formulation (ready to use parenteral administration), and thus requires no reconstitution with any diluent. However, if required, it can be diluted with 5% dextrose (intravenous infusion fluid) either glass bottles or Polyolefin bags / PVC bags. Should the Drug Product be diluted to concentrations below 15mcg / ml, human serum albumin should be added to a final concentration of 2 mg/mL.

No diluent is supplied with the Drug Product. It is intended that standard 5% dextrose and human serum albumin solutions from hospital pharmacy stocks will be used to prepare the Drug Product for infusion.

Pharmaceutical Development

The Drug Product formulation was established based on knowledge of the formulation excipients and concentrations of the reference medicinal product.

Pre-formulation and characterization studies confirmed that the excipients and concentrations in the formulation of the reference medicinal product are acceptable for the stability and maintenance of the Drug Substance at both 300 mcg/0.5 mL and 480 mcg/0.5 mL in the prefilled syringe presentation. Neither of the two Drug Product strengths employs an overage. Both strengths contain a 0.06 mL overfill (0.56 mL target fill volume) to ensure an extractable volume of 0.5 mL at the time of administration.

Adventitious agents

The manufacture of the Drug Product utilises one excipient of biological origin, Polysorbate 80, which is not animal-derived.

Polysorbate 80 consists of a mixture of fatty acids. The materials used for the manufacturing of Polysorbate 80 are of vegetable and petrochemical origin and do not contain material of bovine, ovine or caprine origin.

Bovine milk sourced from New Zealand is in the composition of the Terrific Broth culture media, used in the production of the MCB or WCB. It is subject to a strong heat treatment, therefore, its viral safety is considered adequate.

Other reagents from biological origin such as L-Cysteine are derived from feathers or human hair but are also processed under very harsh conditions.

Manufacture of the product

The Drug Product manufacturing process consists of mixing the active substance with the excipients (formulation buffer and polysorbate 80) and adjustment of pH followed by sterile filtration and filling into pre-sterilised syringes. The syringes are stoppered, transferred for visual inspection, and are subsequently labelled, packaged and dispatched.

All excipients conform to the requirements of the European Pharmacopoeia.

In-Process Manufacturing Controls

The manufacturing process is controlled by a series of in-process controls. The process control strategy and critical steps were identified and evaluated during manufacturing process development. Critical Process Parameters (CPPs), Key Process Parameters (KPPs) and Non-Critical Process Parameters (NCPPs) were established.

Manufacturing process development

During development, the Drug Product manufacturing process underwent three process changes (Process I, II and III). The different processes resulted from changes that occurred in the manufacturing process, including changes related to scale up, changes to processing aids, addition and deletion of process steps and other changes.

Each Drug Product process used Drug Substance from a different manufacturing process. Process I material was used in the non-clinical studies, process II in the clinical trials and process III is the proposed commercial process.

An extensive comparability study has been performed between process II and III materials.

Process validation

The manufacturing processes for both Drug Product concentrations were qualified during three consecutive Drug Product production batches (for each concentration). All steps have been validated, including the shipping, for which the suitability of the containers and the stability of the product in case of temperature excursions have been demonstrated.

Product specification

Vela pharmazeutische Entwicklung will act as the release testing site of the commercial batches for the EU market. Adequate data has been provided for Vela pharmazeutische Entwicklung to act in that capacity.

Control of Drug Product

There is currently a Ph. Eur. monograph for Filgrastim Concentrated Solution (2206), which served as a basis for the establishment of the Drug Product specification.

Routine testing is performed at release for sterility, bioburden, polysorbate 80. Identity is confirmed by IEF against a reference solution. Purity is tested by SDS-PAGE, RP-HPLC, SE-HPLC and IEF and the biological activity is measured by the same in-vitro bioassay as employed for the control of the Drug Substance.

The same methods as those used in the control of the Drug Substance are employed, except for the determination of polysorbate 80 concentration, which is specifically conducted on the Drug Product.

The applicant will review the DP specifications once data on a pre-determined number of batches is available.

Container closure system

The Drug Product is presented as a solution for injection/infusion in a 1 mL glass, single-use, pre-filled syringe.

The container closure system is a syringe system comprised of a syringe with a glass barrel assembled with a steel needle, an elastomeric needle shield and a polypropylene rigid needle shield; and an elastomeric plunger stopper

The syringe is also assembled with a polypropylene plunger rod. The syringe barrel and stopper are lubricated with silicone oil.

The glass syringe barrel, elastomeric needle shield, plunger stopper and the silicone oil comply with the the Ph.Eur. The needle adhesive is appropriately qualified for use.

Stability of the product

The Drug Product is to be stored at 2°C to 8°C.

Stability data for six batches derived from process II Drug Product have been presented, including 36 months at real time/real temperature conditions 6 months at accelerated and 7 days at stress conditions. Stability data is provided for three Process III drug product batches include up to 24 months at real time/real temperature conditions, 6 months at accelerated and 28 days at stress conditions. Stability data has also been presented for a further six more recently manufactured batches of Process III product stored for 6 months at long terms and accelerated conditions.

In general, the results support the shelf-life of 30 months and the storage conditions (storage under refrigerated conditions at 2-8°C) as defined in the SmPC.

Comparability to the reference medicinal product

The chosen reference medicinal product, authorised in the EU and used for the entire comparability exercise, is Neupogen (Amgen Ltd.).

The comparability exercise was based on 3 biosimilarity studies and included the comparison of a variety of attributes of both the reference medicinal product and Grastofil, such as physicochemical properties, biological activity, purity and impurity profiles, and stability profiles. The physicochemical attributes, including physical properties and primary and higher order structures of the Drug Substance, were evaluated using a combination of analytical procedures. The biological properties were assessed using both *in vitro* and *in vivo* assays. The purity and impurity profiles were evaluated both qualitatively and quantitatively using orthogonal analytical procedures. Finally, the stability profiles were evaluated under accelerated (25°C ± 2°C) and stressed (40°C ± 2°C) conditions. A forced degradation study was also employed to compare the degradation profiles of Grastofil and the reference medicinal product.

The data presented sufficiently demonstrate that the Drug Substance and Drug Product from the clinical stages of process development (process VII Drug Substance / process II Drug Product) and from the commercial process (process IX Drug Substance / process II Drug Product) are comparable with one another and to the reference medicinal product Neupogen.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The upstream Drug Substance manufacturing process and process control strategy has been adequately described and appropriate assurances have been provided with respect to process control and consistency.

Filgrastim Drug Substances destined for use in both EU and non-EU countries will be produced in the same manufacturing facility, using distinct manufacturing processes. Assurance of adequate product/process segregation has been provided. Process-specific consumables including dedicated chromatography resins and ultrafiltration cartridges will be used.

The product-specific GMP inspection of the Drug Substance manufacturing site, requested with this procedure, has confirmed that appropriate GMP measures are in place in order to control segregation and cross contamination of the proposed EU product from the non-EU regulated product.

The drug product is manufactured in 300 mcg/0.5 mL and 480 mcg/0.5 mL pre-filled syringe presentations. No presentation compatible with the safe dosing of Grastofil in the paediatric population has been proposed. The proposed pre-filled syringes are considered to pose a risk of dosing errors in children, and additional presentation(s) such as a vial or a graduated syringe allowing accurate dosing in the paediatric population should be proposed. Paediatric indications may only be accepted following the submission of a post marketing variation at such time when an appropriate presentation is available.

The comparability exercises conducted to support biosimilarity of Grastofil with the reference medicinal product Neupogen are adequate and their conclusions supported. The comparability studies are additionally supported by a phase I study in man comparing the Process IX / III commercial Grastofil with Neupogen.

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

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Based on the review of the data on quality, the manufacture and control of the Grastofil Drug Substance and the Drug Product are considered acceptable.

The Quality of the 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 satisfactory way.

Safety concerning adventitious agents including TSE has been sufficiently assured.

Biosimilarity with the reference medicinal product Neupogen has been sufficiently demonstrated. From a quality point of view, the observed differences and levels of these differences have been well documented and are acceptable.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the review of drug substance specifications once data on a pre-determined number of batches is available.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical development program for Grastofil was performed using Neukine (a non-EU product which, however, contains Process V drug substance). None of the non-clinical studies have been conducted using Apo-Filgrastim (Process IX drug substance, see discussion on non-clinical aspects).

Primary pharmacodynamic studies

In vitro, a potency assay was conducted in a murine myeloblastic cell line. *In vivo* 1) a bioassay for G CSF in mice, 2) restoration of neutrophil blood cell counts by or Neukine vs. Neupogen (Filgrastim) in neutropenic female BALB/c mice and 3) a comparative effect study of Neukine with Neupogen when administered subcutaneously to mice with induced neutropenia, were conducted. Data from a general 28 Day rat study was also used to demonstrate pharmacological changes.

In the *in vitro* potency assay, the results of these studies indicated that both Neukine and the reference product bind to the murine cellular G-CSF receptors with the same affinity and that both preparations are equally effective at inducing cellular proliferation.

In Swiss albino mice given subcutaneous doses of 0.1, 1, 5 and 10 µg of Neukine or a reference standard, relative potency of Neukine was found to be 1.0977 for total leukocyte count and 0.9162 for neutrophil count. The product used in this study was derived from the Drug Substance Process V of the Apo-Filgrastim and the reference product used in this study was Neupogen (Manufactured by Amgen, Marketed by Roche in India).

In neutropenic female BALB/c mice, the restoration of neutrophil blood cell counts by Neukine vs. the reference product, Neupogen" was investigated. The data showed comparability between Neukine and the reference product in terms of increased neutrophil and leukocyte counts.

A comparative study was carried out, in which Wistar rats were given subcutaneous doses of 50, 150 and 500 µg/kg/day Neukine or the comparator Neupogen (150 µg/kg/day) for 28 days. The data showed that Neukine) and Neupogen at 150 µg/kg/day were comparable in terms of the increase in neutrophil counts.

Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were submitted (see discussion on non-clinical aspects).

Safety pharmacology programme

No safety pharmacology studies were submitted (see discussion on non-clinical aspects).

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were submitted (see discussion on non-clinical aspects).

2.3.2. Pharmacokinetics

Reference was made to the toxicokinetic data from the 28 day GLP compliant study conducted in Wistar rats. No studies have been performed to investigate distribution, metabolism, excretion and pharmacokinetic drug interactions (see discussion on non-clinical aspects).

2.3.3. Toxicology

Single dose toxicity

Four non-comparative single dose studies were conducted with Neukine. These studies were conducted in India and were not GLP compliant. Rats and mice were given i.v or s.c doses of 250, 2500 or 5000 µg/kg. In mice and rats both the i.v. and s.c. doses were, according to the Applicant, well tolerated and all animals survived the 14-day observation period without major clinical signs. No effects on body weight or food consumption were observed. No changes attributable to the test article were found during the macroscopic examination.

Repeat dose toxicity

Wistar rats were given subcutaneous doses of 50, 150 and 500 µg/kg/day Neukine or the comparator Neupogen (150 µg/kg/day) for 28 days. Swellings of the hindlegs or only the joints of the hindlegs were noted at all Neukine doses (study number 259.120.897). The same effects were seen in the Neupogen group. A dose dependent increase alkaline phosphatase was seen in all animals given Neukine at the end of the treatment period. Main macroscopic findings in this study were related to the spleen and to the hindlimbs. In the spleen, histiocytosis often combined with increased haemopoiesis was detected histologically. The capsule of the spleen was often thickened due to a fibrosis. Increased spleen weight was noted in all treated (Neukine and Neupogen) males at 500 µg/kg/day. A dose-dependent increase in white blood cells, in particular in neutrophils, was found with Apo-Filgrastim and Neupogen, showing equivalent effects. No new toxicities were observed. The toxicities noted were comparable and in-line with the expected effects of this class of compound.

A NOEL was not defined in this study for Neukine or the reference product due to the expected pharmacological effects. The toxicokinetic data from this study showed comparability between doses of 50 and 500 µg/kg Neukine and reference product.

The design of the GLP-compliant toxicology study 259.120.897 did not include a dose response for Neupogen in order to detect differences in toxicology and toxicokinetics response between Apo-Filgrastim and Neupogen.

Six studies with Neukine alone were conducted (non-comparative). Swiss albino Mice and Sprague-Dawley rats were given s.c or i.v doses of 0, 50, 100, 250 µg/kg/day (all studies) for 28 days. These were conducted as separate studies. A further two 28-day studies with 28 day recovery periods were also conducted in rats and mice (s.c and i.v). There were no test item-related effects on clinical biochemistry, haematology, urinalysis or histopathology changes, except for higher neutrophil counts which were expected in treated animals compared to controls. The NOAEL in all of these studies was considered to be 250 µg/kg/day.

Genotoxicity

No genotoxicity studies were submitted (see discussion on non-clinical aspects).

Carcinogenicity

No carcinogenicity studies were submitted (see discussion on non-clinical aspects).

Reproduction Toxicity

No reproduction toxicity studies were submitted (see discussion on non-clinical aspects).

Toxicokinetic data

Wistar rats were given s.c and i.v doses of 50, 150 and 500 µg/kg/day Neukine s.c or i.v or a comparator Neupogen at 50 and 500 µg/kg/day s.c or 50 and 500 mg/kg i.v for 14 days (as part of a 28-day study). An enzyme-linked immunosorbent assay (ELISA) was used to quantify Filgrastim in rat plasma. According to the Applicant the rHu(met)G-CSF immunoassay is fully validated and the analytics were performed to GLP. Suitable data have been provided on the determination of G-CSF in rat serum using ELISA.

With exception of males at 50 µg/kg, repeated daily i.v administration of Neukine over 14 days compared to single i.v doses resulted in a slightly increased exposure, in terms of AUC of rHu G-CSF in both sexes. No distinct trend could be observed for Cmax. Comparison of the profiles obtained on day 0 and 13, showed gender-specific differences, i.e. plasma exposure in terms of AUClast as well as Cmax were lower in females, whereas the volume of distribution (Vz_obs) and systemic clearance (CLobs) were higher in females. No consistent trend and no consistent gender difference were observed for terminal elimination half-life (t1/2).

With the exception of the low dose (50 µg/kg), repeated daily s.c dosing of Neukine over 14 days compared to single s.c dose resulted in a slightly increased exposure of rHu G-CSF in males. No distinct trend could be observed for females. No clear trend was seen for Cmax in both sexes, whereas systemic clearance CLobs was slightly-to-markedly higher in females. Comparison of the pharmacokinetic profiles obtained on day 0 and 13, respectively, did not show consistent gender-specific differences in terminal elimination half-life (t1/2). Consistent findings after repeated dosing were that Cmax of Neukine were slightly lower in females at all doses and exposure to Neukine was slightly-to-markedly lower in females. Finally, both the volume of distribution (Vz_obs) and systemic clearance (CLobs) of Neukine were markedly increased in females at all doses.

Local Tolerance

A GLP compliant rabbit study that compared the local tolerance of Neukine (480 µg Filgrastim per 0.5 ml acetate buffer pH 4.0) with the reference product (Neupogen) in rabbits after paravenous and intramuscular administration was conducted. Moreover, a non-GLP compliant non-comparative rabbit local tolerance study was performed with Apo-Filgrastim only.

In the comparative study, 3 New Zealand white male rabbits were given Apo-Filgrastim and Neupogen paravenously and intramuscularly at 480 µg. A visual scale for evaluating erythema formation, oedema formation and pain reactions was used. Necropsy was performed on study day 4 (approximately 96 h after administration of the test item or reference item). Intramuscular administration of both test articles did not cause any erythema formation in all three animals during the observation period of 96 hours. After paravenous administration two animals developed well defined point-like erythema (grade 2) at the application site (punctures) within two hours after treatment with Apo-Filgrastim. Within 24 hours, one animal demonstrated a very slight erythema formation (grade 1) of the treatment area. 96 hours after administration, two of three application sites recovered and only animal showed a very slight erythema formation. Administration of Neupogen caused well defined (grade 2 one animal) to moderate (grade 3, one animal) point-like erythema formation at the application site (punctures) in two of three animals within 24 hours after treatment. Over the observation period, erythema formation lessened to very slight (grade 1). Erythema formation after paravenous administration of both test articles was comparable in terms of intensity and incidence. Most of the erythema were point-like and in the area of the punctures, so they were considered to have been caused by the administration. Neither i.m. or p.v. administration caused visible oedema formation and no signs of pain were noted after treatment with either test article during the observation period of 96 hours. Paravenous administration of Neukine caused several slight red discolorations in 1 animal. After p.v. administration

of the reference Neupogen, 2 animals showed slight hematoma formation and several slight red discolorations were noted in 2 animals.

Histopathological examination showed moderate (grade 3) haematoma at the paravenous administration site in 1 animal with Neukine. In comparison, after paravenous treatment with the reference item Neupogen, 2 animals developed a slight (grade 2) haematoma. These findings were near the injection sites and considered to be caused by the administration volume and / or the route and site of administration. No histopathological findings were noted at the intramuscular administration sites with either test article.

It was concluded based on clinical (in-life), macroscopic and histopathological observations that single intramuscular and paravenous administration of 480 µg Neukine is well tolerated and comparable to 480 µg Neupogen.

Six New Zealand White rabbits were used for evaluating the safety of Neukine by patch test technique on intact skin (non-GLP). The hairs were clipped from the back and flanks one day prior to the application. Two areas on the back, approximately 2-3 cm apart, were designated for the position of control and test product patches in each rabbit. 0.2% SDS was applied as positive control. The patches were removed after 24 hours and the skin sites were scored directly after removal and at 48 hours after removal using a visual scale. There was no erythema/eschar and oedema formation observed in any animal at any time point. Animals treated with positive control patches all showed primary irritation indices of > 5 after 48 hours for erythema/eschar formation.

Other toxicity studies

No other toxicity studies have been submitted.

2.3.4. Ecotoxicity / environmental risk assessment

Apo-Filgrastim is intended to substitute other identical products on the market, so this product is not expected to cause any additional environmental risk. Therefore no environmental risk assessment report is required for this product.

2.3.5. Discussion and conclusion on non-clinical aspects

According to the Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor (EMEA/CHMP/BMWP/31329/2005), safety pharmacology, reproduction toxicology, mutagenicity and carcinogenicity studies are not routine requirements for non-clinical testing of similar biological medicinal products containing recombinant G-CSF as active substance. The absence of secondary pharmacology studies and of studies on pharmacodynamic drug interactions can also be considered acceptable based on the extensive experience with and the well-known properties of filgrastims.

Although non-clinical studies have not been conducted with the intended commercial product using the intended manufacturing process, receptor binding data for the Apo-Filgrastim DP batches from Process VII and IX were submitted for evaluation and comparability was demonstrated. In addition, a recent comparative clinical study that investigated the PK/PD profile of Apo-Filgrastim vs. Neupogen (Phase I 3-arm bridging study) used Process IX (DS) material. Therefore, the CHMP considered that the non-clinical data obtained with Neukine, containing Process V drug substance, can be extrapolated to the Process IX drug substance (Apo-filgrastim) contained in Grastofil. Therefore, from a non-clinical point of view, it can be concluded that there were no significant differences between Grastofil and the reference medicinal product, Neupogen.

2.4. Clinical aspects

2.4.1. Introduction

The clinical development programme for Grastofil spanned from July 2007 to May 2010. Neupogen was the chosen reference product which has been authorised in the Community on the basis of a complete dossier in accordance with the provisions of Article 8 of Directive 2001/83/EC, as amended.

The aim of the Apo-Filgrastim clinical program was to demonstrate biosimilarity of Apo-Filgrastim with the EU-approved reference product Neupogen. Four comparative Phase I studies were conducted in healthy volunteers to demonstrate the equivalence of Apo- Filgrastim with Neupogen in terms of pharmacodynamics and pharmacokinetic parameters:

- A single-dose, randomised, double-blind, two-way cross-over, active-controlled, pharmacokinetic (PK) and pharmacodynamic (PD) study of i.v. Apo-Filgrastim and Neupogen in 36 healthy male and female volunteers. *(Study KWI-300-101)*

- A single-dose, randomised, double-blind, two-way cross-over, active-controlled, pharmacokinetic (PK) and pharmacodynamic (PD) study of s.c. Apo-Filgrastim and Neupogen in 73 healthy male and female volunteers with two fixed dose groups of filgrastim (75µg and 150µg). *(Study KWI-300-102)*

- A repeat-dose, randomised, double-blind, parallel group, active and placebo-controlled, pharmacokinetic (PK) and pharmacodynamic (PD) study of Apo-Filgrastim and Neupogen in 78 healthy male and female volunteers *(Study KWI-300-103)*

- A single dose, randomized, double-blind, active-controlled, comparative three-way crossover PK and PD study of Apo-Filgrastim and EU- approved and US-licensed Neupogen (Amgen) in 48 healthy male and female volunteers with a fixed dose of 300 µg. A single -center study conducted in Canada (Study GCSF-SUIN-05SB01-3FA – also referred to as Phase I 3-arm Study)

GCP

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

- Tabular overview of clinical studies

Table 2: Tabular Listing of All Clinical Studies

Clinical Study	Study Design	Route of Administration	Dose Administered	Comparator Product	Sample Size
Phase I; KWI-300-101	Single dose, cross over PK/PD evaluation	iv	5 µg/kg	Neupogen (EU)	35
Phase I; KWI-300-102	Single dose, cross over, Dose response; PK/PD evaluation	sc	75 µg (~1 µg /kg)		33
			150 µg (~2.0 µg /kg)		35
Phase I; KWI-300-103	Repeat dose, parallel group PK/PD evaluation	sc	5 µg/kg		35 – Apo-Filgrastim; 34 - Neupogen
Phase I; GCSF-SUIN-05SB01-3FA[1]	Single dose PK/PD evaluation	sc	300 µg (~4 µg)	Neupogen (EU) and Neupogen (USA)	43
Phase III; KWI-300-104	Repeat dose, safety	sc	5 µg/kg	None	120

2.4.2. Pharmacokinetics

Study KWI-300-101

Study Design

The study design was a single-dose, randomised, double-blind, active-controlled, two-way cross-over study. Subjects were to be randomized to receive either Neukine (5µg/kg) or the market reference filgrastim, Neupogen (5µg/kg, Amgen).

Healthy subjects were to receive the test product or the reference item intravenously. After a washout period, subjects were to receive the alternative G-CSF product.

The cross-over design was expected to minimise inter-subject variability and therefore lowered the required sample size.

The two subsequent treatments were separated by a sufficient wash out period.

Statistical and Analysis Plan

An analysis of variance (ANOVA) with the fixed factors treatment, period, and sequence and the random factor subject within sequence was applied for the loge-transformed endpoints AUC_{0-32}, $AUC_{0-\infty}$, C_{max} of filgrastim (PK) and C_{max} of the ANC (PD). A 90% confidence interval (CI) for the ratio of geometric means Neukine/Neupogen was calculated using the back transformed (exponential) 90% CI for the least square mean difference "Neukine - Neupogen". In accordance with the guidance documents, the equivalence margin has been set to 80% - 125% for both the pharmacokinetic and the pharmacodynamic parameters.

Efficacy Analysis

Primary End-point

-Comparison of the plasma area under curve (AUC) between test and reference filgrastim medicinal products.

Secondary End-points

- Comparison of C_{max} and $T_{½}$ of filgrastim

- Comparison of the ANC

Absorption and Distribution

Considering the plasma AUC of filgrastim, the initial assumption that between 0 and 32 h after administration of G-CSF would cover more than 90% of the total AUC was correct. The increment between the AUC_{0-32} and the $AUC_{0-\infty}$ was marginal (< 1%). A statistical analysis of the AUC_{0-32} of filgrastim showed a highly significant difference between the test item Apo-filgrastim and the reference item Neupogen with a probability < 0.0001 (above). With regard to the relevant confidence intervals, however, this difference was within the pre-defined equivalence margins (80% - 125%).

