European Medicines Agency
Evaluation of Medicines for Human Use

EMEA/CHMP/651339/2008

CHMP ASSESSMENT REPORT

FOR

Zarzio

International Nonproprietary Name: filgrastim

Procedure No. EMEA/H/C/000917

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

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1 BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Sandoz GmbH submitted on 06 September 2007 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for Zarzio through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The legal basis for this application refers to Article 10(4) of Directive 2001/83/EC, as amended – relating to applications for biosimilar medicinal products.

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

The applicant applied for the following indications:

- 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 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 are similar in adults and children receiving cytotoxic chemotherapy.
- Mobilisation of peripheral blood progenitor cells.
- In patients (children or adults) 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 is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.
- Treatment of persistent neutropenia (ANC $\leq 1.0 \times 10^9$/L) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other therapeutic options are inappropriate.

Scientific Advice:
The applicant received Scientific Advice from the CHMP on 24 June 2005. The Scientific Advice pertained to quality, 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.

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:
Rapporteur: **Ian Hudson** Co-Rapporteur: **Barbara van Zwieten-Boot**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 06 September 2007.
- The procedure started on 26 September 2007
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 19 December 2007. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 14 December 2007. In accordance with Article 6(3) of Regulation (RC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.
- During the meeting on 21-24 January 2008, 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 25 January 2008.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 23 July 2008.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 05 September 2008.
- During the CHMP meeting on 22-25 September 2008, the CHMP agreed on a list of outstanding issues to be addressed in writing and by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 20 October 2008
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the CHMP List of Outstanding Issues to all CHMP members on 03 November 2008.
- During the meeting 17-20 November 2008, 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 Zarzio on 20 November 2008. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 14 November 2008.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

The current treatment of cancer with combination cytotoxic therapy targeting proliferating cells usually leads to bone marrow damage, anaemia, thrombocytopenia and, most importantly, neutropenia resulting in impaired host defence. A severe neutropenia will inevitably lead to serious infections. Life-threatening gastrointestinal and pulmonary infections as well as sepsis will occur as long as the severe neutropenia prevails. This leads to delays in subsequent chemotherapy cycles. The recovery of the bone marrow is stimulated by various growth factors. The most important growth factor for the recovery of neutrophils is granulocyte colony-stimulating factor (G-CSF). G-CSF is a 20,000 Dalton glycoprotein hormone that stimulates the proliferation of neutropoietic progenitor cells and their differentiation to granulocytes, and functionally activates mature neutrophils.

Human G-CSF is a single polypeptide chain protein of 174 amino acids with O-glycosylation at one threonine residue. It acts by binding to a specific transmembrane receptor (G-CSF receptor), a member of the class I cytokine receptor family expressed on various haematopoietic cells such as stem cells, multipotent progenitors, myeloid-committed progenitors, neutrophils, and monocytes. This receptor forms homo-oligomeric complexes upon ligand binding. Seven membrane-bound and one soluble isoform of the G-CSF receptor have been isolated; the membrane-bound isoforms arise from alternative RNA splicing leading to differences in the cytoplasmic sequences, but the extracellular, ligand-binding domains are identical. Consequently, the effects of G-CSF (and of recombinant human G-CSF, rhG-CSF) are mediated via a single affinity class of receptors. The same mechanism of action and receptor mediated biological activity operates in mobilization of mature neutrophils into the circulating neutrophil pool and acceleration of granulopoiesis.

Zarzio is a recombinant human G-CSF 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. Moreover, it is not glycosylated.

A marketing authorisation application has been submitted by Sandoz GmbH (Austria) for the product Zarzio (filgrastim) under 2001/83/EC Article 10(4) Biosimilar medicinal product.

The indications claimed are exactly the same as those of the reference product Neupogen.

- 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 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 are similar in adults and children receiving cytotoxic chemotherapy
- Mobilisation of peripheral blood progenitor cells.
- In patients (children or adults) 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 is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.
- Treatment of persistent neutropenia (ANC $\leq 1.0 \times 10^9$/L) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other therapeutic options are inappropriate.

2.2 Quality aspects

Introduction

Two presentations of the medicinal product are provided, 30MU (300 µg/0.5 ml), Solution for Injection/concentration for solution for infusion – pre-filled syringe and 48 MU (480 µg/0.5 ml),

Solution for Injection/concentrate for solution for infusion – pre-filled syringe. The reference product is Neupogen (Amgen GmbH, Germany) also available in the same two presentations, which was used for the entire comparability exercise to demonstrate comparable quality, safety and efficacy.

Active Substance

Manufacture

Filgrastim is manufactured and released at Sandoz GmbH, Kundl, Austria. Filgrastim is produced by recombinant DNA technology in bacteria (*E.coli*) from the full length human sequence for N-(L-Methionyl) granulocytes colony-stimulating factor (r-metHuG-CSF). Native G-CSF is a glycosylated protein but production in bacteria leads to a non-glycosylated product, however, this is still biologically active. 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 chromatographic purification steps to separate filgrastim from other contaminating proteins and impurities. This is followed by further polishing steps. A final buffer exchange is performed to yield the active substance solution.

The *E.coli* are banked in a standard 2 tier banking system consisting of a master cell bank (MCB) and a manufacturers working cell bank (MWCB) to current guidelines. All materials used to manufacture filgrastim are controlled and of a suitable quality. Critical steps and intermediates are controlled by a range of critical process parameters (identified during development), process parameters, action limits and acceptance criteria. Based on an assessment of the critical steps, a validation programme was devised and implemented. Process validation was performed at full scale and the results used to justify the limits set. Within process validation, hold times for intermediates were also validated as was chromatography column re-use. Final active substance filling and shipping was validated.

During manufacturing development of active substance, key steps were investigated and optimised. Development of the purification stages included different chromatographic methods , buffer compositions, and the sequence of steps.

Filgrastim is stored in pre-sterilised non-pyrogenic bottles with screw caps.

Characterisation

Extensive characterisation was undertaken of filgrastim as well as medicinal product and additionally Neupogen medicinal product to confirm identity and purity. Primary structure, secondary and tertiary structures were assessed using appropriate analytical techniques. Charge characteristics were assessed by isoelectric focussing (IEF), as well as cation and anion chromatography. Finally, biological characteristics were assessed by bioassay, western blotting and surface plasmon resonance spectroscopy (to investigate binding affinity). The characterisation programme was based on scientific advice received by the Applicant from the CHMP. Characterisation data supported the correct sequence and folding of the recombinant G-CSF and demonstrated good batch reproducibility. A series of in-house standards were prepared and used throughout characterisation and for batch release. The WHO standard for G-CSF was also used within the bioassay.

Product related impurities were identified, also using stressed samples and characterised by suitable methods in all active substance and DP batches. Appropriate limits were set, based on batch data.

Potential process related impurities were considered and identified. Appropriate analytical methods were selected, and all batches tested and limits set based on batch data and pharmacopoeial specifications.

- Specification

Active substance specifications and shelf-life specifications have been set, supported and justified with batch data and characterisation data. All non-pharmacopoeial methods were suitably validated.

- Stability

Active substance stability has been determined to be 36 months at -20±5°C.

- Comparability Exercise for Active Substance

Medicinal Product composition of Zarzio and Neupogen are quantitatively identical except the buffer system for Zarzio is glutamate and for Neupogen it is acetate.

The data presented confirm Zarzio and Neupogen conform with respect to primary structure, secondary and tertiary structure, molecular mass, hydrophobicity, molecular size, charge, binding and (*in-vitro*) bioactivity.

Medicinal Product

Pharmaceutical Development

The medicinal product composition was developed to be suitable both for subcutaneous injection and for intravenous infusion; furthermore, as a biosimilar medicinal product, it was developed to be similar to the reference product resulting in two formulations 30 MU/0.5 ml and 48 MU/0.5 ml. During development a number of buffer systems were tested for stability of the active substance, concluding that: glutamate = acetate. The final composition was optimised and matched to Neupogen except for the use of glutamate rather than acetate for the buffer. The liquid product is packaged in 1 ml pre-filled syringes and can be used without further reconstitution.

For patients treated with Zarzio diluted to concentrations < 1.5 MU/ml (15 µg/ml), human serum albumin (HSA) should be added to a final concentration of 2 mg/ml.

When diluted in glucose 50 mg/ml (5%) solution, Zarzio is compatible with glass and a variety of plastics including polyvinylchloride, polyolefin (a copolymer of polypropylene and polyethylene) and polypropylene.

Adventitious Agents

No materials of animal or human origin are used to manufacture Zarzio active substance or medicinal product. Some reagents derived from bovine milk protein are used in the manufacture of chromatography columns, but the suppliers certify these comply with requirements.

Both the active substance and medicinal product manufacturing processes are well controlled for microbiological safety. Master and working cell banks have undergone appropriate testing.

Manufacture of the Product

Manufacturing consists of mixing active substance with excipients and adjustment of pH followed by filtration and filling into pre-sterilised syringes. The manufacturing process is controlled by a series of in-process controls. Validation was performed on both presentations 30MU and 48MU. All excipients conform to the requirements of the European Pharmacopoeia.

Product Specification

Medicinal product specification was justified by characterisation and batch data, and analyses performed.

Stability of the Product

Stability data from development batches supports a medicinal product shelf-life of 30 months at 5±3°C for both presentations. Photostability testing confirmed that the pre-filled syringes should be kept in the outer cartons in order to protect from light.

Comparability Exercise for Medicinal Product

Product related impurities were thoroughly investigated; aggregates, and truncated forms showed no significant differences. The data show a consistently lower level of deamidated and oxidised forms. The difference does not appear to impact on bioactivity (*in vitro* bioassay) or stability.

In conclusion, the physicochemical and biological analysis of Zarzio fully supports its biosimilarity to Neupogen. medicinal product composition of Zarzio and Neupogen are quantitatively identical except the buffer system for Zarzio is glutamate and for Neupogen it is acetate.