Table 3: AUC 0-32, AUC 0-inf, Cmax, T ½, Tmax, CL following a single intravenous infusion of 5micrograms /kg Apo-Filgrastim or Neupogen to Healthy Volunteers

Endpoint		Apo-Filgrastim (N=35)	Neupogen (N=35)	Ratio [%]	90% CI [%]	Pr > [t]
AUC_{0-32} [min*pg/mL]	Mean	22047494	24340789	90.6	88.7-92.7	< 0.0001
	SD	4060115	4530366			
	Min	13895600	15376100			
	Median	22587500	24890800			
	Max	29070100	34322900			
AUC_{0-inf} [min*pg/mL]	Mean	22075297	24366534	90.7	88.7-92.7	< 0.0001
	SD	4065640	4535283			
	Min	13917900	15380700			
	Median	22625100	24908100			
	Max	29101400	34348100			
C_{max} [pg/mL]	Mean	103272.4	111567.0	92.5	90.3-94.7	< 0.0001
	SD	15031.9	15688.3			
	Min	71904.5	79926.5			
	Median	103125.0	112086.0			
	Max	142364.0	147204.0			

Endpoint		Apo-Filgrastim (N=35)	Neupogen (N=35)
$T_{1/2}$ [min]	Mean	168.5	165.3
	SD	13.5	13.1
	Min	149.0	136.9
	Median	164.6	164.7
	Max	205.3	198.0
T_{max} [min]	Mean	16.3	16.0
	SD	9.1	5.5
	Min	10.0	10.0
	Median	20.0	20.0
	Max	60.0	30.0
CL [L/min]	Mean	0.0165	0.0149
	SD	0.0040	0.0037
	Min	0.0106	0.0095
	Median	0.0157	0.0143
	Max	0.0267	0.0243

Table 4: AUC (0-32) Filgrastim by treatment group (ITT population)

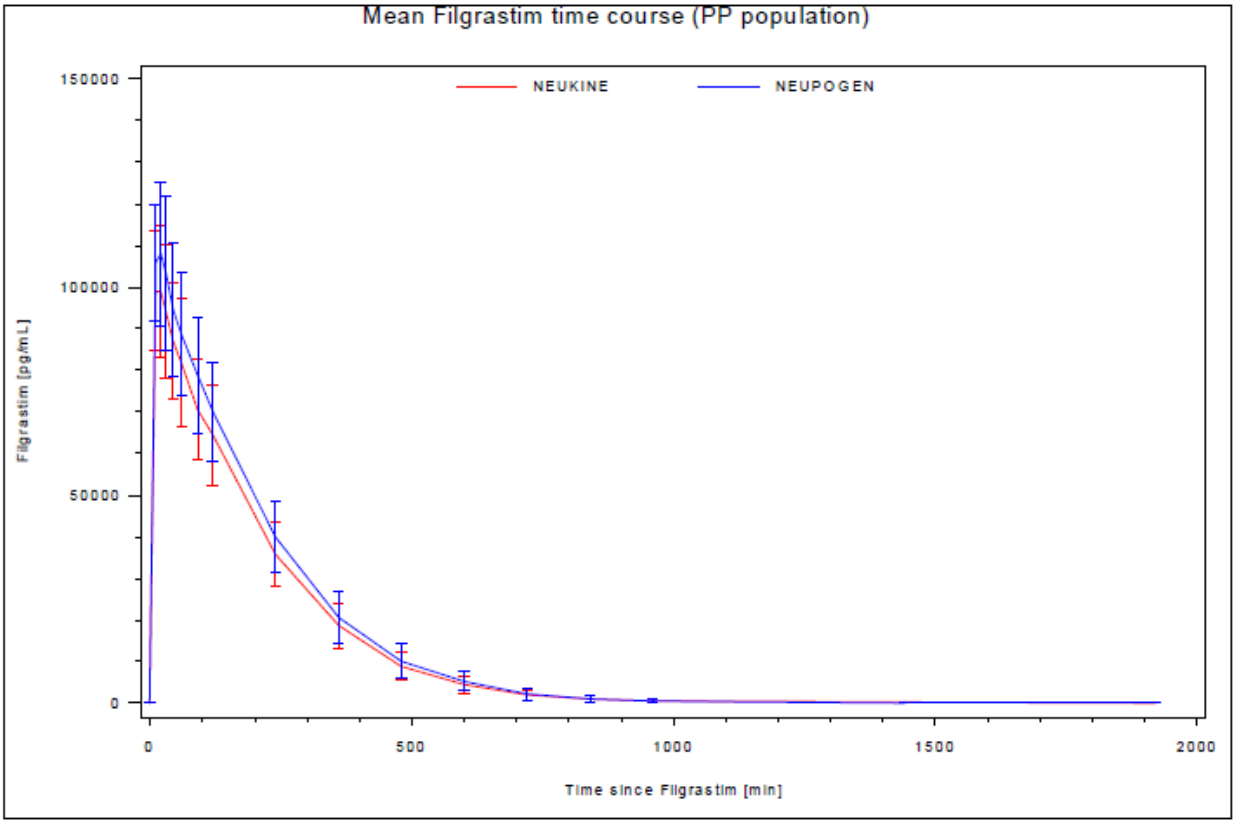
AUC(0-32) [min*pg/mL]								
Treatment	N	Mean	SD	Min	Q1	Median	Q3	Max
NEUKINE	35	22047494	4060115	13895600	18967400	22587500	25038900	29070100
NEUPOGEN	36	24366817	4467908	15376100	19747100	24895700	26749000	34322900

Least square mean difference	Estimate (log-scale)	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Neukine - Neupogen	-0.09836624	<0.0001	[-0.12,-0.08]	90.6	[88.7%,92.7%]

In both treatment groups, mean G-CSF plasma concentrations rapidly increased as expected after i.v. infusion, reached a maximum after 16 minutes, and then decreased to pre-dose values at 24 hours.

With regard to the plasma AUC of filgrastim, the initial assumption that between 0 h and 32 h after administration of G-CSF would cover more than 90% of the total AUC was confirmed. The increment between the AUC_{0-32} and the $AUC_{0-\infty}$ was marginal (< 1%). While the statistical analysis of the AUC_{0-32} and $AUC_{0-\infty}$ of filgrastim showed a significant difference (p=< 0.0001) between the test item Apo-Filgrastim and the reference item Neupogen, this difference, however, was within the pre-defined equivalence margins (80% - 125%) for the relevant confidence intervals.

Figure 1: Mean Filgrastim time course (Per Protocol Population)



Elimination

Table 5: T1/2 Filgrastim by treatment group (ITT population)

T1/2 [min]								
Treatment	N	Mean	SD	Min	Q1	Median	Q3	Max
NEUKINE	35	168.5	13.5	149.0	159.3	164.6	175.3	205.3
NEUPOGEN	36	165.5	13.0	136.9	156.4	165.0	174.2	198.0

Table 6: Tmax Filgrastim by treatment group (ITT population)

Tmax [min]								
Treatment	N	Mean	SD	Min	Q1	Median	Q3	Max
NEUKINE	35	16.3	9.1	10.0	10.0	20.0	20.0	60.0
NEUPOGEN	36	16.1	5.5	10.0	10.0	20.0	20.0	30.0

Table 7: CL Filgrastim by treatment group (ITT population)

Clearance [L/min]								
Treatment	N	Mean	SD	Min	Q1	Median	Q3	Max
NEUKINE	35	0.016477	0.004024	0.010563	0.013216	0.015734	0.019289	0.026702
NEUPOGEN	36	0.014841	0.003725	0.009472	0.012506	0.014226	0.017365	0.024305

Study KWI-300-102

Study design

The study design was a single dose, randomised, double blind, two-way cross-over study. 36 subjects of the 1st cohort received either filgrastim Apotex or Neupogen at a dose of 150 µg. 36 subjects of the second cohort were randomized to receive either 75µg Filgrastim Apotex or Neupogen. After a washout period, subjects receiving one of the filgrastim products then received the other.

Statistical Analysis Plan

An analysis of variance was applied for the comparison of C_{max} of the ANC between treatment groups of the same dosage level (i.e. comparisons between Filgrastim Drug Product 150µg and Neupogen 150µg and between Filgrastim Drug Product 75µg and Neupogen 75µg). The resulting 90% confidence interval for the C_{max} ratio Filgrastim Drug Product/Neupogen was compared with the pre-defined acceptance region of 80% to 125%, biosimilarity in terms of the primary endpoint is postulated if the lower bound is > 80% and the upper bound is < 125%.

Bioequivalence in terms of the co-primary endpoint was postulated if the lower bound of the 90% CI for the AUC_{0-72} ratio of Filgrastim Drug Product/Neupogen was > 80% and the upper bound was < 125%. The same comparison was performed for the co-primary parameter $AUC_{0-\infty}$ and the secondary parameter C_{max}.

Efficacy Analysis

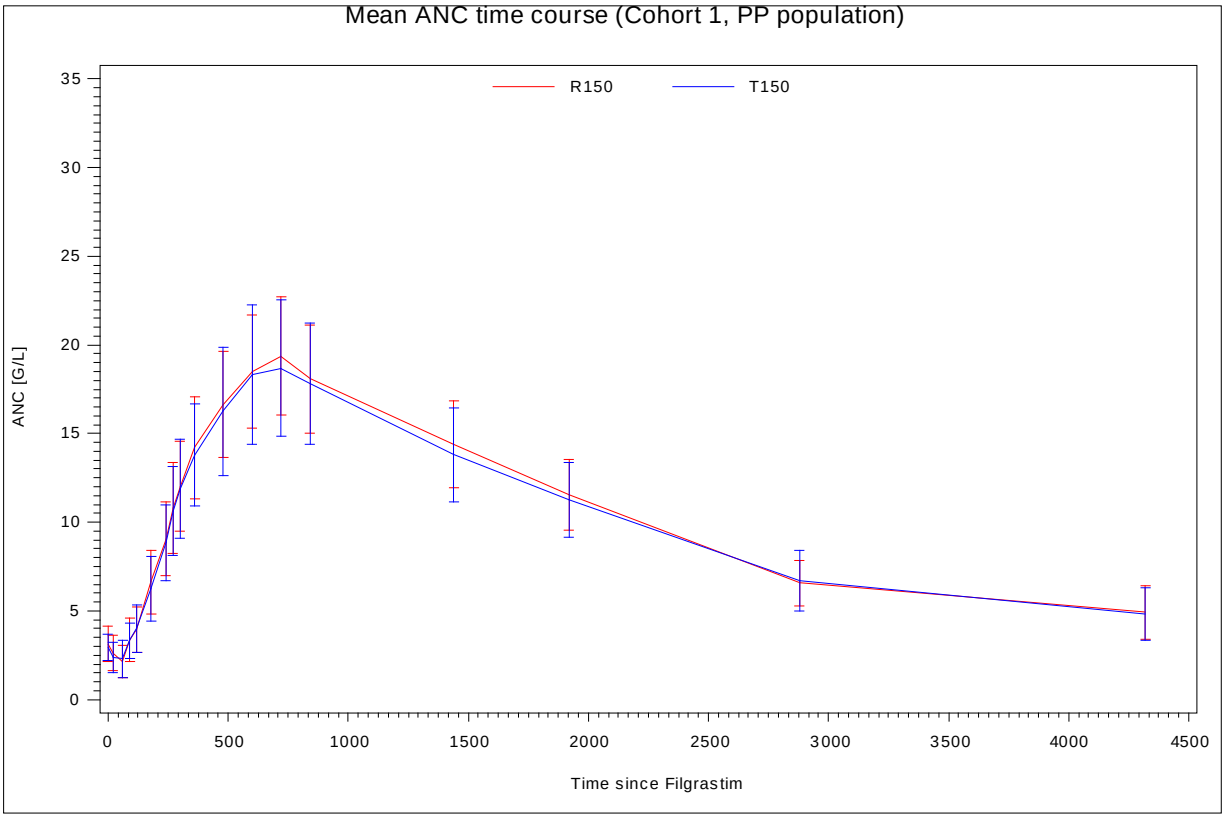
Primary End-point: ANC between test and reference medicinal products.

Co-primary end-point - 150µg dose: ANC AUC between test and reference products

Secondary End-points for the 150µg dose: PK parameters C_{max} and T½

Absorption and Distribution

Figure 2: Study KWI-300-102: Mean ANC-Time Profile Following a Single Subcutaneous Injection of 150 µg of Apo-Filgrastim or Neupogen to Healthy Male and Female Volunteers (PP-Population)



T150 = Apo-Filgrastim 150µg, R150 = Neupogen 150µg

Table 8: AUC (0-infinity) Filgrastim by treatment group (PP population)

AUC(0-infinity) [min*ng/mL]								
Treatment	N	Mean	SD	Min	Q1	Median	Q3	Max
R150	35	3419.7	1093.7	1707.4	2597.7	3411.0	3927.1	6489.9
T150	35	3282.7	920.7	1764.1	2653.5	3231.3	3848.2	5171.3

Comparison of 150 µg treatments

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	-0.03229901	0.03641621	-0.89	0.3815	[-.09,0.03]	96.8	[91.0%,103.0%]

Table 9 Filgrastim levels - 150 µg s.c. (ITT population)

n=36	***Geometric mean***		***Ratio: Test/Reference (%)***	
Parameter	***Test***	***Reference***	***Point estimate***	***90% CI***
AUC_{0-72} (min*ng/mL)	***3190.9***	***3278.4***	***97.3***	***91.59-103.43***
$AUC_{0-\infty}$ (min*ng/mL)	***3197.9***	***3283.9***	***97.4***	***91.66-103.46***
C_{max} (ng/mL)	***7.45***	***7.85***	***95.0***	***86.47-104.28***

Elimination

Table 10: T1/2 Filgrastim by treatment group (ITT population)

T1/2 [min]								
Treatment	N	Mean	SD	Min	Q1	Median	Q3	Max
R150	36	309.3	87.1	166.5	256.2	279.9	314.1	594.0
T150	36	328.4	95.3	160.4	269.8	295.7	374.3	556.7

Table 11: Tmax Filgrastim by treatment group (ITT population)

Tmax [min]								
Treatment	N	Mean	SD	Min	Q1	Median	Q3	Max
R150	36	283.3	53.4	180.0	240.0	300.0	300.0	360.0
T150	36	278.3	41.0	180.0	240.0	285.0	300.0	360.0

Table 12: CL Filgrastim by treatment group (ITT population)

Clearance [L/min]								
Treatment	N	Mean	SD	Min	Q1	Median	Q3	Max
R150	36	0.0481	0.0159	0.0231	0.0358	0.0428	0.0561	0.0879
T150	36	0.0491	0.0155	0.0288	0.0373	0.0463	0.0565	0.0850

Study KWI-300-103

Study design

The study was a randomised, double-masked, active and placebo-controlled, parallel group, multiple dose trial.

78 healthy male or female subjects were randomised to receive either Filgrastim Drug Product s.c. for 4 days (5µg/kg/ per day), market reference filgrastim (Neupogen 5µg/kg/per day) s.c. for 4 consecutive days or placebo (physiological 0.9% NaCl) s.c. for 4 days. A 1:1 randomisation of Filgrastim Drug Product versus Neupogen was performed. Six healthy subjects were randomised to the placebo group to allow a check of the background in the assay of CD34+ cells.

Statistical Methods

The values of the main PD parameters (ANC: C_{max}) and PK parameters (Filgrastim: AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, AUCss) were compared using an ANOVA with the fixed factor treatment and a significance level of $\alpha = 0.05$ after logarithmic transformation of the data. A 90% confidence interval (CI) for the ratio of geometric means Filgrastim Drug Product/Neupogen was calculated using the back-transformed (exponential) 90% CI for the least square mean difference "Filgrastim Drug Product - Neupogen". If this interval is completely contained within pre-defined equivalence margin, biosimilarity was postulated. To demonstrate comparability, the equivalence margin has been set, as defined in the corresponding guidance documents, to 80% - 125% for all PD and PK parameters.

Efficacy Analyses

The following pharmacokinetic parameters were determined:

Table 13: PK endpoint parameters of filgrastim used in the efficacy analysis

Parameter	Trial day	Unit	Description	Endpoint Level
AUC_{0-24} filgrastim	Trial day 1	min*ng/mL	Area under the plasma concentration/time curve, calculated by the linear trapezoidal rule based on filgrastim plasma concentrations from 0 hours up to 24 hours following filgrastim administration	Secondary
$AUC_{0-\infty}$ filgrastim	Trial day 1	min*ng/mL	AUC from time zero to time infinity where $AUC_{0-\infty} = AUC_{0-24} + C_{last}/Lz$, C_{last} is the last measurable drug concentration and Lz is the terminal elimination rate constant	Secondary
C_{max} filgrastim	Trial day 1	ng/mL	Maximum plasma concentration, determined directly from the measured plasma concentrations (last absolute maximum)	Secondary
$T_{1/2}$ filgrastim	Trial day 1	min	Elimination half life	Secondary
T_{max} filgrastim	Trial day 1	min	Time at which Cmax is achieved, determined directly from the measured plasma concentrations	Secondary
CL filgrastim	Trial day 1	L/min	Systemic Clearance	Secondary
V_d filgrastim	Trial day 1	L	Volume of distribution, based on $AUC_{0-\infty}$	Secondary
AUC_{ss} filgrastim	Trial day 4	min*ng/mL	Area under the plasma concentration/time curve in steady state, calculated by the linear trapezoidal rule based on filgrastim plasma concentrations following the last filgrastim administration (trial day 4) up to the last measured filgrastim concentration.	Secondary

Note: Plasma filgrastim concentrations have been determined in pg/mL; calculations of PK parameters have been performed in pg/mL, but results are presented in ng/mL for better clarity.

Absorption and Distribution

Table 14: AUC 0-24 Filgrastim by treatment group (PP population)

AUC(0-24) [min*ng/mL] Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	35	11734.8	2737.0	6809.4	9389.5	11732.0	13543.6	17672.4
R	34	11839.4	3292.4	6356.6	9989.0	11061.9	13459.5	20538.4

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	0.00160776	0.06188996	0.03	0.9794	[-.10,0.10]	100.2	[90.34%,111.05%]

Table 15: AUC 0-infinity Filgrastim by treatment group (PP population)

AUC(0-infinity) [min*ng/mL] Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	35	11803.8	2751.5	6890.5	9447.6	11804.3	13592.2	17828.6
R	34	11917.0	3307.9	6410.1	10025.7	11134.3	13563.3	20624.6

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	0.00097815	0.06184965	0.02	0.9874	[-.10,0.10]	100.1	[90.29%,110.98%]

Table 16: AUC ss Filgrastim by treatment group (PP population)

AUC(ss) [min*ng/mL]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	35	5440.8	1484.6	3441.8	4133.3	5040.9	6458.4	8806.5
R	34	5387.6	1790.2	2711.1	4037.4	5234.5	6173.8	11596.8

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	0.02305057	0.06954855	0.33	0.7414	[-.09,0.14]	102.3	[91.12%,114.92%]

Table 17: Cmax Filgrastim by treatment group (PP population)

Cmax [ng/mL]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	35	25.92	6.95	13.17	20.40	24.54	31.62	41.52
R	34	25.54	7.81	13.88	20.27	24.02	27.77	47.34

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	0.02180455	0.06791959	0.32	0.7492	[-.09,0.14]	102.2	[91.26%,114.46%]

Figure 3: Mean Filgrastim time course on study day 1 (Safety population)

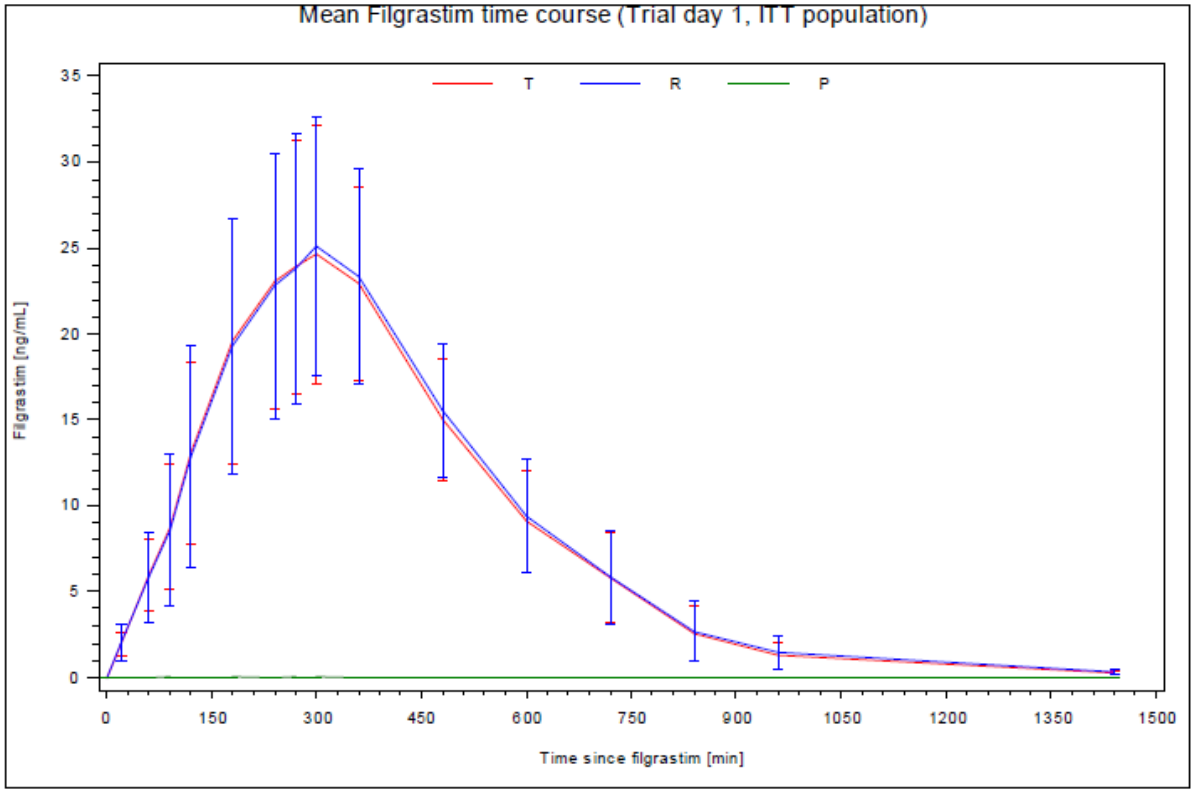


Figure 4: Mean Filgrastim time course on study day 4 (Safety population)

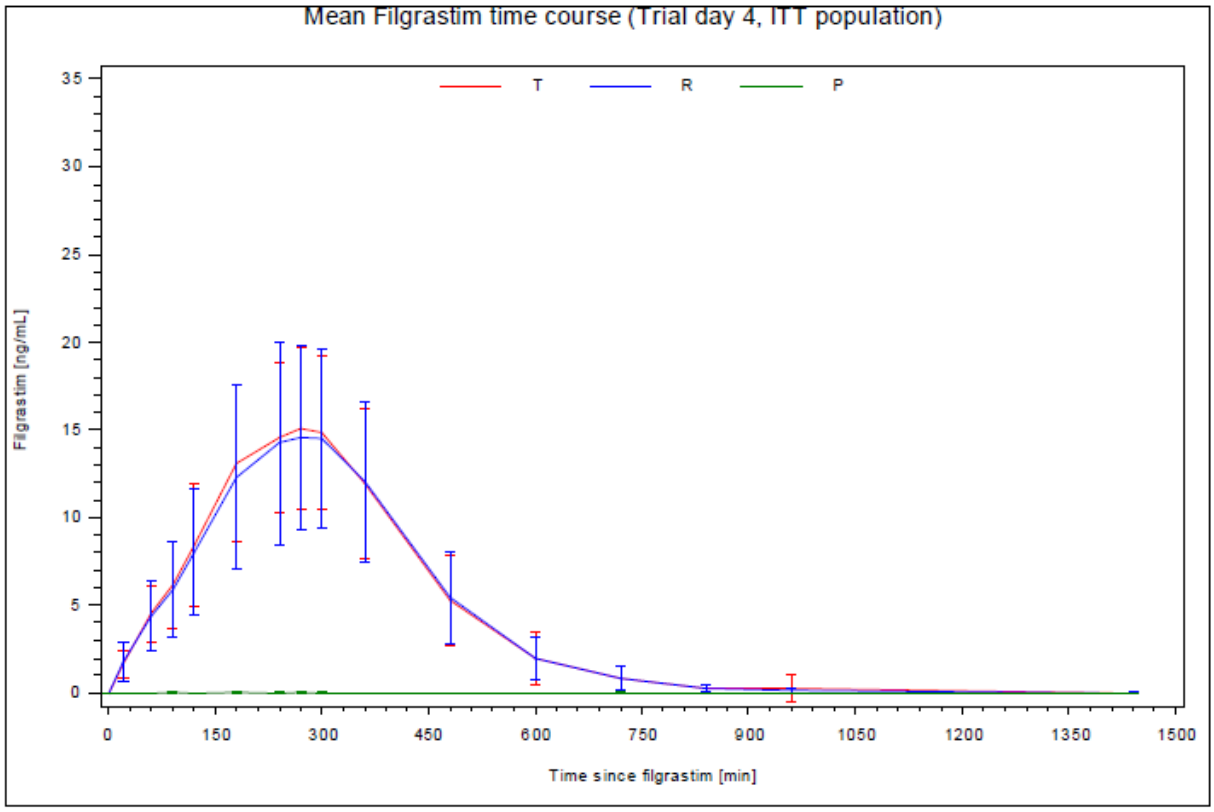


Table 18: Filgrastim levels (ITT population)

	Geometric mean		Ratio: Test/Reference (%)	
Parameter	Test (n=36)	Reference (n=36)	Point estimate	90% CI
AUC_{0-24} (min*ng/mL)	11221.6	11334.7	99.0	89.25-109.82
$AUC_{0-\infty}$ (min*ng/mL)	11289.3	11407.0	99.0	89.22-109.78
C_{max} (ng/mL)	24.39	24.46	99.7	88.74-112.05
AUC_{SS} (min*ng/mL)	5254.1*	5098.6	103.1	92.04-115.37

* n=35

Elimination

Table 19: T ½ Filgrastim by treatment group (ITT population)

Elimination half life [min]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	36	162.2	15.6	135.3	148.2	161.5	172.9	196.4
R	36	162.2	19.4	138.4	148.1	157.7	172.9	222.8

Table 20: Tmax Filgrastim by treatment group (ITT population)

Tmax [min] Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	36	299.2	36.2	240.0	270.0	300.0	300.0	360.0
R	36	305.8	32.7	240.0	300.0	300.0	300.0	360.0

Table 21: CL Filgrastim by treatment group (ITT population)

Clearance [L/min] Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	36	0.0317	0.0114	0.0149	0.0232	0.0276	0.0413	0.0599
R	36	0.0312	0.0097	0.0158	0.0239	0.0310	0.0360	0.0554

Study GCSF-SUIN-055BOI-3FA

Study Design

As a part of the global development of Apo-Filgrastim and following the manufacturing changes in the Apo-Filgrastim drug substance and drug product in terms of the manufacturing scale and addition of the a mixed mode chromatography for additional purification, the applicant conducted this study to further demonstrate the lack of meaningful differences in the clinical performance of Apo-Filgrastim DP from Process II, Apo-Filgrastim DP from Process III and the reference product, EU-approved Neupogen. The Phase I 3-arm study was designed as a comparative, single center, randomized, three-way crossover double-blind study with single-dose subcutaneous administration of 300µg Apo-Filgrastim, EU-approved Neupogen and US-licensed Neupogen.

The pharmacokinetic (PK), pharmacodynamic (PD) and safety endpoints analysed in the study were as below:

- Primary PK endpoints: AUC_t and C_{max} of filgrastim
- Primary PD endpoints: AUC_t and C_{max} of ANC
- Secondary endpoint: T_{half} of filgrastim
- Tertiary endpoints: AUC_{inf}, T_{max}, K_{el} of filgrastim
- Safety endpoints: Adverse events, lab tests, vital signs and immunogenicity.