GMO

Not applicable

Discussion on chemical, pharmaceutical and biological aspects

The quality dossier for Zarzio is well presented fulfilling the requirements of a biosimilar application. The physico-chemical and biological comparability studies using Neupogen from the German market as reference product were performed using a large set of state of the art analytical methods showing no significant differences. The composition of Zarzio is identical to the reference product Neupogen except for the buffer system. Development studies using a number of buffer systems led to the conclusion that both buffer systems are equally suitable for filgrastim formulations. Pharmaceutical development led to a suitable product.

2.3 Non-clinical aspects

Introduction

The non-clinical testing strategy was aimed at comparing the marketed reference product Neupogen with filgrastim. In an *in vitro* NFS-60 cell assay, filgrastim and the reference drug Neupogen were compared for ability to interact with the G-CSF receptor. Filgrastim was also compared with Neupogen in four animal studies assessing pharmacodynamics, toxicity, toxicokinetics, and local tolerance (table 1).

Table 1 Nonclinical study program

Type of Study	Species and Strain Number of animals (n)	Method of Administration	Duration of Dosing	Doses	GLP Comp-liance	Study Number
Pharmaco-dynamic study (part A: normal rats)	CD rat n= 60	Subcutaneously	4 days dosing plus 8 days recovery	Filgrastim: 10, 20, 40, 80 or 160 µg/kg/day Neupogen: 10, 20, 40, 80 or 160 µg/kg/day Placebo controls	Yes	LPT: 19819/06 Sandoz: [Study EP06-004]

Type of Study	Species and Strain Number of animals (n)	Method of Administration	Duration of Dosing	Doses	GLP Comp-liance	Study Number
Pharmaco-dynamic study (part B: neutropenic rats)	CD rat n=60	Subcutaneously	4 days dosing plus 8 days recovery	Filgrastim: 30, 60, 100 µg/kg/day Neupogen: 30, 60, 100 µg/kg/day Placebo controls	Yes	LPT: 19819/06 Sandoz: [Study EP06-004]
Repeat-dose toxicity	Wistar rat n=172	Subcutaneously	28 days dosing plus 42 days recovery	Filgrastim: 20, 100, 500 µg/kg/day Neupogen: 20, 500 µg/kg/day Placebo controls	Yes	Aurigon: 085.121.39 3 Sandoz: [Study EP06-001]
Toxicokinetics	Wistar Rat n=50	Subcutaneously	14 days dosing	Filgrastim: 20, 100, 500 µg/kg/day Neupogen: 20, 500 µg/kg/day Placebo controls	Yes	Aurigon: 085.151.39 4 Sandoz: [Study EP06-002]
Local Tolerance	New Zealand White Rabbit n=36	Intravenously Paravenously Subcutaneously Intramuscularly Intraarterially	Single dose	Per application route 480 µg/day in 0.5 ml: Filgrastim (acetate buffer) Filgrastim (glutamate buffer) Neupogen Contralateral placebo controls	Yes	Aurigon: 085.143.39 5 Sandoz: [Study EP06-003]

Batches used in nonclinical studies were released with the same analytical and quality control procedures established for batches in clinical studies. All batches released for the non-clinical and clinical studies were stated to meet the criteria of equal relative dose potency compared to the G-CSF standard.

All non-clinical studies were stated to be conducted in GLP –certified contract institutions. However, an additional study on the determination of G-CSF level 'Determination of rhG-CSF in rat serum using ELISA' (additional experiments), performed by BioProof AG, was not conducted in compliance with GLP.

Pharmacology

Primary pharmacodynamics

The *in vitro* pharmacological evaluation of filgrastim was performed using the NFS-60 cell proliferation assay. This *in vitro* cell assay is based on the ability of murine myeloblastic NFS-60 cells to proliferate in response to G-CSF. The *in vitro* potency of all recombinant G-CSF samples produced was evaluated by a parallel-line assay format according to the European Pharmacopoeia 1997, Chapter 3.5 (Statistical analysis of results of biological assays and tests). The estimates for relative dose potency of filgrastim and Neupogen are presented in Table 2.

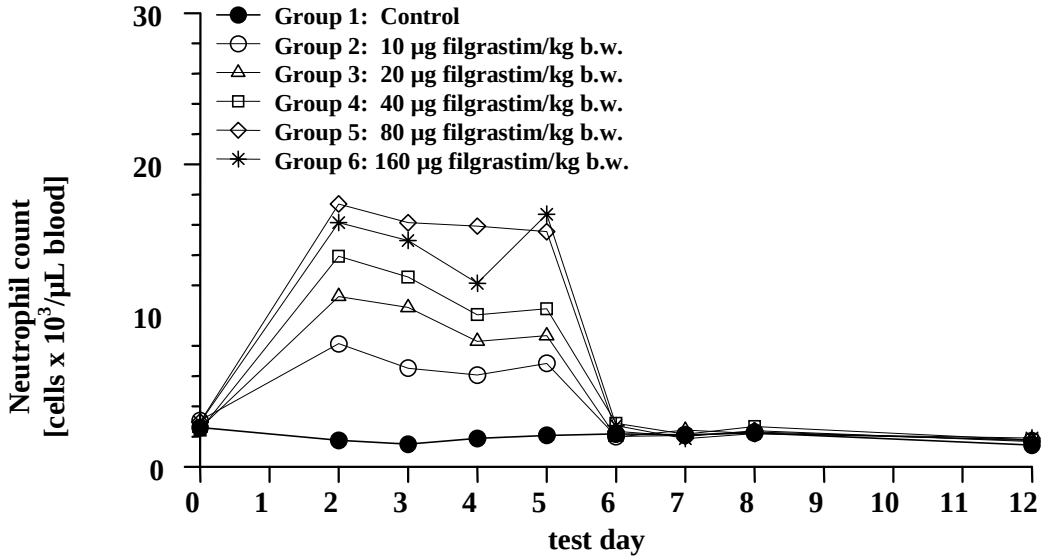
Table 2 Relative potency of filgrastim and Neupogen batches measured by the NFS-60 cell assay

Study Code	Batch	Activity [%]	95% Lower Limit	95% Upper Limit	Activity [U/mg]
EP06-101	#003941609F	110	94	126	1,1 E+08
	#N0875AA	106	98	115	1,1 E+08
EP06-102	#000675111G	103	94	112	1,0 E+08
	#N1114AJ	108	102	114	1,1 E+08
EP06-103	#000675011G	101	73	130	1,0 E+08
	#000675211G	111	100	122	1,1 E+08
	#N1144AE	108	98	118	1,1 E+08
	#N1179AB	106	99	113	1,1 E+08
EP06-301	#000657409G	102	96	109	1,0 E+08
	#000675011G	101	73	130	1,0 E+08
	#000675111G	103	94	112	1,0 E+08
	#000675211G	111	100	122	1,1 E+08
EP06-004	#000675011G	101	73	130	1,0 E+08
	#N1144AE	108	98	118	1,1 E+08
EP06-001	#RS21	99	95	103	1,0 E+08
	#N0577AA	Not analyzed			
EP06-002	#RS21	99	95	103	1,0 E+08
	#N0577AA	Not analyzed			
Loc. Tol.	#0304016S	108	94	122	1,1 E+08

***** Batches starting with #N refer to Neupogen batches, all other batches are filgrastim batches. The third column depicts the activity in percent of the activity of the reference standard, whereas the sixth column lists the specific activity in units per mg. The activity of the reference standard is declared as 1.0×10^8 U/mg.

The *in vivo* potency of filgrastim was investigated in normal (part A of Study EP06-004) and neutropenic rats (part B of Study EP06-004). In Part A of Study EP06-004, filgrastim, Neupogen or control solution were administered subcutaneously on four consecutive days (day 1-4) to male CD rats at dose levels of 10, 20, 40, 80, and 160µg/kg. Blood samples were taken at approximately the same time in the morning before dosing on day 1 and on test days 2, 3, 4, 5, 6, 7, 8 and 12. The kinetics of the neutrophil response for filgrastim and Neupogen treatment are shown in Figure 1. The integrated effect over time of five different dose levels on neutrophil count for filgrastim and Neupogen is shown in Figure 2.

Figure 1 Mean ANCs obtained in normal CD rats treated with four subcutaneous doses of filgrastim or Neupogen



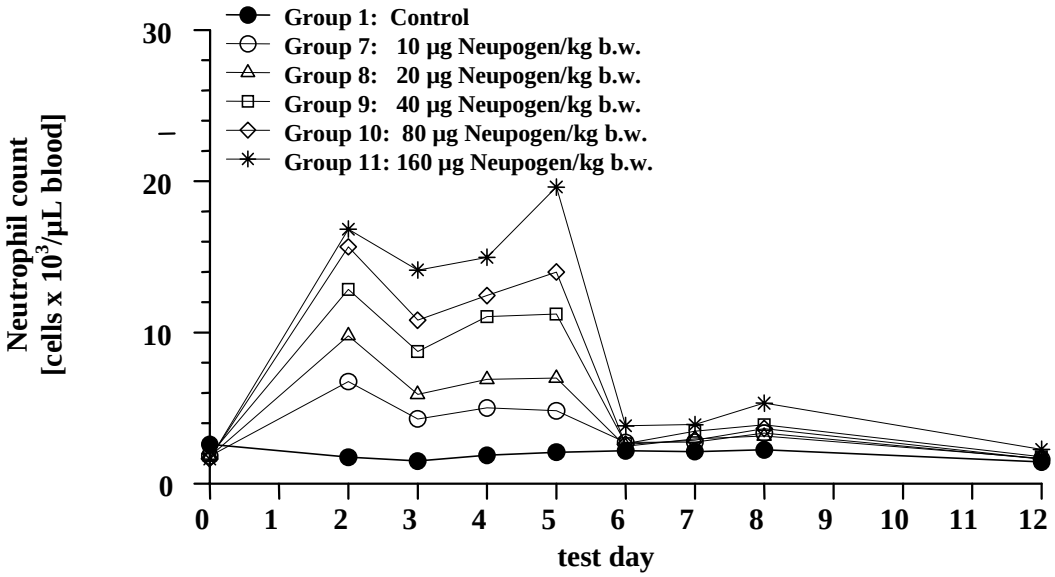
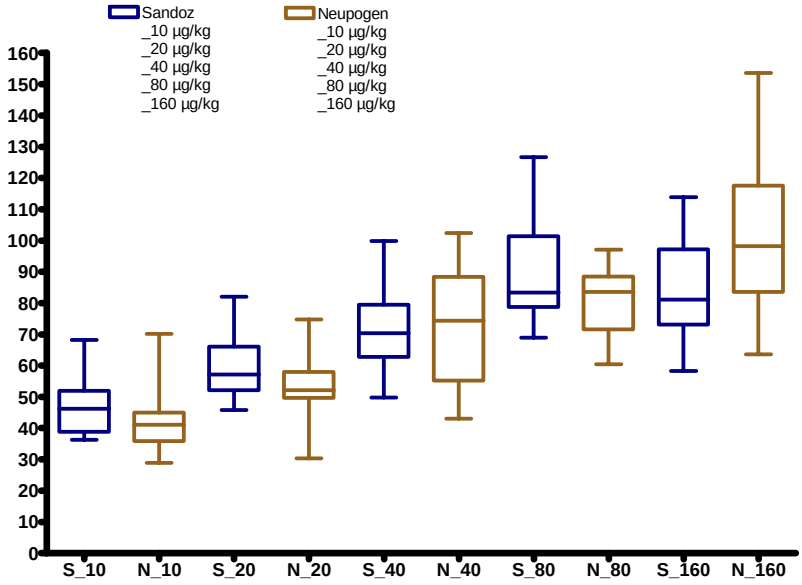


Figure 2. Areas under the effect curve 0-12 days ($AUEC_{0-12}$) of neutrophil counts following four doses of filgrastim or Neupogen



* Sandoz: filgrastim

The corresponding areas under the effect curve (AUEC days 0-12) for neutrophil count of filgrastim (S) and Neupogen (N) at each dose level (10 to 160 µg/kg) are plotted side by side.

The number of eosinophilic and basophilic granulocytes increased slightly in all filgrastim and Neupogen treated groups 2-5 days after the first dosing, with the increase being more pronounced at 160 mg/kg. Monocyte counts increased in all filgrastim and Neupogen treated groups in a dose-dependent manner 2 to 5 days after the first dosing. While the number of LUCs increased in all filgrastim- or Neupogen-treated groups, the absolute numbers of LUCs remained low.

The descriptive comparison of the AUEC by means of 95% confidence intervals for the ratio of the means for ANC and maximal neutrophil counts (E_{max}) are shown in Table 3.

Table 3. Comparison of neutrophil count between treatment groups of equal strength in normal rats

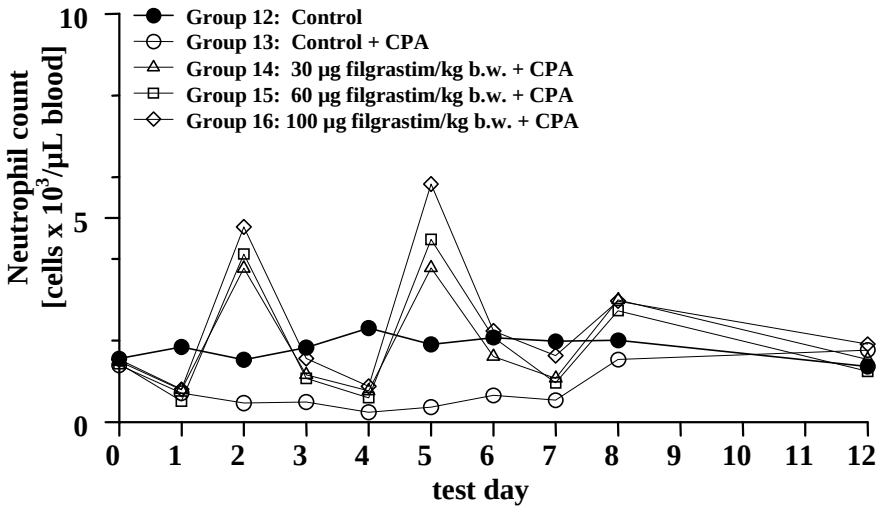
Filgrastim (Test)	Neupogen (Reference)	AUEC [cells x 10^3 x days / l]		E_{max} [cells x 10^3 / l]	
		Ratio*	95% CI for ratio* of means	Ratio*	95% CI for ratio* of means
Filgrastim 10 µg/kg	Neupogen 10 µg/kg	1.12	0.95 - 1.33	1.28	1.08 - 1.51
Filgrastim 20 µg/kg	Neupogen 20 µg/kg	1.15	0.97 - 1.36	1.17	0.99 - 1.39
Filgrastim 40 µg/kg	Neupogen 40 µg/kg	1.02	0.86 - 1.21	1.06	0.90 - 1.25
Filgrastim 80 µg/kg	Neupogen 80 µg/kg	1.12	0.95 - 1.33	1.14	0.96 - 1.35
Filgrastim 160 µg/kg	Neupogen 160 µg/kg	0.85	0.72 - 1.01	0.94	0.80 - 1.11

* ratio test / reference

In neutropenic rats (Part B of Study EP06-004), male CD rats were allocated to three dose groups (30, 60, and 100 µg/kg). On day 0 of the study, animals received a single intraperitoneal dose of 50 mg/kg cyclophosphamide (CPA) inducing neutropenia. Filgrastim, Neupogen or control solution (0.9% NaCl = vehicle/diluent solution for filgrastim) were subcutaneously administered on four consecutive days (days 1-4). An additional control group of normal rats received neither CPA nor rhG-CSF. In analogy to definitions used in humans, neutropenia in rats was defined as ANC that was two standard deviations below the normal mean ANC for untreated control animals, and the duration of neutropenia was compared between the groups.

Induction of neutropenia by CPA produced a reduction of ANCs and WBC counts with a maximum on test days 3-4 followed by recovery to normal values by days 8 (ANC) and 12 (WBC). Groups treated with either filgrastim or Neupogen showed a dosage-dependent increase in mean ANCs on days 2, 3, 5 and 6 compared to neutropenic control animals that did not receive rhG-CSF (Figure 3). Duration of neutropenia is shown in table 4.

Figure 3 Number of neutrophils (mean values per group; neutropenic rats)



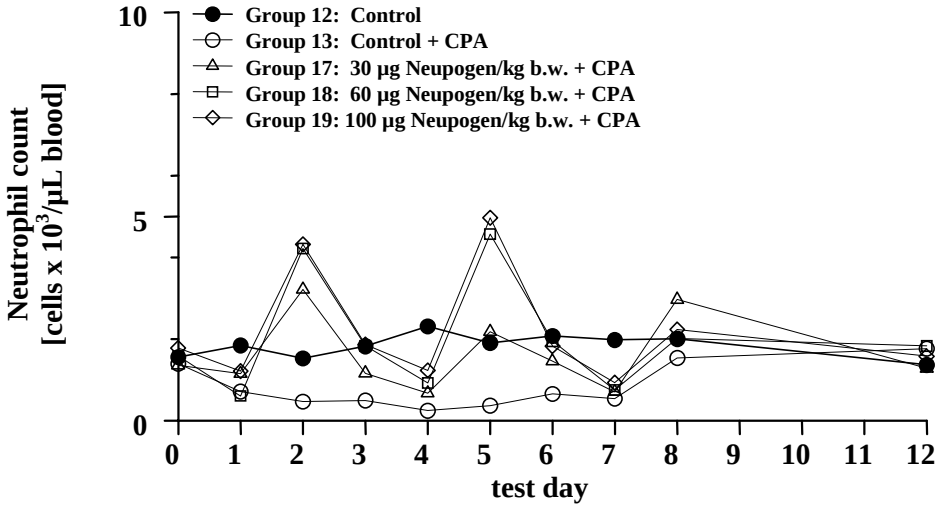


Table 4. Duration of neutropenia (ANC < 1000/l) in days

Group	Mean	STD	Min	Median	Max
CPA Control	7.67	2.10	5.00	7.00	11.00
Filgrastim 30 µg/kg	1.17	0.39	1.00	1.00	2.00
Filgrastim 60 µg/kg	1.00	0.00	1.00	1.00	1.00

Group	Mean	STD	Min	Median	Max
Filgrastim 100 µg/kg	1.09	0.30	1.00	1.00	2.00
Neupogen 30 µg/kg	1.17	0.39	1.00	1.00	2.00
Neupogen 60 µg/kg	1.00	0.00	1.00	1.00	1.00
Neupogen 100 µg/kg	1.00	0.00	1.00	1.00	1.00

- Secondary pharmacodynamics

No secondary pharmacodynamics studies were submitted.

- Safety pharmacology programme

No safety pharmacology studies were submitted.

- Pharmacodynamic drug interactions

No pharmacodynamic drug interactions studies were submitted.

Pharmacokinetics

No pharmacokinetics studies were submitted.

Toxicology

- Single dose toxicity

No single dose toxicity studies were submitted.

- Repeat dose toxicity (with toxicokinetics)

A 4-week repeated-dose toxicity study with a 6-week recovery period (study EP06-001) was conducted in male and female Wistar rats to compare the toxicological endpoints of filgrastim and Neupogen. Both products were given s.c. once daily over a period of 28 days. Three groups of rats were treated with three different doses of filgrastim (20, 100 and 500 µg/kg b.w.) and 2 groups were treated with Neupogen doses corresponding to the low and high doses of filgrastim. One group was treated with vehicle (formulation buffer) and served as a control.

The low dose (20µg/kg/day) was chosen to correspond to the highest human dose (24µg/kg/day) recommended for patients with severe chronic neutropenia. The high dose (500µg/kg/day) was chosen as approximately equivalent to 20-fold the highest human dose (24µg/kg/day) recommended for severe chronic neutropenia and about 50-fold above the dose for mobilization of autologous peripheral blood progenitor cells (10µg/kg/day). The intermediate dose was chosen as the logarithmic mean between the high and the low dose.