The cross-over study was comprised of three periods involving 6 dosing sequences of the investigational product, Apo-Filgrastim, and the comparators US-licensed Neupogen and EU-approved Neupogen. A total number of 48 healthy volunteers, in the age range of 18-55 years were dosed in the study. Forty-five (45) subjects completed at least two periods of the study and of these, forty (40) subjects completed all three periods of the study. Consequently, the safety population included all forty-eight (48) randomized subjects since all subjects received at least one administration of the study treatment. The pharmacokinetic/pharmacodynamic (PK/PD) dataset included forty-five (45) subjects, which is in accordance with the protocol that defined the PK/PD population as subjects who completed at least two periods of the study. The study duration included three periods, a washout between doses and the collection of a blood sample for immunogenicity testing at 240 hours (10 days) post-dose in each period. Passive safety surveillance was performed for the duration of 4 months after the completion of last study period.

Blood sampling was scheduled for filgrastim estimation at 0.5, 1.25, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11.5, 13, 15, 18, 24, 30 and 36 hours after dosing. For absolute neutrophil counts (ANC), blood samples were drawn prior to dosing time (0 hours) and at 0.5, 1, 2, 4, 6, 8, 10, 11, 12, 14, 18, 24, 36, 48, 72 and 96 hours after dosing.

Pharmacokinetic/Pharmacodynamic Analysis

A 2-way analysis of variance (ANOVA) was performed for each comparison. 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.

For filgrastim, ANOVA was performed on the log-transformed AUC_t, AUC_{inf} and C_{max} parameters and on the untransformed T_{max}, K_{el} and T_{half} parameters of filgrastim and for ANC data, ANOVA was performed on the log-transformed AUC_t and C_{max} parameters. In addition, ANOVA was performed on the untransformed T_{max} parameter (PROC GLM of SAS® v8.2 software).

The two one-sided hypothesis was tested at the α=0.05 level of significance for the AUC_t, AUC_{inf} and C_{max} parameters of filgrastim and for the AUC_t and C_{max} parameters of ANC by constructing the 90% confidence interval for the ratio between the test and reference means. Additionally, a supplementary analysis conducted on the ANC data using a higher alpha level of significance (a = 0.025) leading to the calculation of a 95% confidence interval has been performed.

Methods

- **Analytical methods**

The analytical reports used for the PK and immunogenicity analysis of the Apo-Filgrastim clinical studies submitted were:

1. ELISA for measurement of G-CSF in plasma
2. Immunogenicity assays
    a. Screening ELISA
    b. Confirmation of positive samples (depletion with G-CSF)
    c. Neutralising cell based proliferation assay

ELISA for PK estimation

A commercially available Human G-CSF sandwich enzyme immunoassay (EIA) kit was used.

A complete validation of analytical method has been provided. The validation parameters of this study include, intra and inter-assay precision and accuracy of the back calculated concentration for the calibration standards, the inter- and intra-assay precision and accuracy of the QCs and the method total error as well as the short-term stability analysis.

The in-study validation for the three Phase 1 studies was submitted and shows acceptable calibrations standards and QCs values.

The PK sample analysis was completed within less than five months of the sample collection for all three studies. The long-term stability for GCSF was at least five months to cover the above period of storage.

Immunogenicity assays

The anti-drug antibody (ADA) analysis involved the following steps:

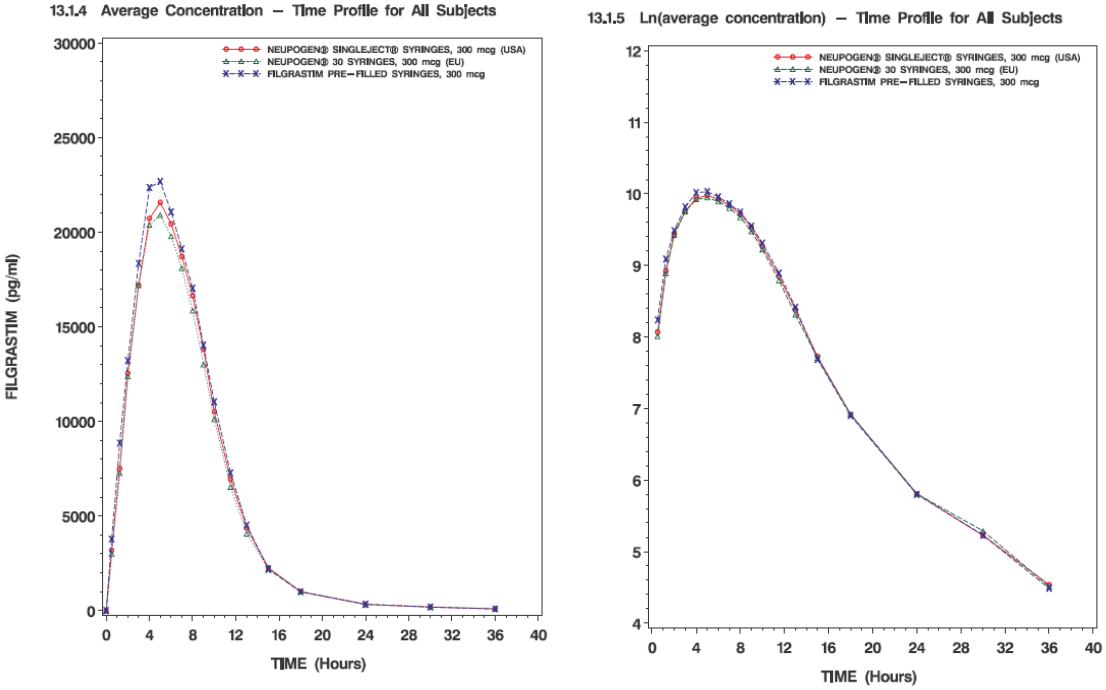
Step 1: A Screening assay performed on all human serum samples for detecting the presence of the IgG and IgM antibodies.

Step 2: A Confirmation assay to confirm the positive results obtained in the Screening assay.

Step 3: A Neutralising assay on confirmed results from Step 2, to evaluate the positive samples for their G-CSF neutralising potential in a Bioassay.

Mean G-CSF concentration time profiles obtained after the single subcutaneous administrations of Apo-Filgrastim, EU-approved Neupogen and US-licensed Neupogen are presented in linear and log plots in Figures below.

Figure 5: Average Concentration – Ln (Average Concentration) / Time Profile for All Subjects



A summary table of the PK Parameters following a Subcutaneous Injection of 300µg Apo-Filgrastim and Comparator Neupogen Products, in Healthy Volunteers are presented in table 24 below:

Table 22: PK Parameters following a Subcutaneous Injection of 300µg Apo-Filgrastim and Comparator Neupogen Products

Parameter	Apo-Filgrastin Mean (CV %) (N=43)	US-Neupogen Mean (CV %) (N=43)	EU-Neupogen Mean (CV %) (N=43)
AUC_t (pg*h/mL)	200720.00 (34%)	192379.97 (31%)	186404.48 (34%)
C_{max} (pg/mL)	24212.80 (44%)	22756.87 (36%)	21835.92 (38%)
AUC_{inf} (pg*h/mL)	202126.78 (34%)	193710.54 (31%)	187937.67 (34%)
T_{max} (h)	5.00 (33%)	5.00 (23%)	5.00 (23%)

K_{el} (1/h)	0.11551 (58%)	0.10586 (44%)	0.10331 (44%)
T_{half} (h)	7.17 (35%)	7.30 (29%)	7.62 (33%)

A summary of all statistics estimated for all pharmacokinetic endpoints of filgrastim for the all three comparison is presented the table below

Table 23: summary of all statistics estimated for all pharmacokinetic endpoints

Endpoint		Apo-Filgrastim vs EU-Neupogen (N=43)	Apo-Filgrastim vs US-Neupogen (N=43)	US-Neupogen vs EU-Neupogen (N=43)
AUCt [pg*h/mL]	Relative Mean#	1.10	1.08	1.01
	90% CI	1.04-1.16	1.02-1.14	0.96-1.06
Cmax [pg/mL]	Relative Mean#	1.11	1.10	1.02
	90% CI	1.02-1.21	1.01-1.20	0.94-1.09
AUC_{inf} [pg*h/mL]	Relative Mean#	1.09	1.08	1.01
	90% CI	1.04-1.15	1.02-1.14	0.96-1.06
T_{max} [h]	Relative Mean#	0.99	0.97	1.01
	90% CI	0.91-1.07	0.88-1.06	0.95-1.07
Kel [1/h]	Relative Mean#	1.14	1.07	1.02
	90% CI	1.03-1.25	0.99-1.16	0.94-1.11
T_{half} [h]	Relative Mean#	0.85	0.90	0.96
	90% CI	0.85-1.01	0.90-1.06	0.88-1.04

Based on the least squares estimates of the geometric means of AUCt, Cmax, AUCinf and based on the least squares estimates of the arithmetic means for Tmax, Kel, Thalf.

The 90% confidence intervals of the relative mean AUC_t and C_{max} for filgrastim, the primary pharmacokinetic endpoints of the study, were contained within the pre-defined acceptance range of 0.8–1.25 for all comparisons.

Dose proportionality and time dependencies

No studies on dose proportionality and time dependencies were submitted.

Special populations

No special population PK studies were submitted.

Pharmacokinetic interaction studies

No pharmacokinetic interaction studies were submitted.

Pharmacokinetics using human biomaterials

No pharmacokinetic studies using human biomaterials were submitted.

2.4.3. Pharmacodynamics

Mechanism of action

Filgrastim exercises its action by binding to specific G-CSF receptors on the cell surface. Binding studies indicate two types of receptors: low affinity 100-130 kDa monomer receptors and high-affinity oligomeric receptors. Its action on G-CSF receptors promotes the growth, proliferation, differentiation, and maturation of neutrophil precursors and enhances the function of mature neutrophils by increasing phagocytic activity and antibody-dependent cell-mediated cytotoxicity. Its activity also mobilises haemopoietic progenitor cells from bone marrow into peripheral blood.

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Figure 6: Mean ANC time course (PP population)

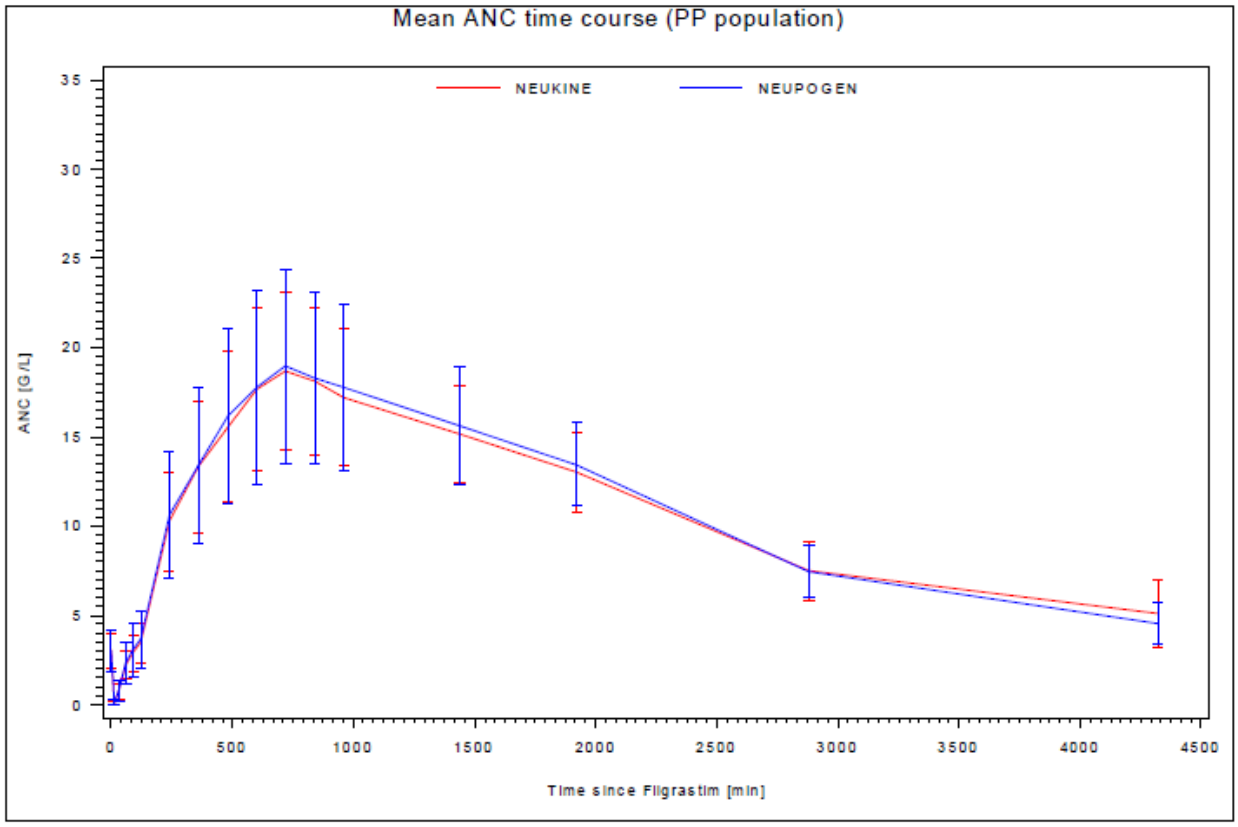

Figure 7: Mean ANC time course (ITT population)

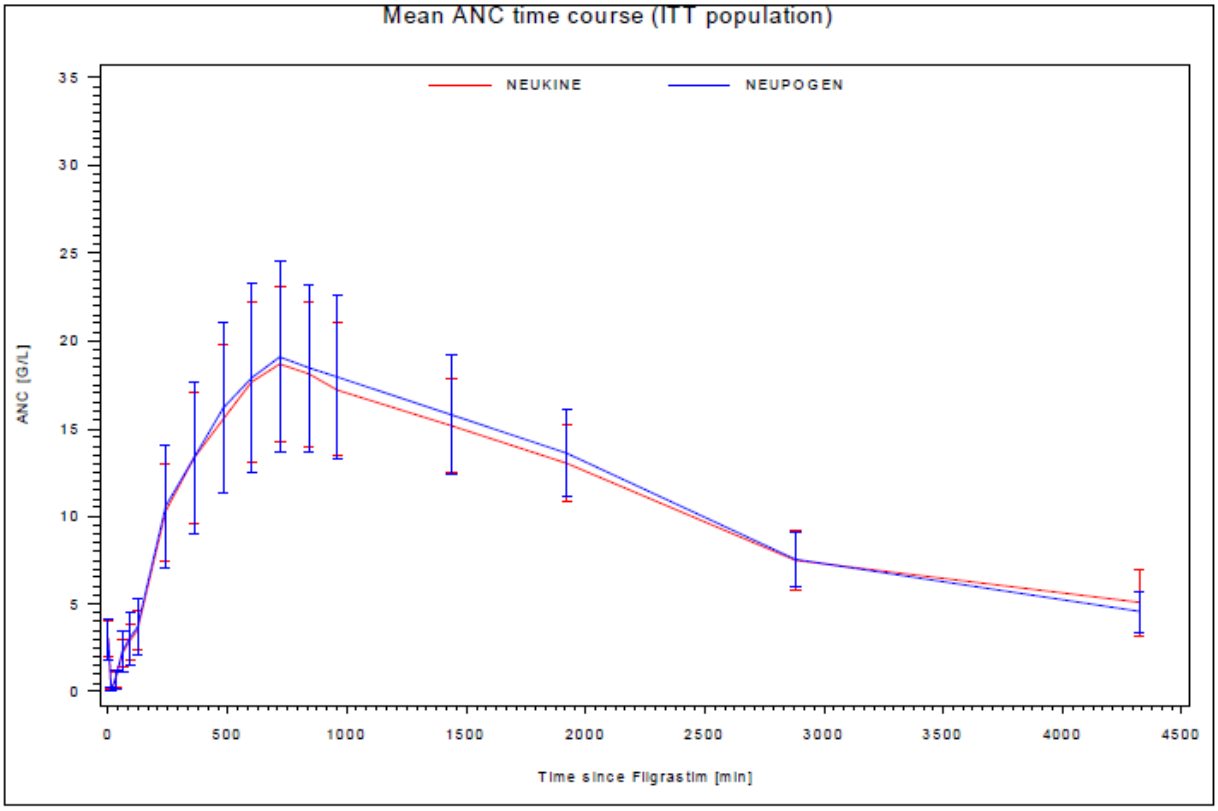

Table 24: Cmax ANC by treatment group (PP population)

Cmax [G/L]								
Treatments	N	Mean	SD	Min	Q1	Median	Q3	Max
NEUKINE	35	19.02	4.35	11.42	15.55	18.48	22.17	29.86
NEUPOGEN	35	19.28	5.21	11.43	15.40	18.74	22.85	32.55

Least square mean difference	Estimate (log-scale)	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Neukine - Neupogen	-0.00469831	0.8972	[-0.07,0.06]	99.5	[93.6%,105.8%]

Table 25: AUC (0-72) ANC by treatment group (PP population)

AUC(0-72) [min*G/L]								
Treatments	N	Mean	SD	Min	Q1	Median	Q3	Max
NEUKINE	35	46137.4	8608.3	31838.0	41535.4	46256.5	51986.2	62765.8
NEUPOGEN	35	46601.5	9321.6	29727.7	39006.4	44899.3	51965.5	63213.4

Table 26: Absolute neutrophil count - 5µg/kg i.v.

n=35	Geometric mean		Ratio: Test/Reference (%)	
Parameter	Test	Reference	Point estimate	90% CI
C_{max} (G/L)	18.55	18.64	99.5	93.63-105.80
AUC_{0-72} (min*G/L)	45314	45714	99.1	95.48-102.91

Considering the PD parameter C_{max} of ANC, there was no statistically significant difference between both study medications (probability = 0.8972) and the confidence intervals were within the pre-defined equivalence margins (80% - 125%). It is therefore concluded that the observed difference in the pharmacokinetics between the test item Neukine and the reference item Neupogen of filgrastim is not relevant for the PD effect in terms of ANC stimulation in humans. This result is emphasized when considering the results obtained by univariate statistical analysis of the AUC_{0-72} of the ANC yielding almost identical values for both the test item Neukine and the reference item Neupogen with regard to the PP population and the ITT population too.

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Table 27: Cmax ANC by treatment group (PP population)

Cmax ANC [G/L]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T150	35	19.04	3.83	11.76	17.23	20.00	21.09	26.53
R150	35	19.59	3.29	12.25	17.47	19.99	21.81	25.75
T75	33	17.13	3.74	10.50	14.96	16.61	19.01	26.01
R75	33	18.60	4.11	12.72	16.42	18.32	20.07	32.05

Comparison of 150 µg treatments

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	-0.03745829	0.02782301	-1.35	0.1874	[-.08,0.01]	96.3	[91.9%,101.0%]

Comparison of 75 µg treatments

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	-0.08378525	0.02650357	-3.16	0.0035	[-.13,-.04]	92.0	[87.9%,96.2%]

Table 28: AUC (0-72) ANC by treatment group (PP population)

AUC(0-72) [min*G/L]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T150	35	43209.3	7921.5	24674.0	37203.7	41269.8	49212.2	55836.6
R150	35	43979.6	6866.4	30334.1	38324.4	43898.5	49615.2	56331.0
T75	33	35076.8	6526.3	21989.0	31623.1	34337.2	39244.7	49218.0
R75	33	37009.8	7622.5	25854.5	32497.0	34306.3	40397.1	58019.7

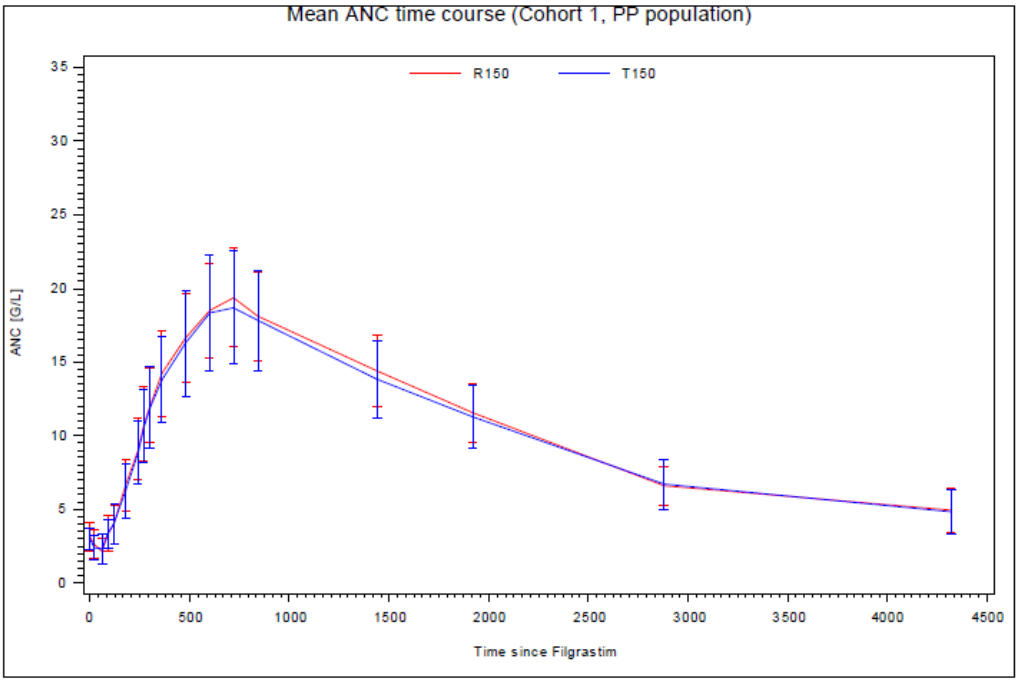
Table 29: Absolute neutrophil count - 150µg s.c. (PP population)

		Geometric mean		Ratio: Test/Reference (%)	
AUC_{0-72} (min*G/L)	35	42455	43461	97.7	93.75-101.79

Table 30: Absolute neutrophil count - 75µg s.c. (PP population)

		Geometric mean		Ratio: Test/Reference (%)	
AUC_{0-72} (min*G/L)	33	34481	36316	94.9	91.72-98.29

Figure 8: Mean ANC time course for the 150ug dose and the 75 ug dose (PP population)



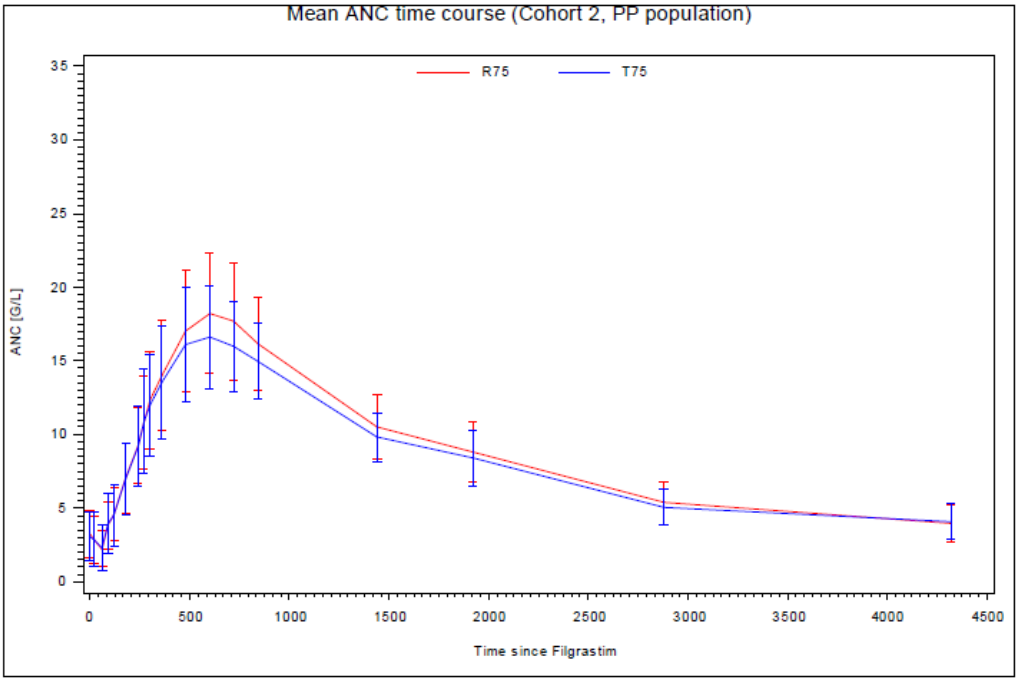


Table 31: Cmax ANC by treatment group (ITT population)

CMax [G/L] Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T150	36	18.88	3.89	11.76	16.65	19.92	21.01	26.53
R150	36	19.65	3.26	12.25	17.48	20.12	21.77	25.75
T75	37	17.25	3.56	10.50	15.10	17.03	19.15	26.01
R75	36	18.44	3.97	12.72	16.38	17.78	19.79	32.05

Comparison of 150µg treatments

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	-0.04782202	0.02893599	-1.65	0.1076	[-.10,0.00]	95.3	[90.78%,100.11%]

Comparison of 75µg treatments

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	-0.07101003	0.02582913	-2.75	0.0095	[-.11,-.03]	93.1	[89.16%,97.30%]

Table 32: AUC (0-72) ANC by treatment group (ITT population)

AUC(0-72) [min*G/L] Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T150	35	43209.3	7921.5	24674.0	37203.7	41269.8	49212.2	55836.6
R150	36	44046.9	6779.6	30334.1	38355.0	44078.3	49345.0	56331.0
T75	36	35373.4	6398.8	21989.0	31915.2	34857.1	39286.7	49218.0
R75	36	36931.6	7339.4	25854.5	32315.6	34406.6	39899.4	58019.7

Table 33: Absolute neutrophil count - 150µg s.c. (ITT population)

		Geometric mean		Ratio: Test/Reference (%)	
Parameter	n	Test	Reference	Point estimate	90% CI
AUC_{0-72} (min*G/L)	35	42455	43461	97.7	93.75-101.79

Table 34: Absolute neutrophil count - 75µg s.c. (ITT population)

		Geometric mean		Ratio: Test/Reference (%)	
Parameter	n	Test	Reference	Point estimate	90% CI
AUC_{0-72} (min*G/L)	35	34793	36221	96.1	92.61-99.64

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Table 35: Cmax ANC by treatment group (PP population)

Cmax [G/l]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	35	30.54	6.15	19.19	27.76	30.57	33.48	46.96
R	34	32.27	7.68	20.47	27.02	29.95	36.38	51.50

Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	-0.04906252	0.05205780	-0.94	0.3493	[-.14,0.04]	95.2	[87.29%,103.85%]

Table 36: Cmax-24h ANC by treatment group (PP population)

Cmax-24h [G/l]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	35	21.04	3.68	13.07	18.64	21.09	24.45	26.01
R	34	21.96	4.62	13.79	18.79	22.05	23.88	34.85

Table 37: AUC 0-24 ANC by treatment group (PP population)

AUC(0-24) ANC [min*G/l]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	35	22974.9	3878.1	14321.4	21027.4	22781.9	26524.6	28634.7
R	34	23873.8	4679.4	16167.1	20837.3	23422.1	25745.5	38997.2

Table 38: Cmax-24h ANC by treatment group (PP population)

AUC(0-24) ANC [min*G/l]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	36	23083.5	3877.5	14321.4	21226.2	22842.6	26541.6	28634.7
R	36	24177.0	4717.4	16167.1	21169.3	23735.3	26019.9	38997.2

Table 39: AUC 0-24 ANC by treatment group (PP population)

Cmax [G/l]								
Treatment group	N	Mean	SD	Min	Q1	Median	Q3	Max
T	35	30.54	6.15	19.19	27.76	30.57	33.48	46.96
R	36	32.55	7.55	20.47	27.26	30.81	36.73	51.50

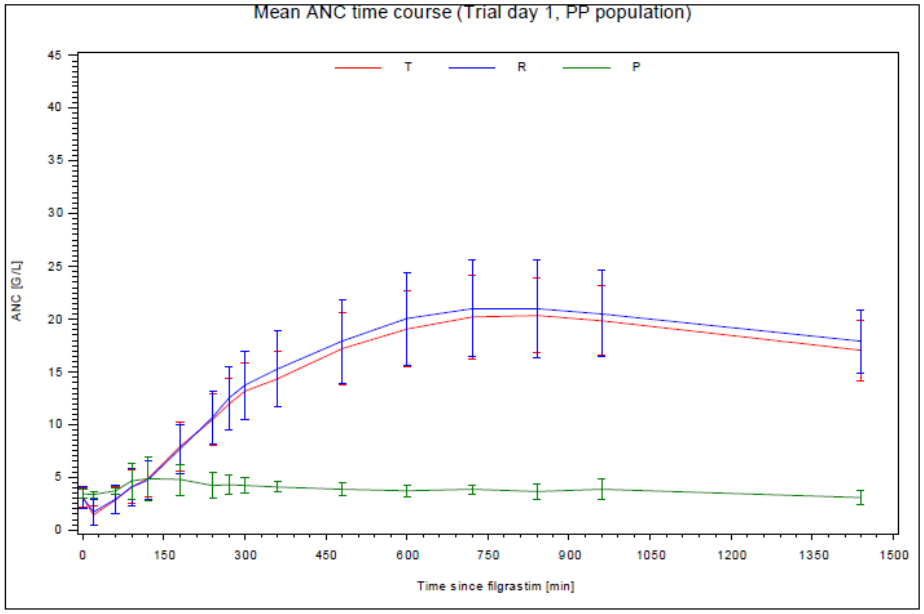
Least square mean difference	Estimate (log-scale)	Standard Error	t Value	Pr > |t|	90% CI (log-scale)	Estimate (%)	90% CI (%)
Test - Reference	-0.05867795	0.05102467	-1.15	0.2541	[-.14,0.03]	94.3	[86.61%,102.67%]

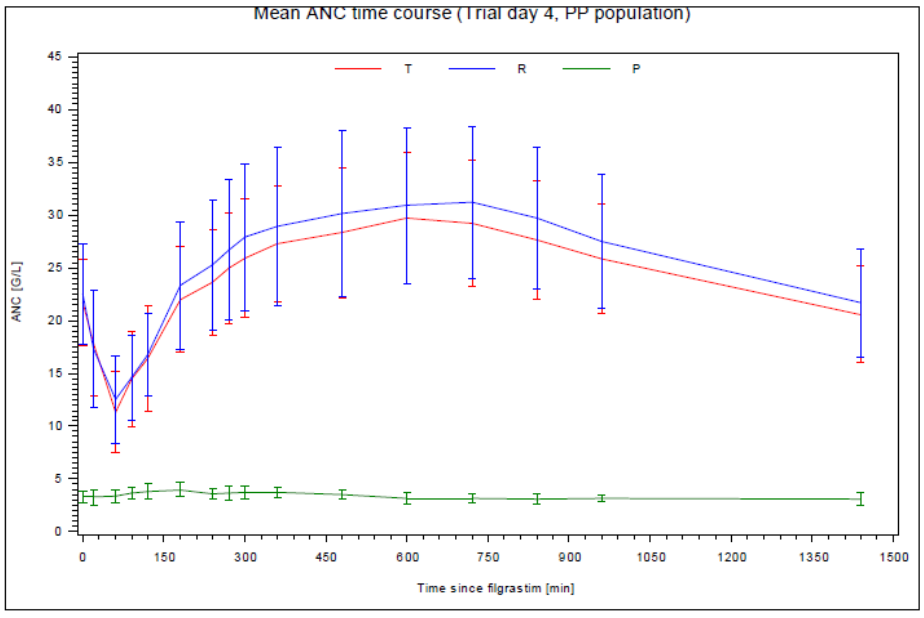
Table 40: Absolute neutrophil count (PP population)

	Geometric mean		Ratio: Test/Reference (%)	
Parameter	Test (n=35)	Reference (n=34)	Point estimate	90% CI
AUC_{0-72} (min*G/L)	22624.9*	23477.2	96.4	89.56-103.69
C_{max-24} (G/L)	20.70*	21.53	96.2	88.94-103.99

The primary endpoint result is emphasized when considering the results obtained by univariate statistical analysis of C_{max}-24h of ANC and the AUC_{0-24} of ANC after first filgrastim application yielding almost identical values for both the test item Filgrastim Drug Product and the reference item Neupogen with regard to the PP population and the ITT population

Figure 9: Mean ANC time course on trial days 1 and 4 by treatment group (PP population)



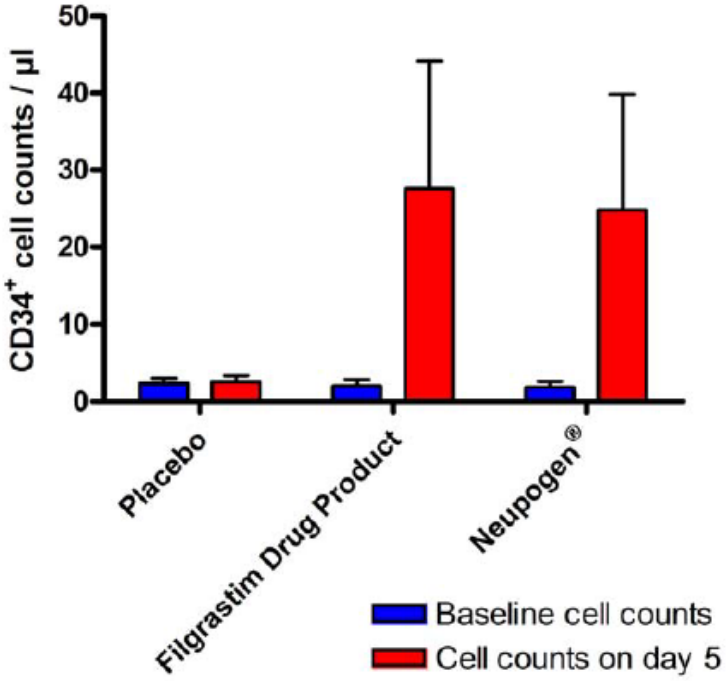


The variation of ANC counts observed in the placebo group was in the expected range of fluctuations (intra-subject and inter-subject variability) usually experienced during sequential ANC counting (mean coefficient of variation: 19%).

Table 41: CD34+ cell count was assessed on study days 1 and 5.

CD34+ [/µl] Treatment group	Trial day	N	Mean	SD	Min	Q1	Median	Q3	Max
T	Screening visit or Day 1	35	2.00	0.81	0.83	1.44	1.86	2.40	3.90
	Trial day 5 (96h)	35	27.65	16.54	6.04	15.30	22.00	40.82	66.72
R	Screening visit or Day 1	34	1.85	0.77	0.62	1.32	1.81	2.16	3.78
	Trial day 5 (96h)	34	24.84	15.03	5.85	12.55	21.30	34.24	72.00
P	Screening visit or Day 1	6	2.34	0.70	1.52	1.83	2.24	2.72	3.50
	Trial day 5 (96h)	6	2.56	0.78	1.59	2.01	2.45	3.30	3.55

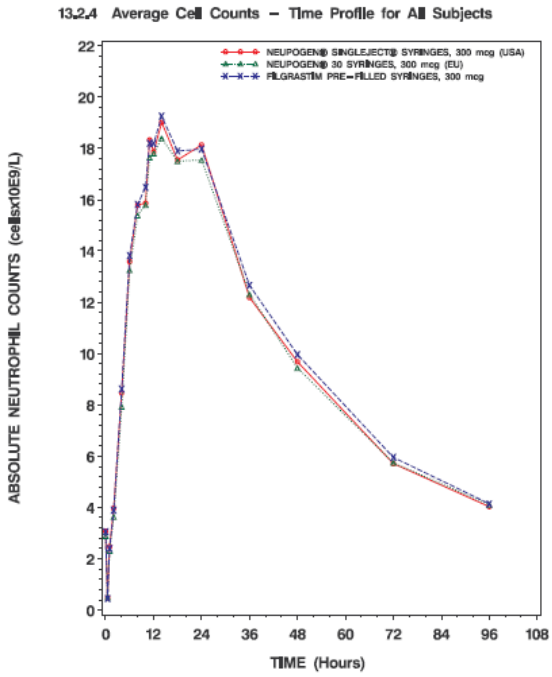
Figure 10: CD34+ cells in peripheral blood after repeated dose of G-CSF

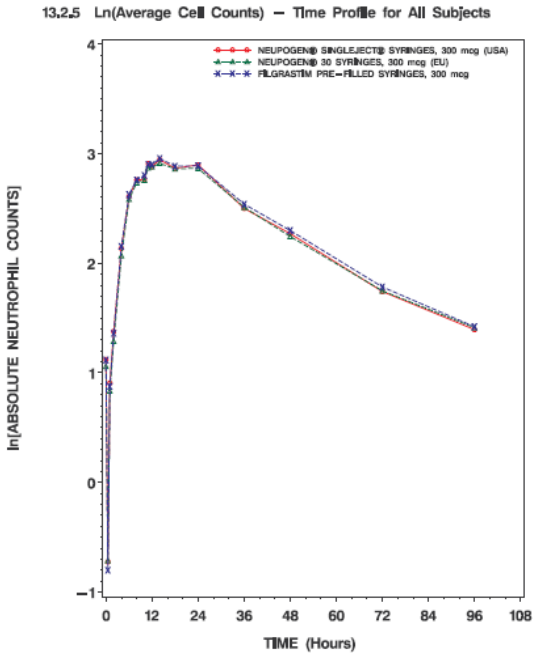


GCSF-SUIN-05SB01-3FA-(5) study

Mean ANC concentration time profiles obtained after the single subcutaneous administrations of Apo-Filgrastim, EU-approved Neupogen and US-licensed Neupogen are presented in linear and log plots in Figures below.

Figure 11: Average Cell Count and Ln (Average Cell Count) / Time Profile for All Subjects





s. US-Licensed are shown in Table below.

Table 42: Results of Apo-Filgrastim vs. EU Neupogen and vs.US license

Evaluation Criteria	Apo-Filgrastim Vs. EU RMP	Apo-Filgrastim Vs. US RMP	US RMP Vs. EU RMP
PHARMACODYNAMIC PARAMETER: ABSOLUTE NEUTROPHIL COUNT (ANC)			
AUCt Ratio (90% CI) (95% CI)	103% (100% - 106%) (99% - 107%)	100% (97% - 104%) (96% - 104%)	102% (99% - 106%) (99% - 106%)
C_{max} Ratio (90% CI) (95% CI)	103% (99% - 108%) (98% - 109%)	100% (96% - 105%) (95% - 106%)	104% (99% - 109%) (98% - 110%)
T_{max} Mean	Apo-Filgrastim: 15.38 hours EU RMP - 13.94 hours	Apo-Filgrastim: 15.38 hours US RMP- 16.05 hours	EU RMP - 13.94 hours US RMP- 16.05 hours

*RMP=Reference Medicinal Product

Comparison across studies

The Applicant submitted a re-analysis of PK parameters from both ITT and PP populations of all phase I PK/PD studies. The results in the PP population are presented below in Table 45.

Table 43: Summary of PD/Statistical Results for ANC Cmax and AUC t Parameters in Clinical Phase I PK/PD Studies with Apo-Filgrastim (Neukine) and Neupogen (PP Population)

Study Dose (n)	Parameter	Ratio T/R%	90% CI[%]	95% CI[%]
KWI-300-101 5µg/kg (n=35)	Cmax (0-72) ANC [G/L]	99.5	93.6 – 105.8	92.5 -107.1
	AUC (0-72) ANC [min* G/L]	99.13	95.5 – 102.9	94.8 - 103.7
KWI-300-102 150 µg (n=35)	Cmax (0-72) ANC [G/L]	96.3	91.9-101.0	91.0-101.9
	AUC (0-72) ANC [min* G/L]	97.7	93.8-101.8	93.0-102.6
KWI-300-102 75 µg (n=33)	Cmax (0-72) ANC [G/L]	92.0	87.9-96.2	87.1-97.1
	AUC (0-72) ANC	95.0	91.7-98.3	91.1-99.0
	[min* G/L]			
KWI-300-103 5µg/kg (n=34)	Cmax (0-24) ANC [G/L]	96.2	88.9-104.0	87.6-105.6
	AUC (0-24) ANC [min* G/L]	96.4	89.6-103.7	88.3-105.2
GCSF-SUIN-05SB01-3FA 300 µg (n=43)	Cmax (0-96) ANC [G/L]	103	99 -108	98 - 109
	AUC (0-96) ANC [min* G/L]	103	100-106	99 - 107

Source: Study reports KWI-300-101 to KWI-300-103, GCSF-SUIN-05SB01-3FA

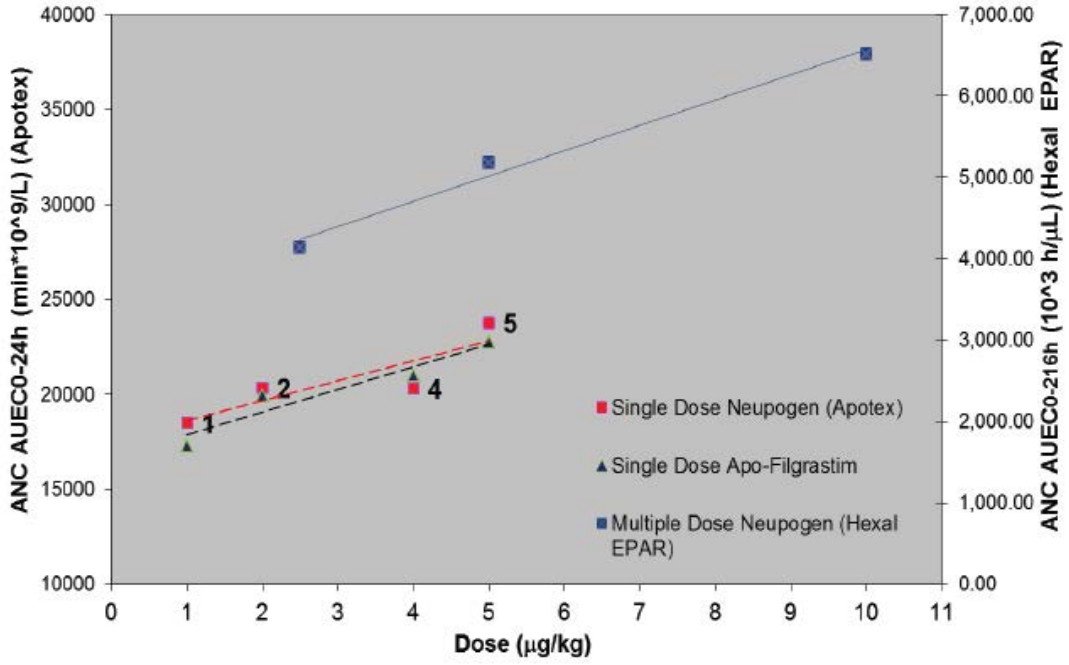
In order to further demonstrate that this dose is indeed on the ascending portion of the dose response curve even for ANC, the ANC AUCs for Neupogen in each of the Phase I studies (and hence at each dose ranging from 1 to 5 µg/kg) in Apo-Filgrastim clinical program were evaluated for trend over dose. Since study KWI-300-103 was a multiple-dose study, in order to have data that is comparable to that of the other single dose studies (KWI-300-102 Cohort 1 and Cohort 2), the ANC AUC0-24h for all studies was used for assessment. The ANC AUC0-24h value for Neupogen in each of these studies is tabulated below in the table below.

Table 44: Summary of AUC 0-24h for ANC following the Single Dose SC administration of Neupogen in Studies KWI-300-102 and KWI-300-103

Study	Dose	ANC AUC0-24h (min*G/L)*
KWI-300-102 Cohort 2	≈ 1 µg/kg#	18,108.32
KWI-300-102 Cohort 1	≈ 2 µg/kg#	21,038.22
KWI-300-103	5 µg/kg	23,766.32

#based on a weight of approximately 75kg.
*geometric mean

Figure 12: Assessment of ANC AUEC* following Single Dose Administration of Neupogen and Apo-Filgrastim and Multiple Dose Administration of Neupogen



*The Y-axes titles of the plot represent the units as identified in the source used for constructing this dose-response-plot. The units used for the Apotex data are' min*10^9/L' and for data reported in Hexal EPAR[10] are '10^3 h/µl'. Given the uncertainty of the units presented in the Hexal EPAR and to ensure that the data presented in the plot was accurate, the data was not converted and thus is not presented in the same units

To demonstrate that single-dose studies were equally as sensitive in detecting PD differences between test and reference products as multiple dose studies, the Applicant presented a review of the data which showed that the difference observed between Apo-Filgrastim and Neupogen following the administration of a single dose (i.e. AUC0-24) is essentially the same as the difference observed after the administration of multiple doses (i.e. 4 daily doses) of filgrastim (AUC0-96); approximately 3.6% and 4.4% respectively. The ANC Cmax data revealed the same. Thus, despite altered levels of expression of G-CSF receptors and altered disposition of filgrastim under a multiple-dose setting, these results suggest that a single dose setting is expected to be as sensitive as a multiple dose setting in ascertaining differences between filgrastim medicinal products. Accordingly, the available single dose data for Apo-Filgrastim at low doses (i.e. 1 µg/kg (75 µg) and 2 µg/kg (150 µg) should provide assurance of clinical similarity following the multiple dose administration of corresponding low doses.

It should be noted that there is limited available data in the literature to compare the response following low dose administration of filgrastim.

Table 45: Summary of PD/Statistical Results for ANC AUEC and Cmax Parameters in Clinical Phase I PK/PD Study KW1-300-103

Study Dose (n)	Parameter	Ratio of the Test and Reference T/R%	Absolute Difference of the Test and Reference LSM (%)	95% CI[%]
KWI-300-103 5µg/kg (n=34)	AUC (0-24) ANC [min*10^9/L]	96.4	3.6	88.3 - 105.2
	AUC (0-96) ANC [min*10^9/L]	95.6	4.4	88.0 - 103.8
	Cmax (0-24) ANC [10^9/L]	96.2	3.8	87.6-105.6
	Cmax (0-96) ANC [10^9/L]	95.2	4.8	85.8-105.6

2.4.4. Discussion on clinical pharmacology

The pharmacokinetics of Apo-filgrastim were initially investigated in three studies in healthy human subjects – a single 5µg/kg intravenous dose study, a 75µg and 150µg single subcutaneous dose study and a repeat subcutaneous dose 5µg/kg/day study. The choice of enrolling healthy subjects in order to minimise variability, which may complicate evaluation of PK equivalence, is endorsed.

In general, the studies were well run and prior to commencement, scientific advice was sought from the CHMP. The main divergence from this advice was to maintain the placebo group in the 3-arm multiple-dose study, KWI-300-103, in order to help demonstrate assay sensitivity instead of replacing it with another study arm where subjects would be administered a different dose of Apo-filgrastim (2.5 or 10µg/kg). This was considered problematic as the Apo-filgrastim development programme then lacked comparative repeated-dose data at two dose levels, which would allow convincing demonstration of PK equivalence.

The methods for quantification of the study treatments in serum included a commercial ELISA and were adequately validated. The validation of the screening, confirmatory and neutralising immunogenicity assays was acceptable.

A new study, GCSF-SUIN-05SB01-3FA (Phase I 3-arm), was submitted during the procedure. The objective of the study was to demonstrate that Grastofil manufactured using drug substance from manufacturing Process IX and proposed commercial drug product from Process III was similar in terms of PK/PD to EU-approved Neupogen. Thus the aim of the study was to bridge the existing clinical data for Apo-Filgrastim (DP process II, and DS Process VII) vs the comparator. The study demonstrated that a single administration sc of 300µg of Grastofil manufactured with the final process intended for the marketed product is comparable, in terms of PK/PD, to the reference test products. In addition, the marketed reference products, Neupogen Europe and USA were comparable as expected. For the primary pharmacokinetic endpoint parameters AUC_t and C_{max} for filgrastim, the 90% confidence intervals were contained within the pre-defined acceptance range of 80-125% for all formulation comparisons.

PK data were analysed using ANOVA on log-transformed data with terms for sequence, subject within sequence, period and treatment. This is the analysis requested in the CHMP bioequivalence guideline. The acceptance limits set for the confidence intervals of the differences were in line with those outlined in the CHMP bioequivalence guideline.

The principle PK issue arose from the results of Study KWI-300-101 and Study GCSF-SUIN-05SB01-3FA. For Study KWI-300-101 the statistical analysis of the AUC_{0-32} and $AUC_{0-\infty}$ of filgrastim showed a highly significant difference (p=< 0.0001) between the point estimates for the test item Apo-Filgrastim and the reference item Neupogen. In Study GCSF-SUIN-05SB01-3FA as in KWI-300-101 there were seen to be statistically significant differences in PK outputs between Neupogen and Grastofil. On the other hand, the CHMP acknowledged that in the comparative healthy volunteer studies, for all the main PK parameters, the 90% confidence intervals of the ratios of the means were fully contained within the 80-125% acceptance limits, in line with the guidance provided in the CHMP Guideline on the Investigation of Bioequivalence (January 2010). Although the usual acceptance criteria for bioequivalence were met, these 'bioassays' constituted a signal of differences between formulations that needed to be investigated prior to a conclusion of biosimilarity being drawn**.**

By way of providing justification for the confidence intervals of the PK ratios not encompassing 100% in the intravenous dose study and in study GCSF-SUIN-05SB01-3FA, the Applicant indicated that the results were likely due to the low variability of the PK data in study KWI-300-101 as a result of having more subjects than required in the study and due to the nature of the intravenous dose model, which is usually associated with lower variability than the subcutaneous dose model. This justification was supported with data from the relevant studies. However, it should be noted that the variability has a significant impact on the width of the confidence intervals and not as great an effect on the point estimate, which showed approximately 10% differences between test and reference products. Therefore, the effect of variability on the differences seen was considered marginal. It was agreed that the differences were unlikely to result from differences in the sensitivities of the PK assay to Apo-filgrastim and Neupogen. Whilst the explanation for the PK differences in the two studies has not been definitively identified, it should be noted that for the majority of the PK output comparisons, differences as regards the point estimates were smaller and not statistically significant. In an exercise consisting of multiple comparisons of PK end points it is conceivable that for a few, significant or somewhat larger differences may be demonstrated, going against trends otherwise seen in the data. Ultimately and importantly, the lack of significant differences with regard to PD outcomes in the studies in question provided significant reassurance that for studies KWI-300-101 and GCSF-SUIN-05SB01-3FA the noted PK differences were unlikely to be indicative of clinically meaningful differences between the test and reference products or to lead to differences in PD response and clinical effect.

Process II material tended to provide PK concentrations lower than Neupogen (particularly in study KWI-300-101). For process III product (study GCSF-SUIN-05SB01-3FA) the values were statistically significantly higher. While it is difficult to directly compare the products of the two processes using these data, it seems possible that they would not be bioequivalent to each other, creating concern that the efficacy and in particular, given the higher levels, safety data from trial KWI-300-104, cannot be extrapolated to process III product. However, there are a number of uncertainties surrounding this observation. For the majority of the PK readouts the differences between test-reference ratios for process II and III products were modest. Also, the lowest ratios occurred in the 5mcg/kg intravenous dose study i.e. a different method of administration and dose from the new GCSF-SUIN-05SB01-3FA study. Further, the comparisons discussed above were made across studies. This can be associated with significant uncertainties with regard to data interpretation. Most importantly, PK evaluation of products of both processes showed the confidence intervals of the ratios of test and reference products to fall fully within the agreed acceptance limits and in the end, the aforementioned differences could simply reflect biological variability and not indicate true differences between materials. Lastly, it is acknowledged that a reasonable Quality comparability exercise was undertaken by the Applicant. The exercise sufficiently demonstrated that Apo-Filgrastim drug substance and drug product from the clinical stages of process development and from the proposed commercial process were comparable with one another and to the reference product, Neupogen. In addition, there were similar receptor binding data presented in the non-clinical section of the dossier for the process II and III products,

further supporting the comparability of the two products. Accordingly, given the positive PK/PD data from study GCSF-SUIN-05SB01-3FA suggesting similarity of Grastofil (process III product) to Neupogen and supportive data from the Quality and non-clinical comparability exercises, comparability of process II and process III products could be accepted.

As regards any uncertainty in the parameters used to determine bioequivalence, data for the parameters AUC_{0-24}, AUC_{0-inf}, C_{MAX} for all 3 PK/PD studies showed the CIs for ratios to be contained within 80-125% limits regardless of analysis population (PP, ITT, sensitivity). Any concerns therefore that in study KWI-300-101 AUC_{0-32} rather than AUC_{0-inf} was primarily used for the comparability exercise could be rested as there was shown to be <1% difference between the two AUC parameters, with confidence intervals for the ratios of both falling within the standard acceptance limits.