During the treatment period, the numbers of WBC and in particular the number of neutrophils were increased above the normal range in filgrastim and in Neupogen dose groups (Table 5). For both filgrastim and Neupogen, the increase in the WBC number was dose-dependent on days 14 and 28 and was more marked in males than in females. At the end of the recovery period (study day 70), WBC levels had returned to the normal range of variation for this species. Mean serum G-CSF levels decreased from more than 300 pg/ml in the high dose groups (500µg/kg) to non-detectable levels at day 70. A relatively small but significant increase in neutrophil count was observed on day 3 in all animals treated with filgrastim, with the exception of the low-dose group females. On study day 14, the number of neutrophils had further increased dose-dependently reaching levels 10- to 12-fold higher than controls. No difference was found between the genders. On study day 28, a further increase in the number of neutrophils in males was found in the high dose filgrastim group (20-fold higher than controls), with no change in respective females. At the end of the recovery period, the number of neutrophils had returned to levels within the normal range of variation for this species. The same pattern of neutrophil kinetics was found in Neupogen treated animals.

Table 5 Overview of haematology results

Treatment		Day 14				Day 28			
		male		female		male		female	
		WBC [x10^3/µl]	ANC [x10^3/µl]	WBC [x10^3/µl]	ANC [x10^3/µl]	WBC [x10^3/µl]	ANC [x10^3/µl]	WBC [x10^3/µl]	ANC [x10^3/µl]
Placebo	**Mean**	13.1	1.7	14.2	1.6	17.2	2.3	12.5	1.4
	SD	1.6	0.5	3.1	0.7	2.2	0.9	2.7	0.5
Filgrastim 20 µg/kg	**Mean**	19.5*	7.0**	16.5	4.8**	26.9**	11.3**	14.5	4.4**
	SD	3.6	2.7	3.7	1.5	5.0	3.5	2.5	0.4
Filgrastim 100 µg/kg	**Mean**	25.9**	12.2**	20.9	8.5**	29.9**	17.2**	24.3**	9.2**
	SD	5.2	3.6	6.5	2.9	5.8	5.4	2.3	0.7
Filgrastim 500 µg/kg	**Mean**	30.2**	16.6**	33.3**	19.4**	63.5**	44.3**	36.6**	19.9**
	SD	7.9	6.9	8.7	5.6	9.7	8.7	3.5	1.8
Neupogen 20 µg/kg	**Mean**	20.8**	7. 0**	16.3	5.1**	27.7**	9.8**	17.7	2.6*
	SD	5.0	1.9	1.1	0.6	4.9	3.7	4.0	1.0
Neupogen 500 µg/kg	**Mean**	31.9**	19.3**	33.9**	19.5**	70.6**	45.7**	29.9**	16.7**
	SD	4.1	2.8	3.1	2.9	32.8	25.6	5.4	3.5

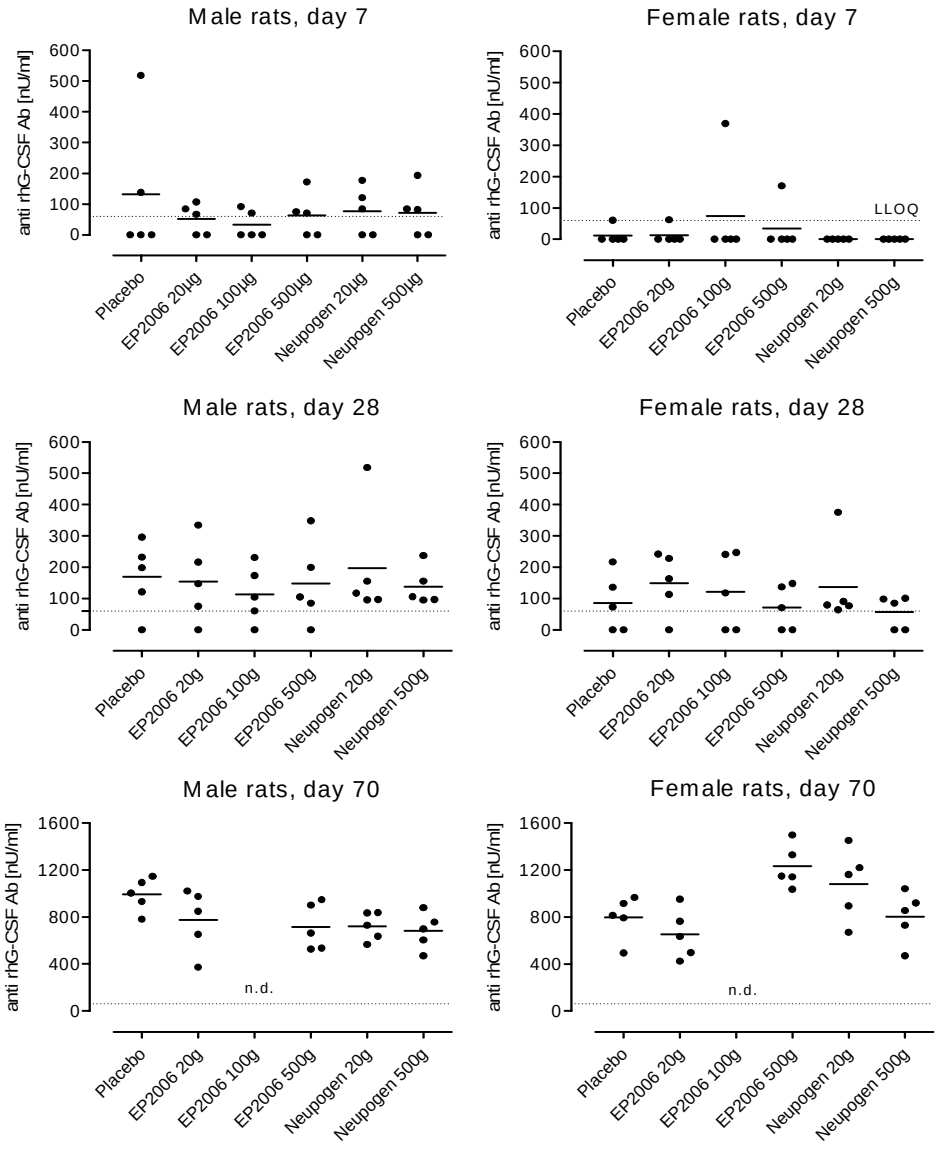
Asterisks indicate significant differences vs. placebo with * $p<0.05$ and ** $p<0.001$.

On day 28 all rhG-CSF levels of test items from the control group and those animals treated with 20µg/kg rhG-CSF (either Neupogen or filgrastim), as well as all from the second withdrawal on study day 70 were below the limit of quantification. Four of ten test items from the animals treated with 100µg/kg filgrastim withdrawn on study day 28 yielded results between 88 and 159pg/ml rhG-CSF. Analysis of all test items from those animals treated with 500µg/ml rhG-CSF (either Neupogen or filgrastim) yielded results within the calibration range of the ELISA: Neupogen-treated animals had rhG-CSF serum concentrations between 243 and 569pg/ml, whereas the concentration of rhG-CSF in serum from filgrastim-treated rats ranged from 266 and 1376pg/ml.

Increased IgG levels were observed with a similar frequency in the filgrastim, Neupogen and control groups. Immunoglobulin A, E, G, and M levels were determined in 110 samples at the end of treatment and at the end of recovery. Samples including those from the control group showed similar IgA, IgE and IgM concentrations on study day 70 compared to study day 28. Increased IgG levels were found in the samples of day 70 compared to the samples of study day 28. This increase was independent of the dose of filgrastim or Neupogen administered and was claimed to be in accordance with the age-dependent increase in IgG reported for rats aged 6 to 30 weeks (Salauze, Serre and Perrin 1994).

Antibodies against rhG-CSF were analyzed on day 7, at the end of the treatment period (day 28) and at the end of the recovery period (day 70) using a validated ELISA assay for the detection of anti-rhG-CSF antibodies. The lower limit of quantification (LLOQ) of the assay was 60 nU/ml. At the end of the 6-week recovery period it could be excluded that active substance was present which might have interfered with the ability to detect anti-drug antibodies. No rhG-CSF was detectable at the end of the recovery period. In Figure 4 the results of the rhG-CSF antibody ELISA in serum of male and female rats on study day 7, 28 and 70 in all groups are shown.

Figure 4 Results of anti-rhG-CSF antibody ELISA at study day 7, 28 and 70 (end of recovery period) in male and female rats.



***EP2006: filgrastim**

n.d. = not determined. Dotted line: lower limit of quantification (LLOQ) = 60 nU/ml.; Note the differences in scale graph day 7 and day 28 versus graph day 70.

The mean concentration detected with the anti-rhG-CSF antibody ELISA in serum of non-treated six week old rats was 330 nU/ml (baseline), a value reflecting a non-specific signal in the antibody assay. All results shown in Figure 4 were corrected by subtraction of this baseline concentration. A signal above the LLOQ was detected in 117 out of 170 samples tested on study days 7, 28 and 70. At the end of treatment week one (day 7) the signals detected in the anti-rhG-CSF antibody ELISA were below the LLOQ in most of the female animals in all treatment groups, whereas a signal above the LLOQ could be detected in serum of two to three males in all groups. However, with a maximum of 200 nU/ml the signal intensity of these male rats was relatively low. During the course of the study, the signal in the anti-rhG-CSF antibody ELISA increased in all tested groups, including the control group although this control group was clearly not treated with the rhG-CSF as the number of neutrophils did not increase during the course of the study. On day 28 the ELISA signals were only slightly higher than on study day 7. Increased levels were independent of drug, dose or gender and were also found in controls of both genders. At the end of the recovery period (day 70) all test items yielded results between 372 and 1499 nU/ml, including the control group. The signal intensities in the anti-rhG-CSF ELISA were higher in females than in males, especially in the high dose group of filgrastim and the low dose group of Neupogen. Throughout the study, slightly increased signals in the

anti-rhG-CSF antibody ELISA were found in both the filgrastim and the Neupogen treatment groups. However, signal intensities were comparable in both treatment groups and were also comparable to those in serum of animals from the control group.

Pre-incubation of serum samples with rhG-CSF in a confirmatory assay did not result in a reduction of the signal in the anti-rhG-CSF antibody ELISA compared to serum samples without pre-incubation.

- Genotoxicity

No genotoxicity studies were submitted.

- Carcinogenicity

No carcinogenicity studies were submitted.

- Reproduction Toxicity

No reproduction toxicity studies were submitted.

- Toxicokinetic data

In the toxicokinetic study EP06-002 fifty animals were treated daily for 14 days with filgrastim or Neupogen and serum kinetics were evaluated after the first and last treatment day to assess the systemic availability of the two compounds. Three groups were treated with filgrastim at dose levels of 20, 100 or 500ug/kg b.w. The concentration of rhG-CSF in rat serum samples taken after single and repeated dosing was determined using a commercially available ELISA kit (Quantikine human G-CSF, R&D Systems) without modifications. In this study, no mortality and no significant alterations in body weight gain were found after administration of filgrastim or Neupogen. At all three dose levels of filgrastim, no significant differences in serum concentrations (C_{max}) and a small increase of the $AUC_{(0\text{-}14d)}$ were found between single and repeated dosing. After repeated treatment with the two Neupogen doses**,** an increase in both serum concentrations (C_{max}) and $AUC_{(0\text{-}14d)}$ was found compared to single dosing (Table 6). Dose-exposure linearity was found for filgrastim. Serum exposure under Neupogen increased with repeated dosing as compared to study day 0.

Table 6 Tabulated summary of main toxicokinetic parameters

Treatment	**Filgrastim**						**Neupogen**			
Study day	0	13	0	13	0	13	0	13	0	13
Dose µg/kg	20		100		500		20		500	
C_{max} [ng/ml]	94	97	425	459	2527	2378	44	80	2458	2817
t_{max} [h]	1.0	3.0	1.0	3.0	1.0	1.0	1.0	3.0	1.0	1.0
$AUC_{0\text{-}14d}$ [ng*h/ml]	574	691	2848	3184	14838	15788	281	438	12681	19129

- Local tolerance

In study EP06-001, a local tolerance test performed in 36 female rabbits, the local tolerability of two formulations of filgrastim containing acetate buffer (Neupogen-like) and containing glutamate buffer (filgrastim-final formulation) was compared to the reference product Neupogen. Two groups of animals were treated with 480 µg/0.5 ml filgrastim in two different formulations and with saline 0.9% as contralateral control and the third group was treated with 480 µg/0.5 ml Neupogen and saline 0.9% as control. The dose was administered as a single bolus injection by the i.v., paravenous (p.v.), s.c., intramuscular (i.m.), or intraarterial (i.a.) route. This dose corresponds to the highest concentration of

rhG-CSF administered to humans as bolus injection. Both formulations of filgrastim were equally well tolerated as the reference product Neupogen and showed similar local tolerability to the control (data not shown). No clinically relevant reactions were observed.

- Other toxicity studies

No other toxicity studies were submitted.

Ecotoxicity/environmental risk assessment

No environmental risk assessment was submitted.

Discussion on the non-clinical aspects

The non-clinical evaluation of filgrastim was performed in full accordance with the EMEA guideline on biosimilar G-CSF (EMEA/CHMP/31329/05. In line with the guideline, no secondary pharmacodynamics, safety pharmacology, pharmacokinetics, single-dose toxicity, carcinogenicity or reproductive toxicity studies were performed.

For the repeat-dose toxicology (study EP06-001) and toxicokinetic (study EP06-002), filgrastim drug substance was formulated in the reference (Neupogen) formulation. The local tolerance (study EP06-003) as well as the pharmacology (study EP06-004) were done with the glutamic acid containing formulation. All clinical studies were done with the glutamic acid formulation.

Filgrastim and Neupogen showed comparable ability to interact with the G-CSF receptor in an *in vitro* NFS-60 cell assay. In normal and neutropenic rats, at all dose levels tested, filgrastim and Neupogen resulted in a dose-dependent increase of ANC. Similar pharmacodynamic response to the two compounds was noted in the comparative animal study across a wide dose range.

The toxicity of filgrastim was assessed in a 4-week repeated dose study conducted at doses of 20, 100 and 500 µg/kg. There were no deaths. There were overt signs of toxicity and body weight was reduced in only a limited number of animals. These effects were dose dependent and gradually disappeared during the 6 weeks recovery period. The treatment related effects were limited to those anticipated after G-CSF administration such as slight increase in spleen weight and myeloid hyperplasia in the bone marrow and probably represent an exaggerated pharmacological response.

The toxicokinetics confirmed that the test animals were systemically exposed to the test compounds, and there was dose-linearity in the case of filgrastim.

Both filgrastim formulations showed identical local tolerance results and since both formulations are composed of well known and well characterised excipients it can be expected that the tolerability of the glutamate formulation will be similar to that of the acetate formulation.

The immunogenicity of filgrastim was assessed as part of the toxicity study. Increased signals in the anti-rhG-CSF antibody ELISA were detected both in filgrastim and Neupogen treated and control rats in the 28-day study. Signal intensities were found to increase with time in both filgrastim and Neupogen as well as in the control group and were comparable in all treatment groups including the control group. This effect was considered to be a combined phenomenon of unspecific binding and normal age-dependent increase of IgGs in rats and it may be concluded that the observed increase in ELISA reactivity was due to the assay design and no formation of anti-rhG-CSF antibodies occurred during the study period. Whilst immunogenicity results in the rat might not be fully predictive for human risk assessment, it is worth noting that no difference between filgrastim and Neupogen and control animals was detected in this study. No antibodies against filgrastim were reported in the clinical studies.

Other toxicity studies are not required for a biosimilar G-CSF development according to the respective EMEA guideline (EMEA/CHMP/31329/05).

The amino acid sequence of human filgrastim occurs naturally and is composed entirely of naturally occurring amino acids and would be expected to react like a naturally occurring protein both *in vivo*

and in the environment. According to the CHMP guideline on 'Environmental risk assessment of medicinal products for human use' (EMEA/CHMP/SWP/4447/00), an environmental risk assessment is not required in the cases of vitamins, electrolytes, amino acids, peptides, proteins, carbohydrates and lipids because they are unlikely to result in significant risk to the environment."

In conclusion, the pharmacodynamic and toxicologic studies performed by the applicant were in accordance with the EMEA guidance for biosimilar rhG-CSF development. The preclinical program confirmed that the activity and toxicity is equivalent between filgrastim and Neupogen.

2.4 Clinical aspects

Introduction

Four PK/PD studies were conducted in healthy volunteers in order to demonstrate comparability of pharmacokinetic characteristics of both products as well as pharmacodynamics, in accordance to CHMP scientific advice. Two routes (intravenous and subcutaneous) and four doses were tested, which span the steep part of the dose-response curve: 1, 2.5, 5 and 10 µg/kg. The design of the three PK/PD phase I studies is summarised in Table 7.

Table 7 PK/PD studies

Study	Design	Study population	Dose and regimen	Objectives
EP06-101 Phase I	Randomized, double blind, 2-way crossover	40 Healthy volunteers	Multiple s.c. doses EP2006 and Neupogen® 10 µg/kg/day	Primary: PK bioequivalence Secondary: PD, safety
EP06-102 Phase I	Randomized, double blind, 2-way crossover	26 Healthy volunteers	Single i.v. dose EP2006 and Neupogen® 5 µg/kg	Primary: PK bioequivalence Secondary: PD, safety
EP06-103 Phase I	Randomized, double-blind, 2-way crossover, with two dose groups	2 x 28 Healthy volunteers	Multiple s.c. doses EP2006 and Neupogen® two different doses 2.5 µg/kg/day 5 µg/kg/day	Primary: PD equivalence Secondary: Safety, PK
EP06-105 Phase I	Randomized, double blind, 2-way crossover	24 Healthy volunteers	Single s.c. dose EP2006 and Neupogen® 1 µg/kg	Primary: PD equivalence Secondary: PK, safety

*EP2006: filgrastim

GCP

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

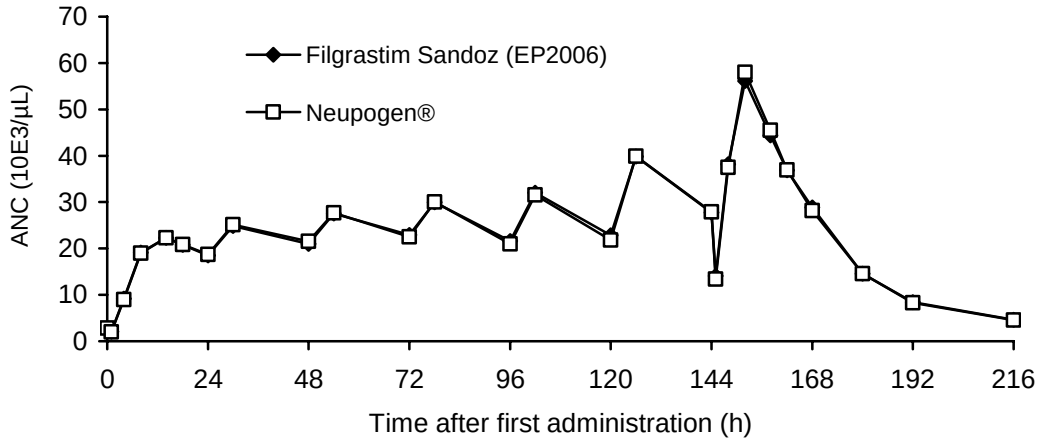
The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Pharmacodynamics

PD activity was based primarily on ANC peak response and ANC exposure, i.e. the whole AUC over 10 days. The results of these studies support the comparability of the test and reference products with respect to their pharmacodynamic effect since absolute neutrophil count (ANC) curves are superimposable whatever the route and the dose.

An example is shown in figure 5 for a 7-day treatment course at the dose of 5 µg/kg/d subcutaneously.

Figure 5. Geometric Mean of ANC after repeated sc injection (5 µg/kg/d)



The results for the primary endpoint of the area under the effect curve (AUEC) calculated from the first time of administration to the last blood sampling are summarised in Table 8 for the different routes and doses. AUEC increases with increasing dose but the dose response curve is rather flat for both products; while doubling the dose from 2.5 to 5 µg/kg, AUEC only increases by about 20%.