The $T_{1/2}$ and clearance summary statistics did not suggest any important differences between the test and reference products. There were some gaps however in the PK data presentation. Most noticeable was the lack of comparison of $C_{MAX,}$ $AUC_{0-\infty,}$ and AUC_{0-72} parameters for the 75µg dose in study KWI-300-102. It was clarified by the Applicant that PK sampling for patients administered this dose was not undertaken in the study. This was noted in the study protocol. Time dependency was evaluated with the repeat dose study KWI-300-103, where similarity for the main PK parameters was demonstrated at only one dose level on study Days 1 and 4 and from 1 through 4.

Given that the conditions of PK similarity have been met, in line with the Guideline on the Investigation of Bioequivalence, the Applicant's justifications of the small but statistically significant differences in PK measures in studies KWI-300-101 and GCSF-SUIN-05SB01-3FA and assertions that these are unlikely to result in significant differences in PD or clinical effect between Grastofil and Neupogen were considered acceptable by the CHMP.

ANC C_{MAX} was selected as the primary pharmacodynamic outcome measure for the three initial studies in healthy subjects. For study GCSF-SUIN-05SB01-3FA AUC_t was selected. In the CHMP biosimilar guideline for recombinant G-CSF products evaluation of comparability of ANC is mandated. However, a specific parameter has not been recommended. Whilst the use of ANC C_{max} as the parameter of choice in the comparability exercise can be supported, it was expected that the relevant ANC AUC parameters would also be presented.

In the three initial PK/PD studies, as was seen for the PK outcomes, the estimates for the PD outcomes of the test product usually trailed those of the reference product. For the majority of the presented outcomes the differences were not statistically significant and the confidence intervals of the differences between the means were contained within the 80-125% limits set by the Applicant.

Notably, in study KWI-300-02, where lower Apo-filgrastim doses were administered, significant differences for ANC C_{MAX} and ANC AUC_{0-72} were seen between test and reference cohorts for the 75µg dose. It has previously been noted that PD differences between G-CSF products may be heightened in comparability exercises involving low G-CSF doses.

The data presented by the Applicant seemed to suggest that similarity between test and reference products, with regard to PD outcomes, had been demonstrated. However, the acceptance limits of 80-125% used had not been fully justified and were considered too wide for demonstration of PD similarity within this biosimilar application; and more so for a package where comparative clinical efficacy and safety data from patients were not available. The Applicant was asked to re-present the PD data across the 4 Apo-filgrastim PK/ PD studies using 95% confidence intervals, as is recommended for evaluation of PD data for biosimilar applications, and tighter acceptance limits. +/- 10%, which was acceptable, was discussed by the Applicant and it was agreed that such limits would be stringent enough to ensure PD equivalence. As expected, the 95% confidence intervals of the ratios were slightly wider than the corresponding 90% confidence intervals, with the majority straddling 100% but

comfortably contained within the narrower 90-111% acceptance limits; a fact which strongly suggested similarity of the PD data. However, for ANC Cmax and ANC AUC0-inf (where the effect of whole curve is considered) of the 75µg dose in study KWI-300-102 and for the key PD data of multiple-dose study KWI-300-103 (both 0-24 and 0-96 estimates), the lower bounds of the 95% confidence intervals fell below 90%. Further, the CIs for ANC Cmax and ANC AUC0-inf of the 75µg dose fell entirely below 100% (≈87-97% and ≈87-98%, respectively).

The Applicant argued that the excursions past the lower limit of the acceptance margins were minimal and that in both cases these were likely due to the lack of sufficient study power, as the more stringent limits were applied retrospectively and therefore not taken into consideration when calculating the study sample size. Analyses using coefficient of variation data from the study were provided, showing the larger sample sizes required to provide adequate power for the CIs to be contained within the more stringent acceptance limits. For the study KWI-300-103 results, the variability of response was further compounded by the inter-individual variability associated with the parallel group design (and not associated with a cross-over study design), hence the larger and more frequent excursions past the lower acceptance margin in the multiple-dose study. Again, some data from study KWI-300-103 was provided to support this assertion. However, it was not considered to conclusively explain the apparent lack of PD similarity in the Apo-filgrastim studies, as larger studies, where sample sizes would have been calculated based on narrower PD acceptance limits, have not been conducted (particularly in a repeated-dose setting). As discussed below, these PD data did not automatically preclude the demonstration of PD similarity. Reassuringly, despite the studies not being powered such that 95% confidence intervals of the PD ratios would be contained within narrow limits, for three of the five dosing regimens evaluated this was the case. For the remaining regimens (noted above), the excursions past the lower bound of the narrower acceptance limit were small, as were the mean differences seen between the PD outputs of the test and reference products.

The Applicant argued that the PD differences seen will not have relevant consequences in daily clinical practice. The main justification was that in the KWI-300-104 study the objective endpoint of DNS in breast cancer was in line with the results expected for G-CSF treatments evidenced by data from published literature. However, the data lacked direct objective comparison to the reference product and therefore, for the purposes of this exercise, were of limited value. More persuasive was the discussion of factors which render the small apparent differences in PD between Grastofil and Neupogen irrelevant in clinical practice; for instance, pharmacodynamic sequelae of increased neutrophil counts in the low dosage repeated-dose setting, the practice of dosing to response and the safety of G-CSF over a broad range of doses.

The discussion above, particularly regarding the extent of the confidence interval excursions past the lower bound of the narrower acceptance limit in a minority of dosing regimens and the robustness of the estimates of the PD ratios in the other regimens, supported (in this case) the adoption of a more flexible approach with regard to the recommended width of the acceptance limit for the CIs of PD ratios. It is important to note that comparability of the physicochemical characteristics and functional attributes of the molecules (critical parts of the comparability exercise for filgrastims) had already been evaluated and confirmed in the Quality and Non-clinical sections of the dossier, providing further reassurance that PD differences seen between the test and reference products were unlikely to reflect clinically significant differences between the products.

The Applicant provided data on geometric means of the test and reference products along with the corresponding ratios of the means and confidence intervals for Apo-Filgrastim, Neupogen and Filgrastim Hexal, as was requested in order to justify that the 5 µg/Kg dose sits on the most sensitive part of the dose-response curve. In addition, a revised dose-response plot was provided for the three products.

Regarding the plot, the rationales for plotting ANC AUC0-24 (AUEC0-24) rather than 0-72, and for omitting the single dose IV data were provided. Whilst the choice of PD parameter to present had to suffice, due to limitations resulting from the limited amount of data from the development programme, it should be noted that AUEC0-24 data only relates to approximately 50% of the ANC vs time curve in the single-dose model. Therefore, it is unclear whether the shape of the Apo-Ffilgrastim dose response curve would have been noticeably altered if the total ANC response (e.g. AUEC0-inf or 0-72) was plotted and if data from a single subcutaneous dose 5µg/kg study had been available. Somewhat reassuringly, data from the Neupogen arms in the Grastofil dossier and from multiple-dose filgrastim Hexal studies were plotted, with the gradients (and shape) of their dose-response curves approximating those of Apo-filgrastim. Overall, the plots seemed to suggest that the 5 µg/kg dose sits on as steep a part of the dose-response curve as the 1µg/kg dose. Further, there was no clear suggestion that the dose-response plateaued at 5µg/kg. These conclusions were supported by the dose-response plot for Filgrastim-Hexal which mirrored that for Apo-filgrastim and was based on robust data.

The Applicant also outlined analyses from study KWI-300-103 which appeared to demonstrate that the difference observed between Apo-Filgrastim and Neupogen following the administration of a single dose (i.e. AUC_{0-24}) was essentially the same as the difference observed after the administration of multiple doses (i.e. 4 daily doses) of filgrastim (AUC_{0-96}); approximately 3.6% and 4.4% respectively. The same effect was seen for C_{max}. Previous comments regarding suitability of using the $AUEC_{0-24}$ for these analyses notwithstanding, the data from the study seemed to suggest that the $AUEC_{0-24}$ parameter may be at least equally sensitive at detecting differences in PD between the test and reference products as the $AUEC_{0-96}$ parameter from a multiple-dose study. This was supported by a plausible and well-reasoned pharmacodynamic rationale regarding receptor activation, ANC response and receptor mediated uptake of G-CSF within the dossier.

Given the above, it could be concluded that the single-dose studies in healthy volunteers evaluating the 4 µg/Kg and 2 µg/Kg doses were equally sensitive as the 1 µg/Kg single-dose and 5 µg/Kg multiple-dose studies in detecting PD differences between test and reference products. Therefore, the significance of excursions of the PD ratio CIs past narrow limits in the latter studies should not be overstated.

The evaluation of CD34+ response to Apo-filgrastim and Neupogen was inadequate. The Day 5 outcomes in study KWI-300-103 were presented without statistical comparison. In addition, sampling in that study was sparse. Therefore, robust comparison of test and reference for this outcome did not occur. In addition, no further comparative CD34+ data were available from the development programme. It is agreed that the available data for CD34+ from studies KWI-300-103 and KWI-300-104 demonstrated the response to Apo-Filgrastim with regard to CD34+ mobilisation and that the response to Apo-filgrastim and Neupogen appeared close. However, the robust data from statistical evaluation of comparability of response, as would normally be expected for a key secondary PD outcome measure within a biosimilar package, could not be provided. Lack of robust data on CD34+ comparability was considered a significant deficiency in the PD similarity exercise. However, PD similarity is strongly supported by pivotal ANC AUC (AUEC) and ANC C_{max} data from a number of studies (as discussed above). Furthermore, the available CD34+ data, whilst not assessed by formal criteria to determine similarity, were considered to be in keeping with a similarity conclusion based on the ANC endpoints. Therefore, based on current knowledge of G-CSF, biosimilar filgrastims and G-CSF analogue activity at the G-CSF receptor, given that test-to-reference comparability has been determined in quality, non-clinical and clinical comparability exercises, the CHMP did not expect CD34+ response to Grastofil and Neupogen to differ in a clinically significant manner in healthy individuals and patients.

2.4.5. Conclusions on clinical pharmacology

Overall, the differences seen between the PD outputs of the products were quite small. Also the evidence suggested that doses 1-5µg/kg sit on the steep and linear part of the dose response curve and that the single-dose studies were equally sensitive as the multiple-dose study (based on data in the dossier) in detecting PD differences between the test and reference products. Given the above, the supporting points outlined by the Applicant and the overall PD data, it may be concluded that PD similarity has been demonstrated between Grastofil and Neupogen and that the differences seen in the multiple-dose study were unlikely to have consequences in clinical practice.

2.5. Clinical efficacy

2.5.1. Dose response studies

No dose-response studies in the target population were submitted.

2.5.2. Main study

KWI-300-104

A non comparative, multicentre, repeat dose safety in use study of Neukine (Filgrastim) in patients receiving chemotherapy known to induce neutropenia

Methods

Study Participants

Inclusion Criteria

- Patients had to fulfil all of the following criteria for inclusion in the study:
- Female, ≥18 of age, suitable and intended to undergo adjuvant TAC chemotherapy
- Body weight of subject must be within 40 and 120 kg
- Subjects are within 60 days after the complete surgical resection of the primary breast tumour: either lumpectomy or mastectomy with sentinel lymph node biopsy or axillary dissection, with clear margins for both invasive and DCIS
- Subjects with stage IIA, IIB or IIIA breast cancer,
- Subjects must have an ECOG performance status ≤ 2
- Subjects who are chemotherapy naïve
- Subjects must have an ANC ≥1.5 x 10^9/l; platelet count ≥100 x 10^9/l
- Subject must have an adequate renal (serum creatinine <1.5 x upper limit) and hepatic function (bilirubin < upper limit of normal, transaminases <1.5 x upper limit and ALP within 1.5 x ULN)
- Has no evidence of metastatic disease outside of breast by physical examination and chest x-ray.
- Has had baseline bilateral mammography

Exclusion Criteria

- Any of the following was regarded as a criterion for exclusion from the study:

- Has any evidence of metastatic disease following surgical resection of the primary tumour including: positive surgical margins, staging work-up, or physical examination suspicious for malignant disease
- Has bilateral breast cancer (concomitant or prior)
- Has had neoadjuvant chemotherapy for this breast cancer
- Has ever had a myocardial infarction or has a history of heart failure, uncontrolled angina, severe uncontrolled arrhythmias, pericardial disease, or electrocardiographic evidence of acute ischemic changes,
- Is receiving concurrent immunotherapy, hormonal therapy (e.g. tamoxifen, gonadal hormone replacement therapy, Herceptin (trastuzumab)), or radiation therapy
- Is receiving concurrent investigational therapy or has received such therapy within the past 30 calendar days,
- Has peripheral neuropathy >Grade 1
- Has a serious uncontrolled intercurrent medical or psychiatric illness, including serious viral (including clinically defined AIDS), bacterial or fungal infection; or history of uncontrolled seizures, or diabetes, or CNS disorders deemed by the investigator to be clinically significant, precluding informed consent
- Is receiving antibiotic treatment 3 days within chemotherapy administration.

Treatments

Repeated doses of Neukine (Apo-Filgrastim) 5 µg/kg/day rounded by the nearest prefilled syringe size were administered subcutaneously (s.c.) as daily injection. Correspondingly, study patients received 300 µg (if body weight 40-75 kg) or 480 µg (if body weight 76-120 kg) Neukine daily.

Treatment with Neukine began on day 2 of every chemotherapy cycle (at least 24 hours after chemotherapy) and was continued up to 14 days or until post-nadir ANC recovery to normal or near-normal values by laboratory standards, whichever occurred first.

After day 10, blood sampling may have been continued daily until a documented ANC of up to 10.0 x 10^9/L after the expected nadir or for up to a maximum of 14 days, whichever occurred first if clinically indicated. Neukine was administered daily for a maximum up to study cycle Day 15, but must have been stopped if patients had an ANC > 10 x 10^9/L.

Dose Reduction or Alteration

Chemotherapy dose reduction by 25% was permitted, as per the Protocol, if subjects experienced grade 3/4 non hematopoietic toxicities, two grade 3/4 infectious episodes, or grade 4 thrombocytopenia.

Discontinuation of treatment was to be considered for patients in whom non-hematologic grade 4 toxic effects developed or persisted according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC), or grade 3 toxic effects occurred despite a dose reduction, or a clinically significant cardiac event developed.

Premedication for Chemotherapy

Dexamethasone (six doses of 8 mg p.o. BID, starting the day before chemotherapy and ending the evening of the day after chemotherapy) were administered in order to prevent docetaxel-related

hypersensitivity and fluid retention. Ondansetron was administered according to the manufacturer's prescribing information.

Concomitant Therapy

Chemotherapy

- 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, every 3 weeks for six cycles.

Dose of antineoplastic agents was calculated according to Mosteller equation in the Protocol: body surface area (BSA) (m^2) = [Ht(cm) * Wt(kg) / 3600]^{½} Upon consultation with the study medical monitor, investigators were allowed to deviate from the equation limiting the dose to the equivalent of 2 m^2, if this was local site practice. Any intent of dose reduction or deviation from the administration schedule was reported to Medical Monitor before the implementation of deviation.

Other Concomitant Treatment

Any treatment considered necessary for the patient's welfare could be given at the discretion of the investigator.

Primary prophylactic antibiotic therapy was not allowed in line with the recommendations of NCCN, Practice Guidelines in Oncology, Myeloid growth factors, V.1.2008. Secondary antibiotic prophylaxis was allowed upon development of episode of FN and implemented in accordance with the recommendations of NCCN Clinical Practice Guidelines in Oncology: NCCN Clinical Practice Guidelines in Oncology, V.I. 2008.

If the administration of a non-permitted concomitant medication became necessary, participation to the study was discontinued prematurely in this patient. Use of other concurrent hematopoietic growth factors was not allowed.

Objectives

Primary objective:

- To evaluate the safety of Neukine (Apo-Filgrastim) used for the reduction in duration of neutropenia in breast cancer patients undergoing chemotherapy

Outcomes/endpoints

Primary efficacy endpoint

- Duration of severe neutropenia in cycle 1. Severe neutropenia was defined as occurrence of ANC below 0.5 x 10^9/L).

Primary safety endpoint

- Subject incidence of adverse events (AEs) (all severe and serious) classified by body system, preferred term (PT), frequency, and relationship to investigational product. Vital signs, the presence of antibodies and clinical laboratory results were also monitored.

Secondary endpoints

- The duration of severe neutropenia in consecutive cycles (2 through 6);
- The frequency of grade 3 and 4 severe neutropenia (ANC below 1.0 x 10^9/l and 0.5 x 10^9/l);

- The depth of ANC nadir in cycle 1;
- The time to the post nadir ANC recovery (ANC >1,5 X 10^9) in cycle 1;
- The rates of febrile neutropenia (FN) by cycle and across the cycles;
- The definition of FN used for the purpose of the Protocol was an observed or imputed ANC
- <0.5 x 10^9/L and concurrent oral equivalent temperature ≥38.2^{0}C;
- The ANC-time profile in cycle 1 (Time from the beginning of chemotherapy to the
- occurrence of ANC nadir);
- The frequency of a nadir of less than 0.5 x 10^9/l and less than 1.0 x 10^9/l ANC;
- The frequency of (culture-confirmed) infections;
- The incidence of i.v. antibiotic therapy and hospitalization;
- The mobilization of CD34+ cells (in selected sites only).

Sample size

A cohort of 100 eligible patients was expected to provide 95% certainty of detecting one report of a specific AE when there is a 3-4% probability that it occurs. As there was no active comparator in this study, Neukine's spectrum of AEs was compared to the events historically documented for Neupogen in its Summary of Product Characteristics. One hundred patients was considered adequate to detect whether these common effects occur to a similar extent and to detect any other AEs occurring with a frequency of more than 3%. Based on previous publications (Green et al. 2003) dropout rate of less than 20% was expected.

Randomisation

Not applicable

Blinding (masking)

The study was open-label.

Statistical methods

Analysis of Efficacy

The efficacy analysis was purely descriptive and exploratory and based upon descriptive summary statistics. The efficacy analysis was performed for FAS and PP subsets.

The main efficacy endpoint was the duration of severe neutropenia (defined as occurrence of ANC below 0.5 x 10^9/L) in cycle 1. Duration was presented by means of summary statistics. Frequency table was created to summarise the incidences of severe neutropenia by day of onset, and the number of patients experiencing severe neutropenia for 0, 1, 2, 3, 4, 5 etc days.

Analysis of Safety

Extent of Exposure

The number of days until ANC recovery is calculated from the day of TAC administration (Day 1) till date of ANC recovery which is determined according to decision of investigator.

Adverse Events

All AEs (as well as medical history terms) were coded centrally using MedDRA Version 10.0. Summaries and analysis were based on the treatment-emergent AEs (referred to AEs in this document), which are defined as AEs occurring on or after the day of the first study drug administration, or AE present before this day and ongoing after administration with increased severity.

Clinical Laboratory Evaluation

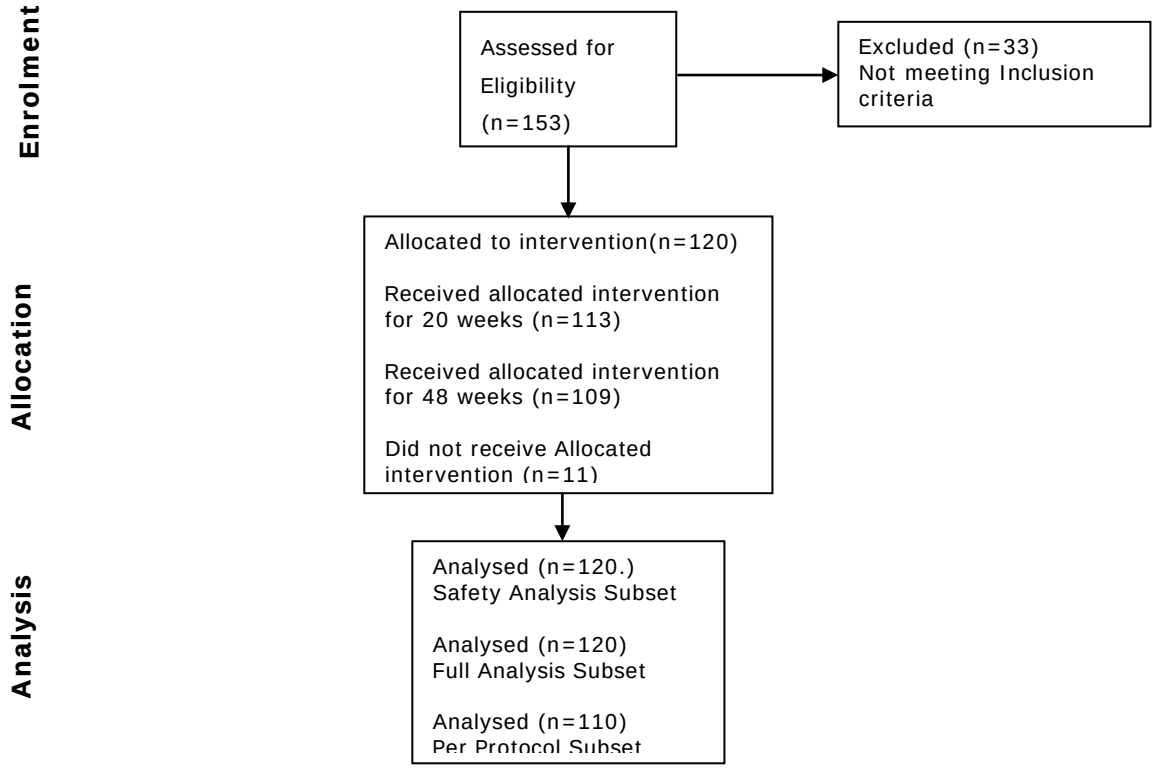
The number of patients who had values outside normal values and by listing values considered clinically relevant by the investigator were presented.

Immunogenicity

Any patient who did not have antibodies at screening, but showed at least one positive sample during the study (which is not a singular positive sample followed by negative samples) was presented as "positive" in data tables. This definition applies to both binding and neutralising.

Results

Participant flow



Overall, 113 patients (94.17%) completed the treatment period and 109 (90.83%) completed the Safety Follow-up period. 11 patients (9.17%) prematurely discontinued the study. The most common reason for study discontinuation was patient's withdrawal of consent and Sponsor's decision. 5 patients (4.16%) discontinued due to withdrawal of consent. Three patients (2.50%) were discontinued by the Sponsor due to serious protocol deviation. Three patients (2.50%) discontinued due to AEs: The first 2 events were reported as SAEs and led to fatal outcomes due to metastases and disease progression. The third was due to a non-serious AE of duodenal ulcer. None of these events were considered related to the study drug

Recruitment

The study (period from 19 September 2009 to 12 May 2010 (date of data cut-off)) was conducted at 29 study sites in Austria, Bulgaria, Byelorussia, Hungary, Macedonia, Poland, Romania, Russia, Serbia and Ukraine.

Conduct of the study

There was one amendment of the study protocol (data not shown).

Baseline data

One hundred and twenty female Caucasian patients were enrolled.

Table 46: Baseline History

	(n=120)
Age, years, mean (SD)	49.97 (9.52)
Prior chemotherapy, n (%)	0(0)
Prior radiotherapy, n (%)	22 (18.33)
Disease stage at entry	
Stage IIa	39 (32.50%)
Stage IIb	44 (36.67%)
Stage IIIa	37 (30.83%)

Table 47: Body Weight, Height, Calculated BMI and BSA at Screening

Visit		Body weight [kg]	Body height [cm]	Calculated BMI [kg/m^2]	BSA [m^2]
SCREENING	N	120	120	120	120
	Mean	71.08	163.37	26.66	1.79
	StdDev	13.46	6.31	4.98	0.18
	Min	46.00	147.00	17.43	1.42
	Median	71.00	164.00	27.01	1.80
	Max	119.80	178.00	40.90	2.40

Numbers analysed

Subsets Analysed

Safety analysis subset (SAS)- all enrolled patients who have received at least one dose of active treatment (n=120).

Full analysis subset (FAS)- all enrolled patients who have received at least one dose of active treatment and who provide any follow-up data for the primary target variables (n=120).

Per protocol subset (PP) - included patients without major protocol deviations or premature termination of the treatment due to reasons that were definitely not related to study medication (n=110).

Outcomes and estimation

Main Efficacy Variable

Table 48: Duration and Incidence of Severe Neutropenia in Cycle 1

	KWI-300-104 (n=120)
Duration of severe neutropenia in cycle 1 mean (SD) days	1.40 (1.07)
Incidence of severe neutropenia in cycle 1 Percentage of total	93 (77.50)

In the PP analysis subset, the duration was even lower with mean (SD) duration of 1.27 (0.95) days. Shorter duration in the PP subset is due to removal of patients with deviations from the study drug administration regimen who were excluded from the FAS.

Subgroup analysis indicated that there is a statistically significant positive correlation between the duration of severe neutropenia and depth of nadir in cycle 1 and earlier day of onset of severe neutropenia. Patients with Neukine dose of 4.5 – 5.2 µg/kg/day had a statistically significant shorter duration of severe neutropenia then patients with either higher or lower Neukine dose. On the other hand there was no correlation with patient age, weight, pre-filled syringe size or dose of chemotherapy.

Secondary Efficacy Variables

Duration of Severe Neutropenia in Consecutive Cycles (2 - 6)

Blood sampling in cycles 2-6 was performed at cycle day 9. As severe neutropenia most often occurred on cycle day 7, the neutropenia had most probably recovered in the majority of patients by the time of blood sampling at day 9. Indeed, severe neutropenia was detected in only 4 out of 114 (3.51%), 8 out of 114 (7.02%), 5 out of 114 (4.38%), 9 out of 113 (7.96%) and 12 out of 113 (10.62%) patients in cycles 2, 3, 4, 5 and 6 respectively.