Table 8 AUEC of absolute neutrophil count - 95% confidence intervals for the ratio of means

Parameter (10^3·h/µl)	Dose (µg/kg)	Route	Filgrastim Geometric mean	Neupogen Geometric mean	Ratio (%)	95% CI Lower bound	95% CI Upper bound
$AUEC_{0\text{-}120h}$	5	i.v.	944.72	950.19	99.42	93.52	105.70
$AUEC_{0\text{-}120h}$	1	s.c.	740.78	725.00	102.29	97.15	107.71
$AUEC_{0\text{-}216h}$	2.5	s.c.	4224.0	4134.5	102.16	99.49	104.91
$AUEC_{0\text{-}216h}$	5	s.c.	5191.8	5176.8	100.61	98.01	103.29
$AUEC_{0\text{-}216h}$	10	s.c.	6474.5	6515.3	99.37	96.30	102.54

* Predefined equivalence intervals: 2.5 µg/kg/day: 87.25% – 114.61%; 5/10 µg/kg/day: 86.50% – 115.61%

The $CD34^+$ cell count after repeated dosing (secondary PD endpoint) showed a similar time profile for filgrastim and Neupogen and $AUEC_{0\text{-}216h}$ data are summarised in Table 9.

Table 9 $AUEC_{0\text{-}216h}$ of absolute $CD34^+$ count - 95% confidence intervals for the ratio of means

Parameter	Dose (µg/kg)	Route	Filgrastim Geometric mean	Neupogen Geometric mean	Ratio (%)	95% CI Lower bound	95% CI Upper bound
$CD34^+$ (h/µL)	2.5	s.c.	2815.1	2694.0	104.49	96.51	113.14
	5	s.c.	2885.5	2898.3	98.99	86.79	112.90
	10	s.c.	5129.3	5023.3	102.11	93.53	111.47

Pharmacokinetics

AUCs and Cmax

The results of the 24-hour AUCs and C_{max} after the first and 7th dose - as estimated by standard non compartmental PK analysis - are presented in Tables 10 and 11, respectively. Repeated dosing led to a decrease in serum G-CSF exposure as shown by a 2-3 fold decrease in C_{max} and 3-5 fold decrease in 24-hour AUC from Day 1 to Day 7. This is in line with the predominant receptor mediated clearance of filgrastim; while neutrophil counts increase in response to repeated dosing of filgrastim, clearance of filgrastim is enhanced.

Table 10 Comparisons of AUC

Parameter	Dose (µg/kg)	Route	Geometric means EP2006	Geometric means Neupogen	Ratio (%)	90% Confidence Interval Lower bound	90% Confidence Interval Upper bound	Intra-ind. CV (%)
$AUC_{0\text{-}24h,sd}$ (ng.h/mL)	5	i.v.	599.1	634.2	94.48	91.89	97.13	5.6
	1	s.c.	58.3	65.7	88.43	83.37	93.81	11.7
	2.5	s.c.	117.7	136.8	86.08	79.68	92.99	17.1
	5	s.c.	354.7	383.7	91.83	86.50	97.49	12.9
	10	s.c.	828.1	908.1	91.84	87.53	96.35	11.4
$AUC_{144\text{-}168h,ss}$ (ng.h/mL)	2.5	s.c.	41.9	49.1	84.78	79.31	90.63	14.7
	5	s.c.	101.6	121.9	83.24	77.49	89.42	15.5
	10	s.c.	172.9	193.1	89.53	83.28	96.25	17.2

*EP2006: filgrastim

Table 11 Comparisons of Cmax

Parameter	Dose (µg/kg)	Route	Geometric means EP2006	Geometric means Neupogen	Ratio (%)	90% Confidence Interval Lower bound	90% Confidence Interval Upper bound	Intra-ind. CV (%)
$C_{max,0\text{-}24h,sd}$ (ng.h/mL)	5	i.v.	176.7	188.7	93.67	90.77	96.66	6.3
	2.5	s.c.	16.6	19.5	85.25	77.39	93.90	21.5
	5	s.c.	46.2	49.9	91.87	84.99	99.30	16.8
	10	s.c.	96.4	110.3	87.61	81.35	94.35	17.6
$C_{max,144\text{-}168h,ss}$ (ng.h/mL)	2.5	s.c.	7.3	9.5	76.53	69.69	84.06	20.8
	5	s.c.	20.9	27.2	76.68	67.23	87.45	28.8
	10	s.c.	34.5	39.1	88.44	80.70	96.92	21.8

*EP2006: filgrastim

The standard acceptance range of 80-125% is recommended in the Guideline to show biosimilarity of G-CSFs. At the lower doses and after a multiple s.c. dose of 5 µg/kg, AUC and C_{max} failed to meet the bioequivalence criteria (shaded values). Serum levels of free G-CSF were lower after the administration of filgrastim than after that of Neupogen; the difference appeared consistent across the routes and doses and was statistically significant since the confidence intervals were entirely below the 100% value.

According to the applicant, this difference was unlikely to be due to a different level of sensitivity of the G-CSF analytical assay or to a systematic difference in baseline neutrophil counts, which were very similar between the two products; it had also been confirmed that both products exhibited the same stability in blood. Furthermore, different batches of drug substance with very similar potency

were used in the trials. The applicant claimed that the observed differences were due to differences in the levels of purity of the two products, leading to a systematic bias toward an apparently increased bioavailability for the reference product.

Table 12 ELISA-detectable content (mg/ml) of the various batches

Study	EP2006	Neupogen	Nominal content
EP06-101	0.996	1.010	0.960
EP06-102	0.983	1.037	0.960
EP06-103 - 2.5 µg/kg	0.626	0.636	0.600
EP06-103 - 5 µg/kg	0.985	1.028	0.960
EP06-105	0.615	0.634	0.600

*EP2006: filgrastim

A recalculation of the bioequivalence ratios using the ELISA-detectable dose (Table 12) is displayed in Table 13.

Table 13 Comparisons of PK parameters adjusted to the ELISA-detectable dose

	Study EP06-	Dose µg/kg	Route	Ratio %	Lower %	Upper %
AUC0-24h,sd						
(ng h/mL)	102	5	i.v.	99.68	96.94	102.47
	105	1	s.c.	91.17	85.95	96.72
	103	2.5	s.c.	87.46	80.95	94.48
	103	5	s.c.	95.87	90.31	101.78
	101	10	s.c.	93.13	88.76	97.70
AUC144-168h,ss						
(ng h/mL)	103	2.5	s.c.	86.14	80.58	92.08
	103	5	s.c.	86.90	80.90	93.35
	101	10	s.c.	90.78	84.45	97.60
Cmax,0-24h,sd						
(ng/mL)	102	5	i.v.	98.82	95.76	101.98
	105	1	s.c.	88.50	81.55	96.03
	103	2.5	s.c.	86.61	78.63	95.40
	103	5	s.c.	95.91	88.73	103.67
	101	10	s.c.	88.84	82.49	95.67
Cmax,144-216h,ss						
(ng/mL)	103	2.5	s.c.	77.75	70.81	85.40
	103	5	s.c.	80.05	70.19	91.30
	101	10	s.c.	89.68	81.83	98.28

Based on the ELISA-detectable dose, the confidence intervals for all AUCs were contained within the standard acceptance interval. As for C_{max}, it remained outside the standard acceptance interval of 80-125% following a single s.c. dose of 2.5 µg/kg (but not 1 µg/kg) and a multiple dose of both 2.5 and 5 µg/kg (shaded values).

Elimination parameters

Equivalence of the elimination half-life was also studied. However, there is no true half-life for G-CSF, due to the saturable capture of the drug by the receptor and the stimulation of the G-CSF receptor by the drug, which is dose and time-dependent. Consequently, there is no log-linear phase over an entire concentration time profile.

At 10 µg/kg after single dose, the log-linear phase was apparent because there was no sampling

beyond 24 hours and the receptor system was always saturated. After multiple dose administration, however, several subjects had only two points that could be used for the estimation of k_{el} since the concentration-time curve did not exhibit a log-linear decay for more than these timepoints. Therefore, the comparison between the two treatments was additionally carried out for the subgroup of subjects (14 out of 32) for whom the estimates of k_{el} and $t_{1/2}$ were based on at least three points. The two treatments were compared with respect to k_{el} and $t_{1/2}$ by means of 90% confidence intervals calculated from an ANOVA model identical to the one for the PK bioequivalence assessment. The ratios and corresponding 90% CIs show the equivalence between the two treatments in terms of the elimination half-lives. The comparison of elimination parameters is shown in table 14.

Table 14 Comparison of elimination parameters at 10 µg/kg/day

Comparison	Para-meter	Point Estimator (%)	90% Confidence interval Lower limit (%)	Upper limit (%)
Single dose with all subjects	$t_{1/2}$	**102.56**	**98.09**	**107.23**
(n = 32)	k_{el}	**97.50**	**93.26**	**101.94**
Multiple dose with all subjects	$t_{1/2,md}$	115.75	95.53	140.25
(n = 28)	$k_{el,md}$	86.38	71.28	104.67
Multiple dose with reduced subject subgroup	$t_{1/2,md}$	**100.53**	**84.33**	**119.83**
(n = 14)	$k_{el,md}$	**99.46**	**83.43**	**118.56**

The results for the 2.5 µg/kg and the 5 µg/kg dose for both single and multiple dose application are shown in Table 15. While the single-dose elimination half-lives were still within the bioequivalence limits the differences between the two treatments seemed to increase with the number of applications, the effect being more pronounced in the lower dose group. However, the applicant claimed that due to the non-linear pharmacokinetics of filgrastim the estimation of the elimination parameters in the non-compartmental analysis did not properly describe the actual elimination of the drug.

Table 15 Comparison of elimination parameters at 2.5 and 5 µg/kg/day

Dose group	Comparison	Para-meter	Mean EP2006	Neupogen®	Point Estimator (%)	90% Confidence Interval Lower limit (%)	Upper limit (%)
2.5µg/kg	Single dose	$t_{1/2}$	6.5257	6.0792	107.19	97.88	117.38
(n = 28)		k_{el}	0.1181	0.1266	93.29	85.19	102.16
	Multiple dose	$t_{1/2,md}$	21.8576	17.1331	126.07	106.58	149.14
		$k_{el,md}$	0.0381	0.0472	79.32	67.04	93.84
5.0µg/kg	Single dose	$t_{1/2}$	4.1257	4.1683	100.28	92.59	108.61
(n = 27)		k_{el}	0.1789	0.17956	99.72	92.08	108.00
	Multiple dose	$t_{1/2,md}$	26.3038	38.8818	90.99	74.29	111.44
		$k_{el,md}$	0.0373	0.0328	109.94	89.76	134.64

Discussion on Clinical Pharmacology

The primary pharmacodynamic endpoint met the predefined comparability criterion and, even if the comparability range calculated by the Applicant may be questioned, the actual results are very close. The predefined equivalence boundaries were derived by the Applicant from published data on the effect observed for Neupogen compared to placebo. It was assumed that the smallest clinically relevant difference in PD response between the test and reference product was 15% of the effect observed for Neupogen compared to placebo in the published study. Decreasing this margin to 10%, which approximately corresponds to half the increase in AUEC between the 2.5 and 5 µg/kg doses, would result in more acceptable equivalence intervals; indeed, the 95% CI for ANC AUEC and E_{max} in study EP06-103 would still fall within these tighter equivalence boundaries.

A statistically significant period effect was reported in all the cross-over studies, the ANC response in the second period being generally greater than in the first period. However, this effect was roughly the same whatever product was administered first. In addition, a post-hoc analysis restricted to the first period also supported the comparability of the ANC response.

It is noteworthy that, in spite of the content correction in pharmacokinetic studies, most results still demonstrate that the subcutaneous route produces significantly lower concentrations of free filgrastim in the serum with filgrastim than with Neupogen. However, it is reassuring that the results after correction provide point estimates for the ratios of the intravenous infusion that are very close to 100% with CIs including 100%.

It is acknowledged that the apparent difference in bioavailability may be overestimated due to the non linear saturable pharmacokinetics of rhG-CSF, which is eliminated for a large part through binding to its target cells, neutrophils and myeloid progenitors. Indeed, the difference in elimination characteristics at different doses may be related to the fact that receptor-mediated clearance (which is saturable) is predominant at lower doses, while renal clearance becomes more important at higher doses.

The Applicant also performed a modelling exercise to interpret serum concentration data in the context of the target-mediated nonlinear pharmacokinetics; this also allowed comparing relevant PK parameters which cannot be estimated through non-compartmental analysis, such as clearances and volumes of distribution (data not shown). First, an individual best fit model was developed and to further test its robustness under multiple dosing, two types of population models were developed: an empirical population model directly derived from the individual best fit model and a mechanistic population model taking into account the target mediated drug disposition. From these population model analyses, it could be concluded that the main findings of the individual best fit model were confirmed, even though these models are quite different and are built based on different assumptions and different parameters. In either approach, all parameters estimated satisfied the common bioequivalence criteria for all doses and for both routes of administration.

The Applicant also provided a comprehensive demonstration of the mechanism underlying the inflation of the difference between the two products as doses are decreased or repeated. Since the greatest inflation occurs when there are high levels of receptors relative to drug, the PK differences observed in healthy volunteers are likely to substantially overestimate differences in the target neutropenic population.

In conclusion, the small differences observed in the pharmacokinetic profile of filgrastim are not expected to translate into significant differences in the PD response, which is related to the amount of filgrastim bound to its target cells.

Clinical efficacy

The comparability of the efficacy based on a PD study in healthy volunteers was considered acceptable by the CHMP in their Scientific Advice to the Applicant. Furthermore, the extrapolation to all indications of the reference products was considered acceptable since the mechanism of action is the same, i.e. direct stimulation of bone marrow cells through one specific type of surface receptor (EMEA/CHMP/BMWP/31329/2005). Thus, the applicant only submitted a supportive clinical efficacy study.

- Supportive studies

Supportive evidence of efficacy was provided by the results of a phase III study, the primary objective of which was the evaluation of the safety, tolerability and immunogenicity of filgrastim. Study EP06-301 was designed as an open, single-arm, multicentre study in chemotherapy-naïve breast cancer patients receiving doxorubicin and docetaxel chemotherapy and filgrastim as primary prophylaxis of severe neutropenia. Treatment consisted of filgrastim from day 2 of each chemotherapy cycle for up to 14 days (or until ANC reached 10 x 10^9/L post nadir), repeated for up to 4 cycles. The total daily dose was 30 MIU for women weighing <60 kg and 48 MIU for women weighing ≥60 kg. Each subject was expected to participate in the study for approximately 6 months, including three months of active treatment (4 treatment cycles) and 3 months of follow-up after the last treatment cycle. The main efficacy variables were the incidence and duration of severe neutropenia in cycles 1 to 4, the incidence of febrile neutropenia, the time to neutrophil recovery. The main results are shown in Table 16

together with data on the available published data for the reference product Neupogen (*Holmes, 2002 and Green, 2003*, see references below).

Table 16 Incidence and duration of grade 4 neutropenia.

	Incidence				Duration (days)			
	EP2006		Neupogen		EP2006		Neupogen	
Cycle	N = 170 N	n (%)	Holmes N=151 n (%)	Green N=75 %	(1) Mean ± SD	(2) Mean ± SD	Holmes Mean ± SD	Green Mean ± SD
1	170	80 (47%)	116 (79%)	83%	2.2 ± 0.9	1.8 ± 1.4	1.8 ± 1.4	1.6 ± 1.1
2	162	25 (15%)	81 (56%)	54%	1.8 ± 0.6	1.3 ± 0.5	1.1 ± 1.1	0.9 ± 1.0
3	159	33 (21%)	86 (60%)	53%	1.9 ± 0.9	1.4 ± 0.6	1.2 ± 1.4	0.9 ± 1.1
4	154	27 (18%)	78 (55%)	49%	2.1 ± 0.8	1.7 ± 0.6	1.3 ± 1.5	1.0 ± 1.3

*EP2006: filgrastim
(1) Recovery to ANC≥1.0x10^9/L
(2) Consecutive days

Green MD et al
A randomized double-blind multicenter phase III study of fixed-dose single-administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy.
Ann Oncol. 2003 Jan;14(1):29-35

Holmes FA et al
Blinded, randomized, multicenter study to evaluate single administration pegfilgrastim once per cycle versus daily filgrastim as an adjunct to chemotherapy in patients with high-risk stage II or stage III/IV breast cancer.
J Clin Oncol. 2002 Feb 1;20(3):727-31

Discussion on clinical efficacy

The comparability of the efficacy based on a PD study in healthy volunteers was considered acceptable by the CHMP in their Scientific Advice to the Applicant. Furthermore, the extrapolation to all indications of the reference products is acceptable since the mechanism of action is the same, i.e. direct stimulation of bone marrow cells through one specific type of surface receptor (EMEA/CHMP/BMWP/31329/2005).
The supportive trial was non comparative and therefore of limited usefulness for the assessment of the comparability of the test and reference products.

Clinical safety

- Safety in healthy volunteers

A comparison of the safety profile of the test and reference products was provided based on the four studies in 146 healthy volunteers. ADRs were consistent with those reported in normal donors as described in AMGEN Neupogen SmPC and were similar for both products (data not shown).

The evaluation of the immune response after rhG-CSF administration was made by a three-step procedure comprising a validated radioimmunoprecipitation assay and a validated cell-based neutralization antibody assay. Serum samples for antibody analysis were taken at baseline (screening), one hour before the start of treatment period II and at follow-up. At these time points, the presence of active substance, which might interfere with the ability to detect anti- rhG-CSF antibodies, may be excluded because rhG-CSF levels would have returned to normal values. Samples were taken in Study EP06-102 – single iv dose, Study EP06-103 – repeated sc dose (2.5 and 5 µg/kg), and Study EP06-101 – repeated sc dose (10 µg/kg). None of the volunteers developed anti-rhG-CSF binding antibodies at any time-point of the studies.

- Patient exposure

The clinical study EP06-301 was primarily designed to assess the safety and immunogenicity of filgrastim in 170 breast cancer patients (see clinical efficacy). The patient exposure is summarised in Table 17. The mean total daily dose was approximately 440 µg (44 MU). The mean cumulative dose ranged from 3323 to 3834 µg across cycles and was 13612 µg for the whole treatment period.

Table 17 Patient exposure to filgrastim in study EP06-301

		All Cycles (n=170)	Cycle 1 (n=170)	Cycle 2 (n=162)	Cycle 3 (n=159)	Cycle 4 (n=154)
Extent of exposure (days)	Mean	31.0	8.7	7.6	8.1	8.3
	SD	7.86	1.94	2.11	2.05	1.67
	Median	33.0	9.0	8.0	8.0	8.0
	Minimum	6	2	2	2	2
	Maximum	48	14	12	14	12

Of the 170 enrolled patients, 154 (91%) patients received 4 chemotherapy treatments. One patient withdrew during the fourth cycle; therefore, 153 patients completed four treatment cycles. The mean extent of exposure to the chemotherapy was 81 days, with a range of 21 to 113 days.

The doses of doxorubicin (60 mg/m^2) and docetaxel (75 mg/m^2) had to be reduced by 25% to 45 mg/m^2 and 56.25 mg/m^2, respectively, in 10 (6%) patients due to febrile neutropenia.

- Adverse events

Treatment emergent adverse events (TEAEs) were defined as AEs reported after the first dose of study chemotherapy through 30 days after the last dose.

A total of 1583 TEAEs were reported during the study affecting all 170 patients. The analysis of the adverse events distinguished between "G-CSF associated adverse events" (i.e. increases in AST, LDH, arthralgia, myalgia, back pain, bone pain, pain in extremities) and all other events identified as "non G-CSF associated adverse events".

Among the latter events (1494), a relationship to filgrastim was suspected for 50 (3%) AEs reported during the treatment period, while a relationship to study chemotherapy was suspected for 1264 (85%) TEAEs.

Of the 89 G-CSF associated events, 44 (49%) were considered to be related to filgrastim while 49 (54%) were suspected to chemotherapy-related. The intensities of the G-CSF related adverse events were mainly mild (89%) or at most moderate (11%) (see Table 18).