Table 49: Duration of Severe Neutropenia in Cycles 2-6

Cycle	Duration of severe neutropenia [days]	n	(%)
2	n	114	100.00
	0	110	96.49
	1	4	3.51
	2		0.00
	3		0.00
3	n	114	100.00
	0	106	92.98
	1	8	7.02
	2		0.00
	3		0.00
4	n	114	100.00
	0	109	95.61
	1	3	2.63
	2	2	1.75
	3		0.00
5	n	113	100.00
	0	104	92.04
	1	8	7.08
	2		0.00
	3	1	0.88
6	n	113	100.00
	0	101	89.38
	1	10	8.85
	2	2	1.77
	3		0.00

Frequency of Grade 3 and 4 Severe Neutropenia (ANC below $1.0x10^9$/l and $0.5x10^9$/l)

Grade 3 neutropenia occurred in 106 (88.33%), while severe (grade 4) neutropenia occurred in 93 (77.50%) patients in cycle 1. Frequency was apparently lower in subsequent cycles, but this is at least partly due to lower frequency of blood sampling and the scheduled sampling time point on day 9 of the chemotherapy cycles.

Table 50: Frequency of Grade 3 and 4 Neutropenia

Cycle	Statistics	n	(%)
1	n	120	100.00
	Grade 3 Neutropenia	106	88.33
	Grade 4 Neutropenia	93	77.50
2	n	114	100.00
	Grade 3 Neutropenia	12	10.53
	Grade 4 Neutropenia	4	3.51
3	n	114	100.00
	Grade 3 Neutropenia	16	14.04
	Grade 4 Neutropenia	8	7.02
4	n	114	100.00
	Grade 3 Neutropenia	13	11.40
	Grade 4 Neutropenia	5	4.39
5	n	113	100.00
	Grade 3 Neutropenia	20	17.70
	Grade 4 Neutropenia	9	7.96
6	n	113	100.00
	Grade 3 Neutropenia	22	19.47
	Grade 4 Neutropenia	12	10.62

The Depth of ANC Nadir in Cycle 1

Mean ANC nadir of 0.37 x 10^9/l was recorded on mean (SD) day 7.20 (0.64).

Table 51: Peak, Depth of Nadir and Recovery of ANC in Cycle 1

	Day on which the peak ANC value was reached*	Peak ANC value [x10^9/L]	Day on which the depth of ANC nadir was reached*	Depth of ANC nadir [x10^9/L]	Day on which recovery of ANC was reached*,**
N	120	120	120	120	117
NMiss	0	0	0	0	3
Mean	2.98	22.37	7.20	0.37	9.11
StdDev	0.47	7.32	0.64	0.51	1.32
StdErr	0.04	0.67	0.06	0.05	0.12
Min	0.00	4.19	5.00	0.00	6.00
Median	3.00	22.36	7.00	0.20	9.00
Max	5.00	41.80	9.00	3.43	18.00

* Day relative to TAC administration, ** Recovery defined as post nadir ANC value >1.5 x10^9/L

Time to Post-nadir ANC Recovery (ANC >1.5 X 10^9) in Cycle 1

Time to ANC Recovery was defined as the number of days until post nadir ANC value > 1.5 x 10^9/l, relative to chemotherapy administration. Recovery occurred after a median of 9 and mean (SD) 9.11 (1.32) days.

Rates of Febrile Neutropenia by Cycle and Across the Cycles

Three study patients experienced FN, all 3 cases being in cycle 1. The rate of FN was 2.5 % in cycle 1 and 0% for all other cycles. Notably, patient 1 was removed from the PP subset due to major protocol deviation of discontinuation of Neukine administration pre-nadir and dosing with marketed formulation of filgrastim. This patient received only 2 doses of Neukine before developing FN. 1 Patient received 10 doses while another received 8 doses of Neukine in cycle 1.

The ANC-time Profile in Cycle 1

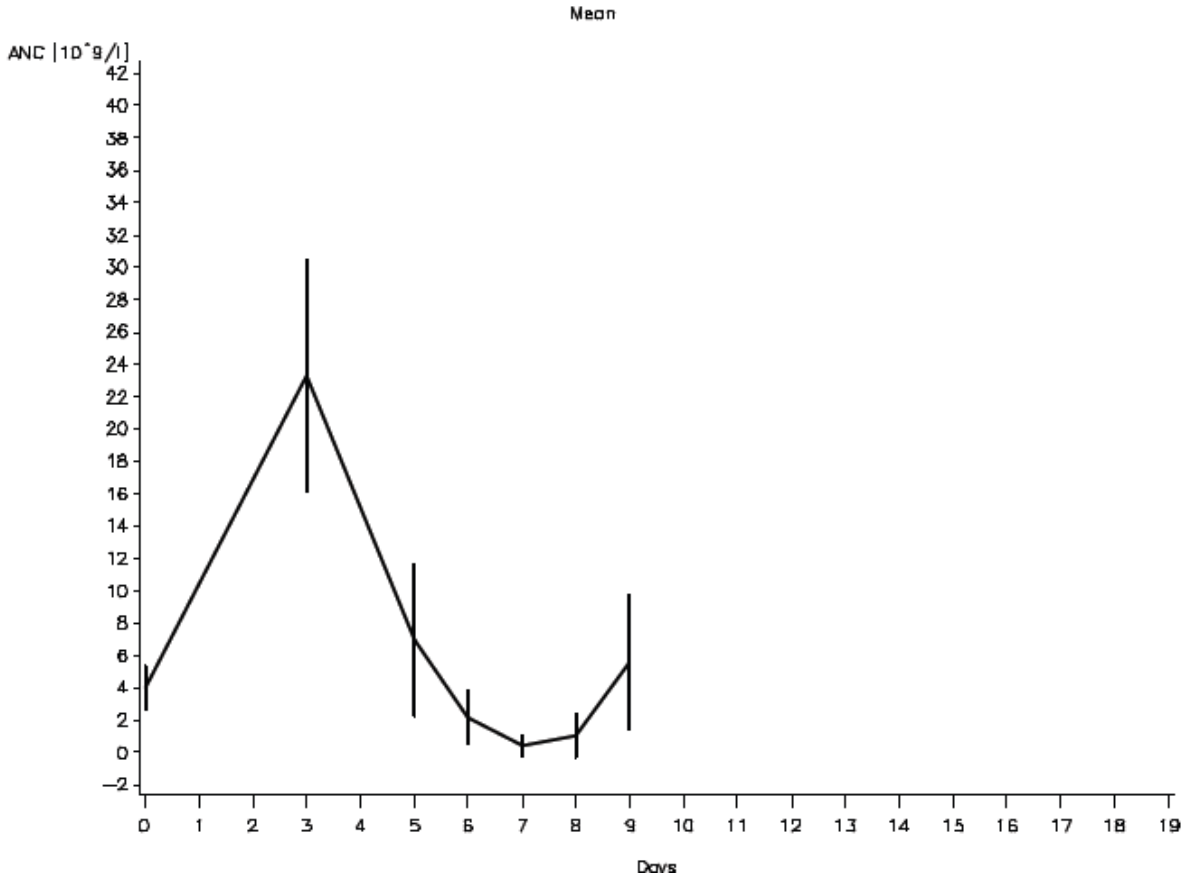
Severe neutropenia occurred most frequently on day 7 of cycle 1, with the day of onset ranging from day 5 to day 9.

Table 52: Incidence of Severe Neutropenia in Cycle 1 by the Day of Onset

Statistics		n	(%)
All patients		120	100.00
Patients experiencing severe neutropenia		93	77.50
Day of Onset	5	3	2.50
	6	11	9.17
	7	71	59.17
	8	7	5.83
	9	1	0.83
Duration of severe neutropenia	0	27	22.50
	1	39	32.50
	2	38	31.67
	3	12	10.00
	4	3	2.50
	5	1	0.83

Absolute neutrophil count peaked at day 3 with a mean (SD) count of 22.73 (7.18) and maximum of 41.80 x 10^9/l.

Figure 13: Absolute Neutrophil Count Time Profile in Cycle 1 (Mean +/- ISD)



Other secondary efficacy variables

Frequency of (Culture-confirmed) Infections

One patient had culture confirmed infections in cycle 1. Three concomitantly occurring infections were culture confirmed: cough, stomatitis and rhinitis. In another patient with FN before initiation of i.v. antibiotics a blood culture result was positive for coagulase-negative Staphylococcus spp. This was taken as sign of contamination and not as bacteraemia, given the general health status of the patient (feeling well, febrile, without signs/symptoms of infection).

Intravenous Antibiotic Therapy and Hospitalisation

Intravenous antibiotics were used in 4 (3.33%) of patients. Febrile neutropenia was the indication for 2 cases. All patients were hospitalized and events reported as SAEs.

Table 53: Intravenous Antibiotic Therapy

ATC4 Term	Drug PT	Subject	Dose	Unit	Frequency	Start date	Stop date	Indication
Penicillins with extended spectrum, J01CA	Piperacillin w/tazobactam	0617	4.5	G	TID	25MAY 2009	29MAY 2009	Empirical therapy of febrile neutropenia
Comb of penicillins, incl. Beta-lactamase inhib., J01CR	Amoxicillin w/clavulanate potassium	1501	1.2	G	TID	23NOV 2008	28NOV 2008	Febrile neutropenia
Second-generation cephalosporin, J01DC	Cefuroxime	1502	750	Mg	BID	27NOV 2008	01DEC 2008	Leukopenia neutropenia
Third-generation cephalosporin, J01DD	Ceftriaxone	1204	2	G	OD	13JAN 2009	17JAN 2009	Subfebrility

Hospitalisation during treatment and follow-up periods was necessary in 8 cases for 7 (5.83%) of patients.

Mobilisation of CD34+ Cells

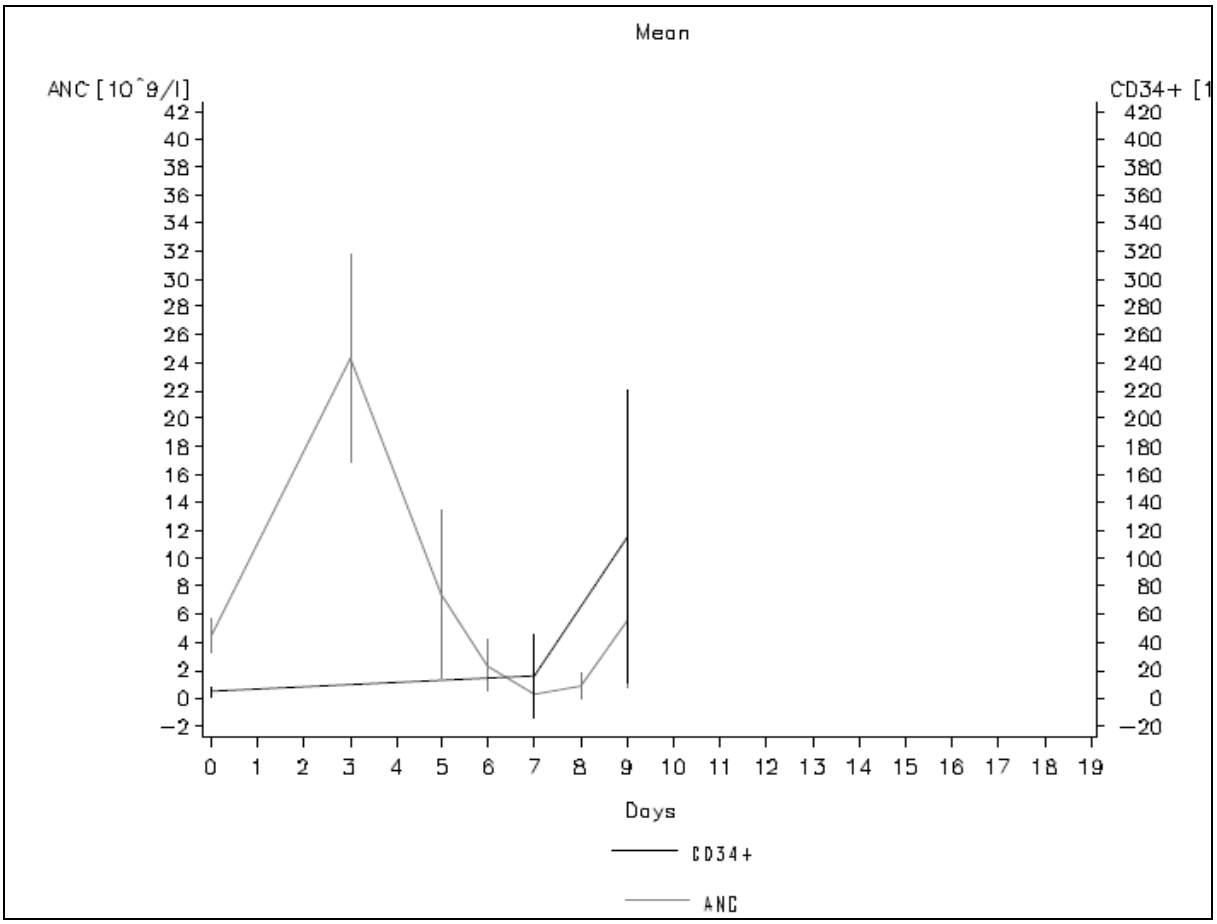
CD34+ cell counts were performed at selected sites and for a total of 39 randomly selected patients.

Three samples were taken from patients in cycle 1. Broad interindividual variation in the capacity of patients to mobilize progenitor cells was noted, a finding that is in line with literature reports.

Table 54: CD34 + [10 6/l] Cell Count

CYCLE 1 DAY 0	N	39
	NMiss	81
	Mean	4.57
	StdDev	3.33
	Min	0.84
	Median	3.00
	Max	14.00
CYCLE 1 DAY 7	N	36
	NMiss	83
	Mean	14.49
	StdDev	28.82
	Min	0.48
	Median	3.00
	Max	127.45
CYCLE 1 DAY 9	N	34
	NMiss	85
	Mean	110.67
	StdDev	101.18
	Min	7.00
	Median	63.00
	Max	375.74

Figure 14: Mean CD34+ Compared with Mean ANC values Population: Full Analysis Subset



Eastern Cooperative Oncology Group (ECOG) Status

At screening, 100 patients had ECOG score 0, 19 patients had score 1 and 1 patient had score 2. Overall improvement was noted by the end of the study (week 48) with the number of patients at score 0 increasing to 106, the number of patients at score 1 decreasing to 3 and no patients having score above 1. It is noted that this does not account for patient who dropped out of the study for whom ECOG status was not assessed.

Transfusions Used to Treat Thrombocytopenia and Anaemia

Transfusion of erythrocytes and blood were required in one patient on 3 occasions. This patient started the study with decreased erythrocyte count of 3.53x 10^{12}/l. She received the first transfusion while in chemotherapy Cycles 3, 5 and 6. Severe anaemia, not related to Neukine was recorded as the indication for the transfusions. No transfusions of thrombocytes were required.

Chemotherapy Dose Delivered

Chemotherapy dose reduction was required in 4 (3.33%) patients, so that 13 out of 688 (1.88%) chemotherapy cycles were delivered at a 25% reduced dose. Two of the patients had dose reduced due to chemotherapy related fatigue while the others due to low ANC, which in one case resulted in FN.

Chemotherapy Cycle Delay

Dose delay was considered as any dose which was more than 23 days after the previous. Sixteen out of 688 (2.3%) cycles were delayed, mostly due to technical and patient personal reasons. The only AEs which may be related to chemotherapy and insufficient ANC recovery are the skin inflammation in one patient and low ANC in another.

Comparison to the Reference Product

The prescribing information (PI) for the reference product Neupogen states that in the phase 3, randomised, double-blind, placebo-controlled study conducted in patients with small cell lung cancer (n = 99) patients were randomised to receive NEUPOGEN (n = 99) starting on day 4, after receiving standard dose chemotherapy with cyclophosphamide, doxorubicin, and etoposide. For patients receiving Neupogen the incidence of at least one infection over all cycles of chemotherapy was 40% (40/99), the incidence of hospitalization was 52% (51/99), the incidence of i.v antibiotic usage in cycle 1 was 38% (38/99) and the incidence of severe neutropenia in cycle 1 was 84% (83/99). Over all cycles, patients had a 57% (286/500 cycles) rate of severe neutropenia. The median duration of severe neutropenia in cycle 1 was 2 days (range 0 to 9 days) and the mean duration of neutropenia in cycle 1 was 2.44 +/- 1.90 days. Over all cycles, the median duration of neutropenia was 1 day. The median severity of neutropenia (as measured by ANC nadir) was 72/mm^3 (range 0/mm^3 to 7912/mm^3) in cycle 1. The mean severity of neutropenia in cycle 1 was 496/mm3 +/- 1382/mm^3. Over all cycles, the ANC nadir was 403/mm^3.

In a randomised, double-blind, placebo-controlled, multi-centre, phase 3 clinical study, 521 patients (median age 54, range 16 to 89 years) were treated for de novo acute myeloid leukaemia. Following a standard induction chemotherapy regimen comprising daunorubicin, cytosine arabinoside, and etoposide15 (DAV 3+7+5), patients received either Neupogen at 5 µg/kg/day or placebo, s.c., from 24 hours after the last dose of chemotherapy until neutrophil recovery (ANC 1000/mm^3 for 3 consecutive days or 10,000/mm^3 for 1 day) or for a maximum of 35 days. In the Neupogen-treated group, the median time from initiation of chemotherapy to ANC recovery (ANC ≥ 500/mm^3) was 20 days, the median duration of fever was reduced by 1.5 days (p = 0.009), and there were statistically significant reductions in the durations of i.v. antibiotic use and hospitalisation. During consolidation therapy (DAV 2+5+5), patients treated with Neupogen also experienced significant reductions in the incidence of severe neutropenia, time to neutrophil recovery, the incidence and duration of fever, and the durations of i.v. antibiotic use and hospitalization. Patients treated with a further course of standard (DAV 2+5+5) or high-dose cytosine arabinoside consolidation also experienced significant reductions in the duration of neutropenia.

The efficacy endpoint outcomes are markedly better than in the studies described in the Neupogen Prescribing information (PI). However, the differences in indications and chemotherapy regimens do not allow meaningful comparison.

Study by Nabholz et al reports on metastatic breast cancer patients receiving TAC chemotherapy regimen, but without prophylactic G-CSF. All patients in that study experienced grade 3 or 4 neutropenia. Neutropenia was observed in 98% of cycles and grade 3 or 4 neutropenia was observed in 95% of chemotherapy cycle.

Studies by Green et al and Holmes et al evaluating Neupogen versus pegfilgrastim were selected as the reference studies due to the similarity in study treatments and endpoints. However, they enrolled patients who were overall older, with a more advanced disease but who received a chemotherapy regimen which is not as myelotoxic. KWI-300-104 study chemotherapy regimen additionally included cyclophosphamide - a chemotherapy component with high myelotoxicity score. Strong predictors of severe/febrile neutropenia include advanced age, performance status, myelosuppressive chemotherapy regimen, early low blood counts, the depth of the absolute neutrophil count (ANC) nadir, and a precipitous, early drop in blood counts of all hematopoietic cell types. Aggressive chemotherapy regimen has been identified as the major predictor of FN [odds ratio 5.2 (3.2–8.4)].

Efficacy outcomes are very similar to the reference studies by Green et a[l7] and Holmes et al. These studies were performed in the same indication and with similar filgrastim dosing regimen, albeit

somewhat different chemotherapy. However, baseline characteristics and differences in Protocol procedures do not allow direct comparison.

Subgroup Analysis

For all interactions, group means of duration of severe neutropenia (cycle 1) were computed and presented.

According to the inferential analysis, there was no statistically significant difference among duration of severe neutropenia in the different age groups, no significant impact of weight on the duration of severe neutropenia (p=0.19) and TAC dose/BSA and absolute Neukine dose have no statistically significant impact on duration of severe neutropenia. However, depth of nadir, dose per body weight and onset day has statistically significant effect on the DSN, as expected.

Ancillary analyses

No ancillary analyses were submitted

Summary of main study

The following tables summarise the efficacy results from the main study 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 55. Summary of Efficacy for trial KWI-300-104

Title: A non comparative, multicenter, repeat dose safety in use study of Neukine (Filgrastim) in patients receiving chemotherapy known to induce neutropenia			
Study identifier	2007-005034-36		
Design	Phase III, open-label, non-comparative, multicenter, repeat dose safety study		
	Duration of screening phase: Duration of active treatment phase: Duration of follow-up phase:		Up to 21 days 18 weeks,6 cycles, each cycle 3 weeks apart 30 weeks
Hypothesis	Safety		
Treatments groups	Neukine (Apo-Filgrastim)		5 micrograms/kg/day, s.c. injection, 120 patients)
Endpoints and definitions	Primary Safety Endpoint	No label	incidence of adverse events (AEs) (all severe and serious) classified by body system, preferred term (PT), frequency, and relationship to investigational product
	Primary Efficacy Enpoint	No label	duration of severe neutropenia in cycle 1. Severe neutropenia is defined as occurrence of ANC below 0.5 x 109/L).
Database lock	12 May 2012		
Results and Analysis			
Analysis description	**Primary Analysis**		

Analysis population and time point description	Full analysis subset (FAS) (n=120)	
Descriptive statistics and estimate variability	Treatment group	Neukine
	Number of subject	120
	Mean duration of severe neutropenia in cycle 1	1.40 days
	SD	1.70 days
	Incidence of severe neutropenia in cycle 1	93 patients
	% total of patients	77.50%
	Treatment Emergent AEs	1216 events
	% not related to treatment	79.28%
Notes	The most frequently reported AE was bone pain, which was reported 267 times by 80 (66.67%) patients. It was most frequently described as mild in 105 (39.33%), moderate in 99 (37.08%) and severe in 63 (23.60%) cases. Nine patients reported 10 serious adverse events (SAEs). All 10 SAEs (described by 14 event terms) reported during the treatment and follow-up periods were considered unrelated to Neukine. Two patients (1.67%) died during the follow-up period due to metastasis and disease progression. There were 99 severe AEs, 42 of which were considered not related, 5 possibly, 30 probably and 22 definitely related to Neukine. Among the 57 severe AEs considered possibly, probably and definitely related, there were 56 bone pain reports and 1 ISR. In addition, there was one life-threatening AE (not related).	
Analysis description	**Secondary efficacy endpoints**	
	FN occurred in 3 (2.5%) patients in cycle 1 and did not occur in subsequent cycles. The mean ANC nadir of 0.37 x 109/l was recorded on mean (SD) day 7.20 (0.64). Post-nadir ANC >1.5 x 109/l, relative to chemotherapy administration, occurred after a median of 9 and mean (SD) 9.11 (1.32) days. Hospitalization during treatment and follow-up periods was necessary for 7 (5.83%), while i.v. antibiotic therapy was administered to 4 (3.33%) patients. Mobilization of CD34+ cells was demonstrated by rise to mean (SD) 110.67(101.18) x 106/l at cycle 1 day 9, over 4.57 (3.33) x 106/l at cycle 1 day 1. Duration of neutropenia in consecutive cycles (2-6) could not be measured reliably due to lower frequency of blood sampling.	

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

Table 56: Studies KWI-300-101, KWI-300-102, KWI-300-103 and GCSF-SUIN-05SB01-3FA. ANC Cmax following intravenous or subcutaneous single or repeat dose administration of Apo-Filgrastim or Neupogen to Healthy Male and Female volunteers.

Study	ANC C_{max} [G/L]		Ratio of Geometric Means [%]	90% CI [%]	95% CI [%]	Pr > [t]
	Apo-Filgrastim	Neupogen				
KWI-300-101 5µg/kg b.w. i.v.	19.02	19.28	99.5	93.6-105.8	92.5 -107.1	0.8972
KWI-300-102 150µg s.c.	19.04	19.59	96.3	91.9-101.0	91.0-101.9	0.1874
KWI-300-102 75µg s.c.	17.13	18.60	92.0	87.9-96.2	87.1 – 97.1	0.0035
KWI-300-103 5µg/kg b.w. s.c. Day 1	21.04	21.96	96.2	88.9-104.0	87.6-105.6	0.4081
KWI-300-103 5µg/kg b.w. s.c. Day 4	30.54	32.27	95.2	87.3-103.9	85.82 – 105.64	0.3493
GCSF-SUIN-05SB01-3FA 300 µg s.c .	20.68	19.92	103	99 -108	98 - 109	0.1793

Table 57: Studies KWI-300-101, KWI-300-102 and KWI-300-103. ANC AUC following intravenous or subcutaneous single or repeat dose administration of Apo-Filgrastim or Neupogen to Healthy Male and Female volunteers

Study	PD Parameter	ANC AUC [min*G/L]		Ratio of Geometric Means [%]	90% CI [%]	95% CI [%]	Pr > [t]
		Apo-Filgrastim	EU-Neupogen				
KWI-300-101 5µg/kg b.w. i.v.	**ANC AUC_{0-72}**	46137.4	46601.5	99.1	95.5 – 102.9	94.8 - 103.7	0.6939
KWI-300-102 150 µg s.c.	**ANC AUC_{0-72}**	43209.3	43979.6	97.7	93.8-101.8	93.0-102.6	0.3240
KWI-300-102 75 µg s.c.	**ANC AUC_{0-72}**	35076.8	37009.8	94.9	91.7-98.3	91.1-99.0	0.0162
KWI-300-103 5µg/kg b.w. s.c. Day 1	**ANC AUC_{0-24}**	22974.9	23873.8	96.4	89.6-103.7	88.3-105.2	0.4027
GCSF-SUIN-05SB01-3FA 300 µg s.c.	**ANC AUC_{0-96}**	57847.8	57127.8	103	100-106	99 - 107	0.1418

In all head-to-head comparisons made, the 90% and 95% confidence interval for ANC Cmax included the 100% value except after s.c. administration of 75µg Apo-Filgrastim or Neupogen in the study KWI-300-102 where it did not include the 100% value (90% CI 87.9- 96.2%). As the 90% and 95% confidence interval of ANC Cmax determined in the same study after s.c. administration of 150µg Apo-Filgrastim or Neupogen included the 100% value (90% CI: 91.9-101.0, 95% CI: 91.02 – 101.93), the lack of inclusion of 100% for the 75µg dose is regarded as a study-specific observation, not indicating a true difference between the two drug products.

As Apo-Filgrastim and Neupogen show bioequivalence for PK parameters and similar effects on ANC, it can be concluded that Apo-Filgrastim has equivalent efficacy to Neupogen.

Clinical studies in special populations

No clinical studies in special populations were submitted.

Supportive studies

No additional studies in the target population were submitted.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The Applicant performed a single-arm phase III study with the primary objective of evaluating the safety profile of Apo-filgrastim. Pharmacodynamic and efficacy endpoints were also evaluated. The Applicant compared the outcome data from this study to data from the literature – specifically to outcomes seen in similar cohorts administered G-CSF products or cohorts for which Grastofil would be indicated.

Primary efficacy endpoint - The main efficacy endpoint was the duration of severe neutropenia (DSN) in cycle 1. The mean (SD) duration of severe neutropenia in cycle 1 was 1.40 (1.07) days. This was seen to be very similar to the filgrastim arms of the reference studies, where the corresponding values were 1.6 (1.1) and 1.8 (1.4) days, respectively. However, the reference studies used a somewhat different chemotherapy regimen which did not include cyclophosphamide.

Secondary efficacy endpoints - Severe neutropenia in cycle 1 occurred in 77.50% of patients and febrile neutropenia occurred in 3 (2.5%) patients in cycle 1 and did not occur in subsequent cycles.

Grastofil is restricted for use in adults only as there is currently no presentation compatible with safe dosing in children (see section 2.2.4).

Efficacy data and additional analyses

The mean ANC nadir of 0.37 x 10^9/L was recorded on day 7.20 (0.64). Post-nadir ANC >1.5 x 10^9/l, relative to chemotherapy administration, occurred after a median of 9 and mean 9.11 days. Hospitalization was necessary for 7 (5.8%), while i.v. antibiotic therapy was administered to only 4 (3.3%) patients. Mobilisation of CD34+ cells was demonstrated to rise to mean of 110.67 x 10^6/L at cycle 1 day 9, from 4.57 (3.33) x 10^6/L at cycle 1 day 1. Duration of neutropenia in consecutive cycles (2-6) was not reliably measured due to lower frequency of blood sampling. Data from repeat-dose studies of marketed G-CSF products in patients have occasionally shown there to be decreases in DSN and incidence of severe neutropenia with subsequent cycles of chemotherapy, especially between cycle 1 and cycle 2. Whilst in study KWI-300-104 decreases were seen between cycle 1 and cycle 2, it was

not clear whether these changes were due solely to reduced blood sampling in cycles 2-6. The clinical study report suggested that blood sampling before Day 9 in cycles 2-6 was not performed. It is unclear in this case how accurate estimation of ANC over time and consequently duration of severe neutropenia in those cycles was assured. This is considered a significant flaw in the study procedures. The comparison of data from these studies to those from the literature highlighted by the Applicant was complicated by differences in baseline disease characteristics and demographics, concomitant chemotherapy, region and clinical practice, such that comparison of these endpoints was not considered to be reliable.

The rate of febrile neutropenia in KWI-300-104 study does not seem to be discordant to that obtained in the other studies. What is remarkable is that 29 patients had a nadir depth $<0.08\ 10^9$/L ANC lasting 2.44 days, which means that a non-negligible number of patients were at very high risk of getting an infectious disease. Also, 77.50% of the patients had severe neutropenia despite all patients being naïve to chemotherapy and therefore having a more responsive bone marrow than patients previously treated with chemotherapy. Moreover, if the four patients who received intravenous antibiotic treatment (only one due to febrile neutropenia) were taken into consideration, the rate of febrile neutropenia would increase only slightly to 5% (6/120), which is still low considering the profound neutropenias experienced by study patients.

The Applicant stated that body temperature was measured by the patients, and that the collection of data may not have been completely objective. Also, the slightly lower dose of doxorubicin could have had an impact on the rate of febrile neutropenia. Regardless of whether these justifications have merit, it should be remembered that the rate of febrile neutropenia is not a pivotal endpoint in the comparison of the G-CSF biosimilar to the reference product, provided that PK and PD biosimilarity is robustly shown. Therefore, undue significance should not be given to these uncertainties.

Other endpoints evaluated include ECOG status, number of transfusions used to treat thrombocytopenia and anaemia, chemotherapy dose delivered, chemotherapy cycle delay and occurrence and/or resolution of chemotherapy-induced mucositis.

2.5.4. Conclusions on the clinical efficacy

Given the issues which arose during the procedure regarding demonstration of pharmacodynamic comparability of Grastofil and Neupogen in the PK/PD studies, a comparative randomised trial in patients would have provided data to adequately support the comparability of the two agents, especially in case sensitive PD endpoints were pursued as well. However, as noted above, in the G-CSF biosimilar clinical comparability exercise, the endpoints of DSN and FN are not considered pivotal and are measures of lesser sensitivity by which clinical comparability may be demonstrated. Overall, the efficacy data from study KWI-300-104 were not considered to provide significant support to the pivotal PD data from the phase I studies.

2.6. Clinical safety

Patient exposure

A tabular summary of the clinical programme that contributed towards safety information is presented below:

Table 58: Summary of Apo-Filgrastim studies in the clinical program

Study Number	Study Design	Study Population	Sample Size	Trial Site
KWI-300-101	Phase I - single-dose, randomized, double-blind, two-way cross-over study - PK and PD evaluation of Apo-Filgrastim and Neupogen (EU)	Healthy Volunteers	N= 36	Vienna, Austria
KWI-300-102	Phase I- single-dose, randomized, double-blind, two-way cross-over study, dose response - PK and PD evaluation of Apo-Filgrastim and Neupogen (EU)	Healthy Volunteers	N=73	Vienna, Austria
KWI-300-103	Phase I- randomized, double-masked, active and placebo-controlled, parallel group study to examine the pharmacodynamic (PD) biosimilarity of repeat dose Apo-Filgrastim and Neupogen (EU)	Healthy Volunteers	N=78	Vienna, Austria
Study GCSF-SUIN-05SB01-3FA;	Phase I, single dose, randomized, double-blind, active-controlled, comparative three-way crossover pharmacokinetic and pharmacodynamic study of Apo-Filgrastim and EU and US Neupogen (Amgen). Apo-Filgrastim product used was from the commercial manufacturing process IX.	Healthy Volunteers	N=48	Toronto, Canada
KWI-300-104	Phase III- a non-comparative, multicentre, repeat dose safety with Apo-Filgrastim to induce neutropenia	Breast cancer patients receiving TAC chemotherapy as routine treatment	N=120	17 centers in Eastern Europe

Cancer patients safety population

A total of 120 women were enrolled into study KWI-300-104. There were six cycles of chemotherapy, each cycle 3 weeks apart for a total of 18 weeks. Treatment with Apo-Filgrastim began on day 2 of every chemotherapy cycle and was continued up to 14 days or until post-nadir recovery of the absolute neutrophil count, whichever occurred first. Apo-Filgrastim was self-administered. Study patients received 300 µg (if body weight was 40-75 kg) or 480 µg (if body weight was 76-120 kg) of Apo-Filgrastim daily for a maximum of six cycles of chemotherapy. 113 patients (94.17%) completed the treatment period and 109 patients (90.83%) completed the safety follow-up period up to week 48. Patient withdrawal is summarised in the following table:

Table 59: Patients withdrawn from study KWI-300-104 (Cancer patients safety population)

Withdrawal from study	N	(%)
Yes	11	9.17
No	109	90.83
Reason for withdrawal		
Withdrawal of consent	5	4.17
Adverse event	3	2.50
Serious protocol deviation	3	2.50
N = number of patients		

Patient disposition is shown in the following table:

Table 60: Patient disposition per chemotherapy cycle and follow-up visit in study KWI-300-104 (Cancer patients safety population)

	N	(%)
Chemotherapy Cycle		
Cycle 1	120	100.0
Cycle 2	114	95.0
Cycle 3	114	95.0
Cycle 4	114	95.0
Cycle 5	113	94.2
Cycle 6	113	94.2
Week 20	113	94.2
Follow-up Visit		
Week 24	113	94.2
Week 36	112	93.3
Week 48	109	90.8
N = number of patients		

The mean (SD) dose of Apo-Filgrastim per cycle was 2880.26 (813.03) µg while the mean (SD) dose per weight was 5.14 (0.73) µg/kg/day. Extent of exposure is summarised in the following table:

Table 61: Extent of Exposure in study KWI-300-104

		Used Syringes	Cumulative dose [ug]	Dose per weight [µg/kg/day]
Average	**N***	688	688	688
	Mean	7.84	2880.26	5.14
	StdDev	1.25	813.03	0.73
	Min	1.00	300.00	3.97
	Median	8.00	2700.00	5.17
	Max	14.00	6720.00	6.67

Healthy volunteers safety population

Studies KWI-300-101 and KWI-300-012 were single-dose studies. Healthy volunteers received a single dose of Apo-Filgrastim and Neupogen on two occasions separated by a washout period. Study KWI-300-103 was a repeat dose study in which healthly volunteers were exposed to a daily administration of the study drug on 4 consecutive days.

In total, 144 subjects were exposed to Apo-Filgrastim and Neupogen (reference medicinal product) and 6 subjects received placebo. Apo-Filgrastim and Neupogen were administered either by (i) the subcutaneous route in fixed doses of 75 µg and 150 µg respectively or by (ii) the intravenous route at 5µg/kg body weight. Overall, 3 subjects did not complete the study: 2 subjects owing to voluntary withdrawal and 1 subject because of pregnancy. Subject withdrawal is summarised in the following table:

Table 62: Subjects withdrawal in studies KWI-300-101, KWI-300-102, KWI-300-103

Withdrawal from Study	Apo-Filgrastim N (%)*	Neupogen N (%)*	Placebo N (%)
Yes	2 (1.07)	1 (0.53)	0 (0.00)
No	142 (75.94)	143 (76.47)	6 (3.21)
Reason			
Voluntary Subject Withdrawal	1 (0.53)	1 (0.53)	0 (0.00)
SAE	0 (0.00)	0 (0.00)	0 (0.00)
Inclusion/Exclusion criteria Violation	0 (0.00)	0 (0.00)	0 (0.00)
Compliance Failure	0 (0.00)	0 (0.00)	0 (0.00)
Other	1 (0.53)	0 (0.00)	0 (0.00)
N = number of subjects			

* percentage are calculated based on the 187 subjects who received at least one dose of study drug and constituted the safety population

Healthy volunteers received a single dose of Apo-Filgrastim and Neupogen on two occasions separated by a washout period in the studies KWI-300-101 and KWI-300-102. In study KWI-300-103, subjects were exposed to a daily administration of the study drug on 4 consecutive days. Mean daily exposure to study drug is displayed in the following table.

Table 63: Mean Extent of Exposure (µg) in studies KWI-300-101, KWI-300-102 and KWI-300-103 (Healthy volunteers safety population)

	KWI 300-101		KWI 300-102				KWI 300-103	
	Apo-Filgrastim 5µg/kg N=35	Neupogen 5µg/kg N=36	Apo-Filgrastim 75 µg N=37	Neupogen 75 µg N=36	Apo-Filgrastim 150 µg N=36	Neupogen 150 µg N=36	Apo-Filgrastim 5µg/kg N=36	Neupogen 5µg/kg N=36
Mean	351.71	349.58	75.00	75.00	150.00	150.00	1356	1381
Min	255.00	255.00	-	-	-	-	980.0	920.0
Max	460.00	450.00	-	-	-	-	1820	1820
For study KWI-300-102, fixed doses were used, i.e. 75µg and 150µg								

The number of subjects and their extent of exposure to study drug is displayed in the following table:

Table 64: Number of Subjects Exposed to Different Daily Doses of Study Drug in Studies KWI-300-101, KWI-300-102 and KWI-300-103 (Healthy volunteers safety population)

Daily Dose Amount	Apo-Filgrastim (N=144) N (%)	Neupogen (N=144) N (%)
<100 µg	37 (25.69)	36 (25.00)
100 µg - <200 µg	36 (25.00)	36 (25.00)
200 µg - 300 µg	16 (11.11)	16 (11.11)
>300 µg	55 (38.19)	56 (38.89)
N = number of subjects		

Adverse events

Cancer patients safety population

In study KWI-300-104, 110 out of 120 breast cancer patients (91.6%) reported 1216 treatment-emergent adverse events. Most treatment-emergent adverse events were assessed as not related to study medication. Results are summarised in the following tables:

Table 65: Overview of treatment emergent adverse events in study KWI-300-104

	N (%)	Event
At least one TEAE	110 (91.6)	1216
Possibly related to Study Drug	70 (58.33)	252
Severe	42 (38.18)	99
Serious	9 (7.50)	10
Withdrawal due to TEAE	3 (2.50)	3
Resulting in Death	2 (1.67)	2
N = number of patients; E = number of Events		

Table 66: Summary of treatment emergent adverse events in study KWI-300-104 according to SOC > 5% (Cancer patients safety population)

		Patients	Events
System Organ Class	**MedDRA Preferred Term**	**N (%)**	**E (%)**
All Patients/Events		120 (100.0)	1216 (100.0)
Ear and labyrinth disorders	Vertigo	11 (9.17)	36 (2.96)
Gastrointestinal disorders	Abdominal pain	6 (5.00)	7 (0.58)
	Abdominal pain upper	7 (5.83)	14 (1.15)
	Diarrhoea	22 (18.33)	36 (2.96)
	Dyspepsia	7 (5.83)	17 (1.40)
	Nausea	64 (53.33)	278 (22.86)
	Vomiting	12 (10.00)	21 (1.73)
General disorders and administration site conditions	Asthenia	6 (5.00)	20 (1.64)
	Fatigue	24 (20.00)	60 (4.93)
	Pyrexia	7 (5.83)	13 (1.07)
Metabolism and nutrition disorders	Anorexia	6 (5.00)	12 (0.99)
Musculoskeletal and connective tissue disorders	Bone pain	80 (66.67)	267 (21.96)
Nervous system disorders	Dizziness	16 (13.33)	59 (4.85)
	Headache	29 (24.17)	84 (6.91)
Respiratory, thoracic and mediastinal disorders	Pharyngolaryngeal pain	9 (7.50)	9 (0.74)
Skin and subcutaneous tissue disorders	Alopecia	36 (30.00)	36 (2.96)
N = number of patients; E = number of Events			

The most common treatment-emergent adverse events were:

- nausea observed with 278 events (22.86%) in 64 patients (53.33%)
- bone pain with 267 events (21.96%) in 80 patients (66.67%).

The most common treatment-emergent adverse event that was possibly related to the study medication was bone pain in 70 patients (58.33%). The mean (SD) duration of bone pain was 9.78 (13.28) days among the patients who reported bone pain. Bone pain was most described as mild in 105 (39.33%) cases. Pain was described as severe in 63 (23.60%) cases, moderate in 99 (37.08%) cases and mild in 105 (39.3%) of cases. The incidence and severity of bone pain were highest in the first cycle with 62 (51.67%) patients reporting bone pain, of which 20 patients (16.67%) reported severe bone pain. Bone pain did not result in any discontinuations.

All other possibly drug related treatment-emergent adverse events were mild or moderate and were observed in less than 5% of study patients. All possibly drug related treatment-emergent adverse events resolved. Injection site reactions were observed in 4 (3.33%) patients, two of which (1.67%) reported injection site pain in addition. One other patient (0.83%) reported pruritus at the injection

site. All reactions were mild except for one moderate swelling, one case of severe warmth, and one event that required analgesia.

Results are summarised in the following table:

Table 67: Summary of possibly related treatment emergent adverse events in study KWI-300-104 according to SOC (Cancer patients safety population)

		Patients	Events
Total no. of subjects		120 (100.00)	
Total no. of subjects with possibly related TEAEs		70 (58.33)	252
System Organ Class	**MedDRA Preferred Term**	**N (%)**	**Event**
Musculoskeletal and connective tissue disorders	Bone pain	70 (58.33)	228
Gastrointestinal disorders	Nausea	4 (3.33)	6
	Abdominal pain	1 (0.83)	1
General disorders and administration site conditions	Injection site reaction	4 (3.33)	4
	Injection site pain	2 (1.67)	4
	Injection site pruritus	1 (0.83)	1
	Pyrexia	1 (0.83)	3
Nervous system disorders	Headache	3 (2.50)	3
	Dizziness	2 (1.67)	2
N = number of patients; E = number of Events			

Healthy volunteers safety population

Back pain (21.53% of subjects in Apo-Filgrastim and Neupogen groups) was the most commonly observed possibly related treatment-emergent adverse events. The incidence of other possibly related treatment-emergent adverse events including fatigue, arthralgia, feeling hot, neck pain, dyspnoea, and pyrexia occurred in between 1 and 5 patients out of 144. The numbers of events were similar between Apo-Filgrastim and Neupogen groups.

Bone pain (considered to be present if the preferred terms arthralgia, back pain, bone pain, neck pain or pain in extremity were reported) was considered to be possibly-related to study drug in a similar percentage of subjects who received Apo-Filgrastim and Neupogen (24.31% and 22.92% respectively).

Two subjects experienced allergic reactions which were considered to be possibly related to study Apo-Filgrastim in one subject. The symptom was pruritic rash occurring on 3 occasions.

One event of injection site erythema, two events of headache and two events of back pain were considered to be severe and related to study medication. All were resolved.

Other adverse events were considered to be mild or moderate.

Serious adverse event / deaths / other significant events

Deaths

Cancer patients safety population

Two patients in study KWI-300-104 died during the follow-up period owing to (i) metastasis and (ii) disease progression. Both patients died about 5 months after the last dose of the study drug. Neither death was considered to be related to study drug.

Healthy volunteers safety population

No deaths were reported.

Other serious adverse events

Cancer patient safety population

8 serious adverse events in addition to the two above mentioned cases of death were recorded during study KWI-300-104 and are summarised in the following table:

Table 68: Serious adverse events in study KWI-300-104 (Cancer patients safety population)

MedDRA Preferred Term	Study Period	Severity	Relation to Study Drug	Outcome	Withdrawal due to AE
Disease progression	Follow-up	Life threatening	Not related	Death	Yes
Breast cancer recurrent	Follow-up	Moderate	Not related	Resolved	No
Primary hypothyroidism	Follow-up	Severe	Not related	Resolved	No
Febrile neutropenia	Cycle 1	Severe	Not related	Resolved	No
Metastases to central nervous system	Follow-up	Severe	Not related	Death	Yes
Depression	Cycle 1	Moderate	Not related	Resolved	No
Panic attack		Moderate	Not related	Resolved	No
Agranulocytosis	Cycle 4	Moderate	Not related	Resolved	No
Abdominal pain	Cycle 1	Mild	Not related	Resolved	No
Asthenia		Mild	Not related	Resolved	No
Leukopenia	Cycle 1	Severe	Not related	Resolved	No
Neutropenia		Severe	Not related	Resolved	No
Neutropenia	Cycle 1	Severe	Not related	Resolved	No
Leukopenia		Severe	Not related	Resolved	No

None of the serious adverse events were considered to be related to study drug. For the 8 serious events other than death, all resolved and none led to withdrawal.

Healthy volunteers safety population

No cases of other serious adverse events were reported.

Laboratory findings

Cancer patients safety population

Blood samples were taken at screening, day 0 of chemotherapy cycle 1, day 7 of chemotherapy cycle 1, day 0 of cycle 4, week 20 and week 24. Day 0 was within 72 hours before administration of chemotherapy.

Liver enzymes

From study start up to the beginning of chemotherapy cycle 4, the mean serum Aspartate transaminase (AST) activity remained stable when assessed. During the follow-up period after the end of treatment with Apo-Filgrastim, the AST values slightly increased to values of 0.49 µkat/L (week 20) and 0.45 µkat/L (week 24).

Alanine aminotransferase (ALT) and Gamma-glutamyl transpeptidase (GGT) activities remained stable over the course of the study up to the beginning of chemotherapy cycle 4 with increases on Day 7 of cycle 1 (ALT mean change from baseline 0.18 µkat/L, GGT mean change from baseline 0.21 (µkat/L)) .

Mean Alkaline phosphatase (ALP) activity remained stable over time. Mean lactate dehydrogenase (LDH) activity remained low in cycle 1 and then slightly increased in cycle 4 and during the follow-up of the study.

Urate

Serum urate concentrations during the course of the study are shown in the following table:

Table 69: Uric acid values and change from baseline (µmol/L) in study KWI-300-104 (Cancer patients safety population)

	Screening	Cycle 1 Day 0	Cycle 1 Day 7	Cycle 4 Day 0	Week 20	Week 24
N	120	119	110	113	111	112
Mean	249.75	246.68	206.29	238.47	268.15	277.20
SD	91.44	67.19	62.09	66.45	70.57	73.14
Minimum	96.00	106.00	89.00	115.00	134.00	112.00
Median	238.96	243.80	203.50	237.00	264.00	268.92
Maximum	738.50	476.50	382.00	437.00	481.79	481.79
Mean change from baseline	N.A.	N.A.	-40.24	-11.04	21.37	29.79
N: Number of patients; SD: Standard Deviation						

Haematology

Platelet counts decreased on Day 7 of cycle 1 compared to baseline (172.91 x 10^9/L versus 276.23 x 10^9/L). On Day 0 of cycle 4, platelet value was 344.25 x 10^9/L which was above the baseline value, indicating a transient decrease during chemotherapy cycles, as shown in the following table:

Table 70: Platelet values and change from baseline (10^9l/L) in Study KWI-300-104 (Cancer patients safety population)

	Screening	Cycle 1 Day 0	Cycle 1 Day 7	Cycle 4 Day 0	Week 20	Week 24
N	120	136	117	129	113	113
Mean	283.57	276.23	172.91	344.25	270.39	226.78
SD	77.00	72.44	52.70	88.35	63.47	54.44
Minimum	152.00	152.00	74.00	177.00	127.00	106.00
Median	271.00	267.50	162.00	332.00	273.00	221.00
Maximum	594.00	521.00	339.00	757.00	479.00	454.00
Mean change from baseline	N.A.	N.A.	-106.20	66.51	-9.27	-52.88
N: Number of patients platelet samples; SD: Standard Deviation						

Haemoglobin values decreased after start of chemotherapy. During the follow-up period after the end of treatment with Apo-Filgrastim, the haemoglobin showed a trend towards return to baseline, as shown in the following table:

Table 71: Haemoglogin values and change from baseline (mmol/L) in study KWI-300-104 (Cancer patients safety population)

	Screening	Cycle 1 Day 0	Cycle 1 Day 7	Cycle 4 Day 0	Week 20	Week 24
N	120	119	117	114	113	113
Mean	7.97	8.00	7.69	7.14	7.34	7.69
SD	0.76	0.78	0.78	0.52	0.63	0.64
Minimum	5.46	5.74	5.33	5.77	5.53	6.02
Median	8.07	8.07	7.70	7.08	7.33	7.70
Maximum	9.69	10.00	9.69	8.57	8.94	9.63
Mean change from baseline	N.A.	N.A.	-0.29	-0.84	-0.65	-0.30
N: Number of patients; SD: Standard Deviation						

Healthy volunteers safety population

In studies KWI-300-101 and KWI-300-012 (single-dose studies), safety laboratory blood samples were taken at baseline, after 24 hours and after 72 hours. In study KWI-300-103, which was a repeat dose study, safety laboratory blood samples were taken at baseline, after 48 hours and after 96 hours. All three studies in healthy volunteers were performed at the same clinical site. The final healthy volunteer study, GCSF-SUIN-05SB01-3FA-(5) was conducted at a different site, with blood sampling performed at multiple intervals until 96 hours after administration of the test dose. There were not any statistically significant differences in measurements of ALT, ALP, LDH, urate, CRP, d-Dimer or aPTT between the Apo-Filgrastim and Neupogen arms of the above studies and at the times chosen.

Safety in special populations

No safety studies in special populations were submitted.

Immunological events

Antibodies to filgrastim were determined in a three-step antibody assay: (i) Screening assay, (ii) Confirmatory assay and (iii) Neutralising assay. Immunogenicity assessment was performed in study KWI-300-104 at (a) the time of patient screening (baseline value), (b) Day 0 of Cycle 2-6 and (c) in the safety follow-up in Week 20, 24, 36 and 48.

Antibodies were detected only in the screening antibody assay in 4 patients. None of the patients had persistent presence of antibodies throughout the study. None of the samples were confirmed as positive in the confirmatory assay. The neutralisation assay was therefore not performed as no positive samples were detected.

Safety related to drug-drug interactions and other interactions

Data on the influence of extrinsic factors such as smoking and diet have not been provided. Drug-drug interactions have not been studied. There are no data on overdose or drug abuse potential. There are no data on the effects of the current product on the ability to drive or operate machinery. There are no data on the effects of the current product on mental activity.

Rebound effects of Apo-Filgrastim were not observed. In cancer patients, absolute neutrophil counts had returned to baseline by the beginning of each subsequent chemotherapy cycle. In healthy subjects, absolute neutrophil counts had returned to baseline by day 4.

Discontinuation due to adverse events

One subject withdrew from study KWI-300-104 because of a non-serious duodenal ulcer. The withdrawal was not considered to be related to study medication.

2.6.1. Discussion on clinical safety

One hundred and forty four healthy subjects were exposed to either Apo-filgrastim or Neupogen (originator) in the initial three phase 1 studies. A further 48 healthy volunteers were exposed to Grastofil and Neupogen in the later completed phase I study, GCSF-SUIN-05SB01-3FA. However, safety data in healthy individuals is only considered supportive, with safety data from clinical trial patients being the main focus of the safety evaluation of G-CSF biosimilar products.

One hundred and thirteen female patients with breast cancer were exposed to Apo-filgrastim over 6 cycles of chemotherapy with follow-up data to 48 weeks for 109 patients in one phase 3 non-comparator clinical study.

The information collected on neutropenia cases and febrile neutropenia comes from two different sources: one the efficacy data and the other the standard adverse event reported in the CRF. Three cases of serious neutropenia were reported, all during cycle 1 and one was a febrile neutropenia. The information provided however, does not appear to match the data provided in the efficacy part of the dossier. It is noted that for cycles 2-6 this information was collected at day 9 (after the day 7 when neutropenia occur most often) when most patients are likely to have recovered i.e. the number of cases are likely to have been under-estimated by the applicant.

The efficacy results show that 93/120 (77%) of the patients had severe neutropenia, mainly starting at day seven and with a duration of 1 day (39%) or 2 days (38%). However, the data from the standard adverse event report are not in line with these percentage as 6 neutropenia events occurred in cycle 1 (4 cases were severe neutropenia) and the duration was longer (4-5 days) in most of the cases. These differences are likely to be due to differences in reporting of FN events as AEs by the investigators but could also be seen as a lack of standardization in the collection of safety events. As the main objective of the study KWI-300-104 was to assess the safety of Apo-filgrastim, the Applicant should have made an effort to establish a standardized protocol of safety data collection. Discrepancies in neutropenia cases are disappointing and highlight the weakness of the methodology and possibly training of study site staff.

The adverse events reported for the current product and the originator for the most part (see musculoskeletal AEs and serum liver enzyme result discussion below) appear similar though the numbers of subjects studied and incidence of adverse events (except for bone pain) are too small to allow meaningful comparison with historical data for the originator.

The Applicant could not confirm similarity of changes in serum liver enzyme measures in the dossier, due in part to the lack of a comparative clinical trial in patients. It could also be that the timing of blood samples and the manner in which results were displayed resulted in a laboratory profile that was noticeably dissimilar to the originator. There was then some residual concern that the apparent differences between originator and the current product were not entirely supportive of claims of similarity of safety profile between Apo-Filgrastim and the originator product.—However, assurance was provided as the lab changes in question are well known to be associated with G-CSF therapies and are not unexpected and not classed as serious. It is not unlikely that the inherent variability of data from a relatively small patient sample may have led to the appearance of dissimilarity with regard to laboratory results between Grastofil and Neupogen. This issue can be appropriately and further evaluated through routine pharmacovigilance measures post-approval; including reviews in the PSUR and reporting & discussion of reports of raised liver function tests (LFTs) (ALP, AST, ALT, gamma GT, SGOT, SGPT, bilirubin).

The rate of musculoskeletal pain was seen to be appreciably higher in the Apo-filgrastim study in patients (66.7%) than in studies the literature where Neupogen was administered to patients with similar disease characteristics and demographics (Holmes et al 26%; Green et al 42%). The Applicant states that the differences between Apo-filgrastim and Neupogen in the percentages of musculoskeletal AE disorders, mainly bone pain, are due to the method of data collection for this AE (specifically documented in the CRF on a specific Bone Pain Assessment Module, in addition to the standard documentation on the Adverse Event page). Although, this is seen as a potential explanation the protocol should have been designed to allow a proper comparability exercise.

Whilst a comparative trial in patients would have been preferred, it is nonetheless considered that the Applicant has complied with advice in “Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor”, EMEA/CHMP/BMWP/31329/2005, February 2006 which states:

“Safety data should be collected from a cohort of patients after repeated dosing preferably in a comparative clinical trial. The total exposure should correspond to the exposure of a conventional chemotherapeutic treatment course with several cycles. The total follow up of patients should be at least 6 months”.

However, “Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor”, EMEA/CHMP/BMWP/31329/2005, February 2006 goes on to state that:

“The number of patients should be sufficient for the evaluation of the adverse effect profile, including bone pain and laboratory abnormalities”.

There is concern that the clinical study only included 120 patients (11 of whom withdrew from the study) and that this number of participants might not be adequate to fully evaluate the adverse effect profile. However, safety data are supplemented by data from four phase I studies in healthy volunteers. Furthermore, considering the biosimilarity with regard to physicochemical characteristics and functions of the molecule as well as the sufficiently similar PK and PD profiles, AEs related to exaggerated PD effects can be expected at similar frequencies for the test and reference product.

A robust post-marketing surveillance programme has been agreed with the Applicant including reviews in the PSUR, reporting & discussion of all important, identified and reported risks and reviews of serious and long-term adverse events from registries. These measures are detailed in the risk

management plan (RMP). This provides adequate reassurance that further evaluation of safety comparability will be undertaken and routinely revisited.

It is uncertain that the immunogenicity studies done during the phase III clinical studies are adequate to fully characterise the immunogenicity of the current product. It is considered that the Applicant should undertake additional pharmacovigilance activities with regard to evaluating the immunogenicity of Grastofil in clinical practice. The activities are detailed in the RMP.

Grastofil is restricted for use in adults only as there is currently no presentation compatible with safe dosing in children (see section 2.2.4).

2.6.2. Conclusions on the clinical safety

The CHMP considers that the overall safety profile of the product is acceptable. Immunogenicity is a rare adverse event which requires the implementation of long-term minimisation measures. The Applicant will undertake additional pharmacovigilance activities post-authorisation including reporting and discussion of all identified and potential risks, and reviews of serious and long-term adverse events from registries. From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics. There are no new adverse reactions observed with Grastofil which are different from what has been described with Neupogen.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The applicant has provided documents that set out a detailed description of the system of pharmacovigilance, V7 dated 17 May 2010. A statement signed by the Applicant and the qualified person for pharmacovigilance, indicating that the Applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country has been provided. The Applicant addressed all concerns presented and has submitted a summary of the PSMF which has been dated and versioned. The PSMF is located where the QPPV operates, in The Netherlands, and the EV-code is MFL 1614.

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements. The Applicant must ensure that the system of pharmacovigilance is in place and functioning before the product is placed on the market.

2.8. Risk Management Plan

A Risk Management Plan (v5.0) has been agreed for the product. The content of the agreed RMP is summarised in the following table:

Table 72: Summary of the EU RMP (version 5.0)

Safety concern	Planned Pharmacovigilance action(s)	Proposed Risk Minimisation Measures
Identified Risks		
Splenomegaly/splenic rupture	Routine Pharmacovigilance Patient follow up through registry Healthy donor follow-up through registry	Routine risk minimisation (labelling). Splenomegaly and splenic rupture are mentioned in Section 1.3.1 Grastofil SmPC, section 4.4 and 4.8. Therefore, spleen size should be carefully monitored. A diagnosis of splenic rupture should be considered in donors and/or

Safety concern	Planned Pharmacovigilance action(s)	Proposed Risk Minimisation Measures
		patients reporting left upper abdominal pain or shoulder tip pain.
Transformation to leukaemia and myelodysplastic syndrome (in patients with severe chronic neutropenia)	Routine Pharmacovigilance Patient follow up through registry	Routine risk minimisation (labelling).Transformation to leukaemia or myelodysplastic syndrome is mentioned in Section 1.3.1 Grastofil SmPC, section 4.4 and 4.8.
Cutaneous Vasculitis	Routine Pharmacovigilance Patient follow up through registry	Routine risk minimisation (labelling). Cutaneous vasculitis is mentioned in Section 1.3.1 Grastofil SmPC, section 4.8.
Osteoporosis in patients with SCN	Routine Pharmacovigilance Patient follow up through registry	Routine risk minimisation (labelling). Osteoporosis is mentioned in Section 1.3.1 Grastofil SmPC, section 4.8.
Exacerbation of rheumatoid arthritis	Routine Pharmacovigilance Patient follow up through registry Healthy donor follow-up through registry	Routine risk minimisation (labelling). Exacerbation of rheumatoid arthritis is mentioned in Section 1.3.1 Grastofil SmPC, section 4.8.
Hypersensitivity (including anaphylaxis)	Routine Pharmacovigilance Patient follow up through registry Healthy donor follow-up through registry	Routine risk minimisation (labelling). Allergic reactions (allergic-type reactions, including anaphylaxis, skin rash, urticaria, angioedema, dyspnoea and hypotension) are mentioned in Section 1.3.1 Grastofil SmPC, section 4.8. Hypersensitivity is mentioned in Section 1.3.1 Grastofil SmPC, section 4.3.
Acute febrile neutrophilic dermatosis (Sweet's syndrome)	Routine Pharmacovigilance Patient follow up through registry	Routine risk minimisation (labelling). Sweet's syndrome (acute febrile dermatosis) is mentioned in Section 1.3.1 Grastofil SmPC, section 4.8.
Acute respiratory distress syndrome	Routine Pharmacovigilance Patient follow up through registry Healthy donor follow-up through registry	Routine risk minimisation (labelling). Pulmonary adverse effects including interstitial pneumonia, pulmonary oedema and lung infiltrates in some cases with an outcome of respiratory failure or adult respiratory distress syndrome (ARDS) which may be fatal are mentioned in Section 1.3.1 Grastofil SmPC, section 4.8. In addition, section 4.4 mentions that patients with a recent history of pulmonary infiltrates or pneumonia may be at higher risk. The onset of pulmonary signs such as cough, fever and dyspnoea in association with radiological signs of pulmonary infiltrates and deterioration in pulmonary function may be preliminary signs of Adult Respiratory Distress Syndrome.
Pulmonary haemorrhage	Routine Pharmacovigilance Healthy donor follow-up through registry	Routine risk minimisation (labelling). Pulmonary adverse events in normal donors (haemoptysis, pulmonary haemorrhage, lung infiltration, dyspnoea, and hypoxia) are mentioned in Section 1.3.1 Grastofil SmPC, section 4.8.
Hemoptysis	Routine	Routine risk minimisation (labelling).

Safety concern	Planned Pharmacovigilance action(s)	Proposed Risk Minimisation Measures
	Pharmacovigilance Healthy donor follow-up through registry	Pulmonary adverse events in normal donors (haemoptysis, pulmonary haemorrhage, lung infiltration, dyspnoea, and hypoxia) are mentioned in Section 1.3.1 Grastofil SmPC, section 4.8.
Lung infiltration	Routine Pharmacovigilance Healthy donor follow-up through registry	Routine risk minimisation (labelling). Pulmonary adverse events in normal donors (haemoptysis, pulmonary haemorrhage, lung infiltration, dyspnoea, and hypoxia) are mentioned in Section 1.3.1 Grastofil SmPC, section 4.8.
Sickle cell anaemia with crisis	Routine Pharmacovigilance Patient follow up through registry	Routine risk minimisation (labelling) Sickle cell crisis in patients with sickle cell disease is mentioned in Section 1.3.1 Grastofil SmPC, section 4.8. In addition, section 4.4, that physicians should exercise caution when considering the use of filgrastim in patients with sickle cell disease and only after careful evaluation of the potential risks and benefits.
Interstitial pneumonia	Routine Pharmacovigilance	Routine risk minimisation (labelling). In Section 1.3.1 Grastofil SmPC, section 4.4 it is mentioned that pulmonary adverse effects, in particular interstitial pneumonia, have been reported after G-CSF administration. Patients with a recent history of lung infiltrates or pneumonia may be at higher risk. The onset of pulmonary signs, such as cough, fever and dyspnoea in association with radiological signs of pulmonary infiltrates and deterioration in pulmonary function may be preliminary signs of acute respiratory distress syndrome (ARDS). Grastofil should be discontinued and appropriate treatment given. In addition, section 4.8 mentions that pulmonary adverse effects including interstitial pneumonia, pulmonary oedema, and lung infiltration have been reported in some cases with an outcome of respiratory failure or acute respiratory distress syndrome (ARDS), which may be fatal.
Increased risk of GvHD	Routine Pharmacovigilance	Routine risk minimisation (labelling). In Section 1.3.1 Grastofil SmPC, section 4.4 and 4.8, it is mentioned that current data indicate that immunological interactions between the allogeneic PBPC graft and the recipient may be associated with an increased risk of acute and chronic graft versus host disease when compared with bone marrow.
Potential Risks		
Immunogenicity	Routine Pharmacovigilance Patient follow up through registry	Section 1.3.1 Grastofil SmPC, section 4.8 states that in clinical studies with cancer patients none of the patients developed anti-rhG-CSF antibodies (neither binding nor neutralizing) following treatment with Grastofil. No additional risk minimisation steps are currently considered necessary.

Safety concern	Planned Pharmacovigilance action(s)	Proposed Risk Minimisation Measures
Interaction with lithium	Routine Pharmacovigilance Targeted questionnaire	Routine risk minimisation (labelling).In section Since lithium promotes the release of neutrophils, lithium is likely to potentiate the effect of Grastofil. Although this interaction has not been formally investigated, there is no evidence in available literature that such an interaction is harmful.
Risks in Off-label use	Routine Pharmacovigilance Post-authorization drug utilisation study (related to off- label paediatric use)	No additional risk minimisation steps are currently considered necessary.
Malignant cell growth (haematological malignancy and myelodysplastic syndrome) in healthy stem cell donors	Routine Pharmacovigilance Healthy donor follow-up through registry	Routine risk minimisation (labelling) In Section 1.3.1 Grastofil SmPC, section 4.4, it is mentioned that transient cytogenetic modifications have been observed in normal donors following G-CSF use. The significance of these changes in terms of the development of haematological malignancy is unknown. Long-term safety follow-up of donors is ongoing. A risk of promotion of a malignant myeloid clone cannot be excluded. It is recommended that the aphaeresis centre perform a systematic record and tracking of the stem cell donors for at least 10 years to ensure monitoring of long-term safety
Risks in Long term use	Routine Pharmacovigilance Patient follow up through registry	No additional risk minimisation steps are currently considered necessary, other than routine pharmacovigilance. It is currently unclear whether long-term treatment of patients with SCN will predispose patients to cytogenetic abnormalities, MDS or leukaemic transformation. It is recommended to perform morphologic and cytogenetic bone marrow examinations in patients at regular intervals (approximately every 12 months).
Missing information		
Risks in pregnancy and lactation	Routine Pharmacovigilance Follow up through registry	Routine risk minimisation (labelling) Grastofil SmPC, section 4.6 states that there are no or limited data from the use of filgrastim in pregnant women. There are reports in the literature where the transplacental passage of filgrastim in pregnant women has been demonstrated. Studies in animals have shown reproductive toxicity with increased incidence of embryo-loss in rabbits, but no malformations have been observed. Although there is no evidence from rats and rabbit studies that filgrastim is teratogenic, the potential risk for humans is unknown. Filgrastim should not be used during pregnancy unless clearly necessary. There are data available in the literature which shows that filgrastim or other

Safety concern	Planned Pharmacovigilance action(s)	Proposed Risk Minimisation Measures
		Granulocyte colony stimulating factors are excreted in human milk. The excretion of filgrastim in milk has not been studied in animals. A decision on whether to continue/discontinue breast-feeding or to continue/discontinue therapy with filgrastim should be made taking into account the benefit of breastfeeding to the child and the benefit of filgrastim therapy to the woman.

2.9. User consultation

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

3. Benefit-Risk Balance

Benefits

Beneficial effects

The pharmacokinetics of Apo-filgrastim were investigated in four studies in healthy human subjects – a single 5µg/kg intravenous dose study, a 75µg and 150µg single subcutaneous dose study, a 300 µg single subcutaneous dose study and a repeat subcutaneous dose 5µg/kg/day study.

PK data were analysed using ANOVA on log-transformed data with terms for sequence, subject within sequence, period and treatment. This is the analysis requested in the CHMP bioequivalence guideline. The acceptance limits set for the confidence intervals of the ratios were in line with those outlined in the CHMP bioequivalence guideline.

Data for the PK parameters AUC_{0-24}, AUC_{0-inf}, C_{MAX} for all 4 PK/PD studies showed the confidence intervals for ratios to be contained within 80-125% regardless of analysis population (PP, ITT, sensitivity). Any concerns in study KWI-300-101 that AUC_{0-32} rather than AUC_{0-inf} was primarily used for the comparability exercise can be rested as there was shown to be <1% difference between the two AUC parameters, with confidence intervals for the ratios of both falling within the acceptance limits. 90% confidence intervals for the PK data from the 300 µg single subcutaneous dose study, GCSF-SUIN-05SB01-3FA-(5), were also comfortably contained within the 80-125% acceptance margin. This study was used to demonstrate PK & PD similarity of the Grastofil product intended for commercialisation (process III) to Neupogen, as it was noted that some changes in manufacturing process proposed for the product originally used in the clinical trials (process II) could cause alterations in the product.

The data presented by the Applicant for PD outcomes suggested that similarity between test and reference products, with regard to PD outcomes, has been demonstrated, as 90% CIs and the 95% CI for the differences fell within the Applicant's pre-specified acceptance margins of 80-125%.

Clinical data from the target population appeared to confirm that PD, haematological and infective outcomes in advanced breast cancer patients administered Apo-filgrastim in combination with

myelosuppressive chemotherapy were similar to those seen in similar population administered Neupogen or other G-CSF products.

Uncertainty in the knowledge about the beneficial effects

Statistically significant PK differences between the products were seen in studies KWI-300-101 and GCSF-SUIN-05SB01-3FA. These 'bioassays' constituted evidence that real differences between test and reference formulations existed that may, in principle, have presented a concern for conclusion of biosimilarity. Although for all key PK parameters, the 90% confidence intervals of the ratios of the means were fully contained within the 80-125% acceptance limits, in line with the guidance provided in the CHMP Guideline on the Investigation of Bioequivalence, the significant differences needed to be addressed. The low variability of the PK data as a result of enrolling more subjects than required in the above studies was thought to only partly explain the significant differences. Ultimately, the reasons for these differences were not definitively identified. However, it was noted that for the majority of PK outcomes in the dossier the trend was for differences between test and reference products to be smaller and not statistically significant. Further, the differences did not lead to clinically or statistically significant differences in PD outcomes, strongly suggesting that clinically, the differences were not meaningful and would not lead to differences in clinical effect.

For the first three studies in healthy volunteers the majority of PK estimates for Apo-filgrastim (process II product) were lower than those of the reference product (significantly so, as already noted for study KWI-300-101); although for the single dose study using process III commercial product (GCSF-SUIN-05SB01-3FA), the estimates were statistically significantly higher. There was suggestion therefore that products of the two processes may not have been bioequivalent to each other, creating concern that efficacy and safety data from trial KWI-300-104, could not be extrapolated to process III product. However, the CHMP considered that there were a number of factors that would significantly undermine such claims; not least the modest differences between test-reference PK and PD ratios for process II and III products, the inherent limitations of interpreting data from across studies and most significantly the confidence intervals of the ratios falling fully within the agreed acceptance limits. Importantly, the clinical comparability exercise is underpinned by Quality and non-clinical comparability exercises which sufficiently demonstrated that Apo-Filgrastim drug substance and drug product from the clinical stages of process development and from the proposed commercial process were comparable with one another and to the reference product, Neupogen. Given the positive PK/PD data from study GCSF-SUIN-05SB01-3FA (process III product) and supportive data from the Quality and non-clinical comparability exercises the CHMP was assured of comparability of process II and process III products.

As regards pharmacodynamics, the 80-125% acceptance margin used for the initial PD comparability exercise during the MAA procedure was considered too wide for demonstration of PD similarity especially for the Grastofil package where comparative clinical efficacy and safety data from patients were not available. A tighter acceptance limit of +/- 10% was agreed. However, a number of confidence intervals of PD outcome ratios did not fall entirely within these limits, especially in the repeated-dose and low dose settings. Reassuringly, despite the studies not being powered such that 95% confidence intervals of the PD ratios would be contained within the narrow limits, for three of the five dosing regimens evaluated this was the case, strongly supporting PD similarity of the products. For the remaining regimens, the excursions past the lower bound of the narrower acceptance limits were small, as were the actual mean differences between the products. Given that the more stringent limits were applied retrospectively and were not factored into the initial sample size calculations for the PK/PD studies, it is not inconceivable that a small number of confidence intervals may fail to fall within the more stringent boundary as the studies would have been underpowered. In this case, the adoption of a more flexible approach with regard to the recommended width of the acceptance limit for the CIs of PD ratios was accepted by the CHMP. Further, it was shown by the Applicant that the doses of 1-

5µg/kg sit on the steep part of the dose response curve and that the single-dose studies, based on data in the dossier, are equally as sensitive as the repeated-dose study in detecting PD differences between the test and the reference products. Therefore, given the overall data it can be concluded that PD similarity has been demonstrated between Grastofil and Neupogen and that the differences seen in the repeated-dose study are unlikely to have consequences in clinical practice.

The supportive CD34+ PD data in the dossier are weak due to the inadequate blood sampling schedule and the poor collection of data. Although robust data from statistical evaluation of comparability of response could not be provided, data from studies KWI-300-103 and KWI-300-104 demonstrated that the CD34+ responses to Apo-filgrastim and Neupogen were alike. Therefore, based on current knowledge of G-CSF, biosimilar filgrastims and G-CSF analogue activity at the G-CSF receptor, given that test-to-reference comparability has been determined in quality, non-clinical and clinical comparability exercises, the CHMP does not expect CD34+ response to Grastofil and Neupogen to differ in a clinically significant manner.

The lack of robust controlled safety and efficacy data from patients requires that PK and PD similarity in healthy volunteer studies be robustly demonstrated. Overall, given the totality of the data available within the clinical development programme, it is considered that the PK and PD similarity of Grastofil and Neupogen has been demonstrated.

Risks

Unfavourable effects

Safety data has been accrued from one phase III non-comparative, repeat-dose study over 6 cycles of chemotherapy in 120 female patients with breast cancer. Bone pain is a known adverse event associated with the originator. For the current product, bone pain was recorded in 66.7% of patients. The bone pain lasted about 10 days and was described as severe in approximately 24% cases, moderate in 37% cases and mild in 39% of cases. The incidence and severity of bone pain were highest in the first cycle of chemotherapy. Other events, including injection site reactions, were recorded in less than 5% patients and were either mild or moderate; they all resolved. There were some fluctuations in the serum activities of liver-derived enzymes such as AST, ALT, ALP, gamma-GT and in the serum activity of LDH and the serum concentration of urate in response to exposure to the current product.

Safety data has also been accrued from four phase 1 single dose studies in a total of 230 healthy volunteers. Bone pain occurred in approximately 23% subjects in both Apo-Filgrastim and originator arms of the three initial phase I studies. Overall, the adverse events reported for the current product and the originator appeared similar in type and frequency though the numbers of subjects studied are too small to be definite. There were not any deaths in the studies submitted.

Uncertainty in the knowledge about the unfavourable effects

There is concern that the number of healthy volunteers who took part in the submitted studies is too small and the exposure to the current product too short to adequately characterise the safety profile of the current product. The non-comparative nature of the phase III study also hinders comparison to the originator. However, the concern is not significant as safety data in healthy individuals is only considered supportive, with safety data from clinical trial patients being the main focus of the safety evaluation of G-CSF biosimilar products. The safety profile of Apo-filgrastim in the pivotal study was as expected for a filgrastim, acknowledging some limitations in the size of the safety database. Furthermore, considering the biosimilarity with regard to physicochemical characteristics and functions of the molecule as well as the sufficiently similar PK and PD profiles between Apo-filgrastim and the

reference medicinal product, AEs related to exaggerated PD effects can be expected at similar frequencies for the two products.

A robust post-marketing surveillance programme has been agreed with the Applicant including reviews in the PSUR, reporting & discussion of all important, identified and reported risks and reviews of serious and long-term adverse events from the registries. These measures are detailed in the RMP.

Exposure to the originator is known to result in marked changes in the serum activities of liver-derived serum enzymes in subgroups of patients. The applicant has not confirmed such changes to the same extent in blood test results taken whilst patients were exposed to the current product and certainly not in the context of a comparative clinical study. It may be that the timing of blood samples and the manner in which results were displayed have resulted in a laboratory profile that is noticeably dissimilar to the originator. There is then some residual concern that the apparent differences between originator and the current product may not be entirely supportive of claims of similarity of safety profile between Apo-Filgrastim and the originator product. However, some assurance is provided as the lab changes in question are well known to be associated with G-CSF therapies and are not unexpected and not classed as serious. It is not unlikely that the inherent variability of data from a relatively small patient sample may have led to the appearance of dissimilarity with regard to laboratory results between Grastofil and Neupogen at certain times during the study. This issue can be appropriately and further evaluated through routine pharmacovigilance measures post-approval; including reviews in the PSUR and reporting & discussion of reports of raised liver function tests (LFTs) (ALP, AST, ALT, gamma GT, SGOT, SGPT, bilirubin).

Whilst the apparent absence of antibody development to the current product would be consistent with the originator, there is concern that the number of patients was too small to adequately characterise its immunogenicity. It is considered necessary for the Applicant to undertake additional pharmacovigilance activities with regard to evaluating the immunogenicity of Grastofil in clinical practice. These activities are detailed in the RMP.

Benefit-risk balance

The benefit/risk balance of Grastofil is considered positive, as a benefit/risk ratio comparable to the reference product can be concluded.

Discussion on the benefit-risk balance

PK similarity between Apo-filgrastim/ Grastofil and Neupogen at and around the main clinical dose (5µg/kg) has been convincingly demonstrated. The totality of the PD data from the development programme supports the PD similarity of the test and reference products. Although the clinical efficacy and safety data submitted were from a single uncontrolled clinical study, it should be noted that in a G-CSF biosimilar MAA, robust PD data in healthy volunteers could be considered pivotal, as in this case.

Overall, the demonstration of biosimilarity should be based on the results of robust quality (analytical structure, potency assays, purity), non-clinical (receptor binding, toxicokinetic studies) and clinical (PK, PD, safety and efficacy) comparability exercises. The totality of the data provided from the quality, non-clinical and clinical comparability exercises support demonstration of biosimilarity of Grastofil to Neupogen.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Grastofil in the in the following indication:

- Grastofil is indicated for the reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes) and for the reduction in the duration of neutropenia in adult patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia.
- Grastofil is indicated for the mobilisation of peripheral blood progenitor cells (PBPCs) in adults.
- In adult patients with severe congenital, cyclic, or idiopathic neutropenia with an absolute neutrophil count (ANC) of $\leq 0.5 \times 10^9$/L, and a history of severe or recurrent infections, long term administration of Grastofil is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.
- Grastofil is indicated for the treatment of persistent neutropenia (ANC less than or equal to 1.0 x 109/L) in adults with advanced HIV infection, in order to reduce the risk of bacterial infections when other options to manage neutropenia are inappropriate.

is favourable and 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).

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and 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 agreeed subsequent updates of the RMP.

When the submission of a PSUR and the update of a RMP coincide, they should be submitted at the same time.

In addition, 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.