Table 18 Overall summary of TEAEs

	G-CSF-associated adverse events	Non G-CSF-associated adverse events
No. of subjects dosed	170	170
No. of subjects with AEs (%)	39 (22.9%)	168 (98.8%)
No. of AEs	89	1494
Severity		
Mild	79 (88.8%)	557 (37.3%)
Moderate	10 (11.1%)	311 (20.8%)
Severe	0 (0.0%)	231 (15.5%)
Life-threatening[#]	0 (0.0%)	208 (13.2%)
Death	0 (0.0%)	0 (0.0%)
Relationship to study drug		

	G-CSF-associated adverse events	Non G-CSF-associated adverse events
Not suspected	45 (50.6%)	187 (12.5%)
Suspected to filgrastim*	44 (49.4%)	50 (3.3%)
Suspected to chemotherapy*	48 (53.9%)	1264 (84.6%)

* An adverse event can be related to both filgrastim and chemotherapy

Life-threatening AEs consisted aside from the events also reported as SAEs of chemotherapy induced changes in the laboratory parameters (neutropenia (161/77%), leucopenia (35/17%), lymphopenia (1/0.5%), granulocytopenia (1/0.5%)

Table 19 provides more details about the G-CSF associated AEs. Musculoskeletal pain was reported in 35 patients (21%) while transient reversible increases in AST and LDH were observed in 4 patients (2%).

Table 19 Protocol identified G-CSF associated AEs

System organ class / preferred term	Patients (N = 170)	Events (N = 1583)
Laboratory evaluations/Investigations	4 (2.4%)	4
AST increased	2 (1.2%)	2
LDH increased	2 (1.2%)	2
Musculoskeletal and connective tissue disorders	35 (20.6%)	85
Arthralgia	7 (4.1%)	9
Back pain	1 (0.6%)	1
Bone pain	18 (8.8%)	31
Myalgia	23 (13.5%)	44

Among the non G-CSF associated adverse events suspected to be related to filgrastim, only "fatigue" was reported in more than a single patient (6 cases); this common event was usually attributed to chemotherapy.

Local tolerance at the injection site was to be summarised by signs/symptoms, relationship to filgrastim and severity for related events. No occurrences of itching, redness, swelling, pain, ulcerations or other signs and symptoms of poor local tolerability of filgrastim were observed.

- Serious adverse event/deaths/other significant events

Three patients died during the follow-up period, two due to disease progression (both these patients had stage IV breast cancer at screening) and one due to injuries suffered in a car accident.

Twenty patients reported 23 SAEs, including the 3 deaths described above. The other 20 other SAEs included: febrile neutropenia (14): grade 4 (11), grade 3 (2), grade 2 (1); diarrhea (2 patients); anaemia (1); atrial fibrillation (1); hepatitis (1); hypertensive crisis (1). Three patients were withdrawn from the study due to AEs: one case of febrile neutropenia in the first cycle, which resolved in 25 days; one case of atrial fibrillation in the 2nd cycle, which resolved in 2 days; one case of non-serious allergic dermatitis in the face beginning on Day 7 of the 3rd cycle, which resolved in 21 days; as the investigator considered the relationship to filgrastim highly probable, the patient did not receive a 4th chemotherapy cycle.

- Laboratory findings

During the treatment period, none of the patients had a CTC grade 4 value for any biochemistry parameter. Grade 3 values were observed only for alkaline phosphatase (1 patient) and gamma glutamyl transferase (4 patients). As expected, all patients had at least one haematological abnormality, in keeping with myelosuppression associated with chemotherapy.

Only a small fraction of laboratory abnormalities were judged by the investigator to be clinically significant at any time during the treatment period: ALT (3 patients; 1.8%), AST (2 patients; 1.2%); LDH (2 patients; 1.2%). Even for haematology parameters, <2% of patients had clinically significant abnormalities during the treatment period.

- Immunogenicity studies

Serum samples for antibody analysis were taken at baseline (screening) or at day 1 of cycle 1 (C1D1), at day 1 of cycle 2 (C2D1), at day 21 of cycle 4 (C4D21) and at study termination (day 91). At the chosen time-points, the presence of active substance, which might interfere with the ability to detect anti- rhG-CSF antibodies, is not expected. Table 20 summarizes the assessment of antibody formation in serum samples collected during the study.

Table 20 Results of antibody measurements in study EP06-301

			Results above cut-off, n (%)	
Time point	**Number of patients**	**Samples available**	**Screening RIP assay [1]**	**Confirmatory RIP assay [2]**
Baseline / Cycle 1	170	170	2 (1.2)	0 (0)
Cycle 2 (Day 1)	162	158	4 (2.5)	0 (0)
Cycle 4/End of treatment (Day 21)	162	160	1 (0.6)	0 (0)
Study termination (90 day follow-up)	153	151	7 (4.6)	0 (0)

Four unscheduled visits were additionally analysed in the screening RIP and were found to be negative for anti-rhG-CSF binding antibodies.
[1] Study-specific cut-off: 2.27% total binding determined by measuring sera of 170 patients in the screening RIP assay before treatment with rhG-CSF (baseline/cycle 1)
[2] Positive if depletion rate >20%

All samples were first analysed in the screening RIP assay. Of the 643 analysed samples, 629 were negative. The fourteen samples with total binding values >2.27% were re-analysed in a confirmatory RIP assay. As all depletion rates were <20%, the samples were qualified as negative. Thus, none of the patients developed anti-rhG-CSF binding antibodies at any time-point of the study.

- Discussion on clinical safety

A direct comparison of the safety profile of the test and reference products is possible from the four studies in 146 healthy volunteers (data not shown). ADRs were consistent with those reported in normal donors as described in the Neupogen SmPC and were similar for both products. Overall, these data support the comparability of the products.

The clinical study EP06-301 was primarily designed to assess the safety and immunogenicity of filgrastim in 170 breast cancer patients. As for efficacy evaluation, the type of patients included in the trial and the differences in exposure hamper a proper comparison with historical data of Neupogen; therefore, these safety data are considered of limited value to support the comparability of the test and reference products.

Overall, filgrastim was very well tolerated by these cancer patients although their exposure to filgrastim was not consistent with the literature data for the reference product. Indeed, since the dose was not adjusted to bodyweight, only 22% of the patients received doses included between 4.5 and 5.5 µg/kg while 75% received higher doses up to 8 µg/kg.

In addition, due to misunderstanding of the treatment protocol by some investigators, a small number of patients received unduly short treatment courses.

Immunogenicity data in healthy volunteers and in cancer patients showed no evidence of IgG antibody formation against rhG-CSF, a finding consistent with the known low immunogenicity of Neupogen. Since PK and PD results are considered sufficiently comparable to support the biosimilarity of the test and reference products, a robust (head to head) comparison of their long-term safety is less critical. The small single-arm trial submitted by the Applicant allows, to a certain extent, to rule out any

unexpected safety issue and suggests low immunogenicity of the test product. Additional safety and immunogenicity data will be collected post-marketing within the frame of two registries included in the Risk Management Plan.

Ongoing safety follow-up will be performed on healthy subjects included in the Phase I studies (EP06-103) and two post-marketing studies: a phase IV study in patients with severe chronic neutropenia (12 months of treatment) with an extended follow-up within the frame of the SNC European registry (5 years in total) and a follow-up of healthy stem cell donors undergoing PBPC mobilisation in co-operation with apheresis centres (5 years after mobilisation). The Applicant has committed to submit the protocols and study reports of these three studies and they have been included as follow-up measures.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan (see summary in Table 21).

Table 21 Summary of the Risk Management Plan

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Severe splenomegaly / splenic rupture	**Routine** Routine pharmacovigilance reporting **Additional** 1) Pharmacovigilance program in patients with severe chronic neutropenia a) Phase IV study b) Safety follow-up of study patients in co-operation with the SCN European registry 2) Co-operation with apheresis centres for healthy stem cell donors	**Routine (labelling)** • Splenomegaly and splenic rupture are mentioned in section 4.8 of the SPC • Statement in section 4.4 of the SPC that splenic enlargement is a direct effect of the treatment with filgrastim Therefore, spleen size should be carefully monitored (e.g. clinical examination, ultrasound). A diagnosis of splenic rupture should be considered in donors and/or patients reporting left upper abdominal pain or shoulder tip pain.
Serious pulmonary adverse events: Interstitial pneumonia, adult respiratory distress syndrome (ARDS)	**Routine** Routine pharmacovigilance reporting **Additional** 1) Pharmacovigilance program in patients with severe chronic neutropenia a) Phase IV study b) Safety follow-up of study patients in co-operation with the SCN European registry 2) Co-operation with apheresis centres for healthy	**Routine (labelling)** • Pulmonary undesirable effects including interstitial pneumonia, pulmonary oedema and pulmonary infiltrates in some cases with an outcome of respiratory failure or adult respiratory distress syndrome (ARDS) which may be fatal are mentioned in section 4.8 of the SPC • Statement in section 4.4 of the SPC that patients with a recent history of pulmonary infiltrates or pneumonia may be at a higher risk.

	stem cell donors	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 (ARDS)
Cutaneous vasculitis	**Routine** Routine pharmacovigilance reporting **Additional** 1) Pharmacovigilance program in patients with severe chronic neutropenia a) Phase IV study b) Safety follow-up of study patients in co-operation with the SCN European registry 2) Co-operation with apheresis centres for healthy stem cell donors	**Routine (labelling)** In section 4.8 of the SPC it is mentioned that events of cutaneous vasculitis have been reported in patients treated with filgrastim.
Exacerbation of rheumatoid arthritis and arthritic symptoms	**Routine** Routine pharmacovigilance reporting **Additional** 1) Pharmacovigilance program in patients with severe chronic neutropenia a) Phase IV study b) Safety follow-up of study patients in co-operation with the SCN European registry 2) Co-operation with apheresis centres for healthy stem cell donors	**Routine (labelling)** According to section 4.8 of the SPC exacerbations of rheumatoid arthritis and arthritic symptoms have been observed.
Allergic reactions	**Routine** Routine pharmacovigilance reporting **Additional** 1) Pharmacovigilance program in patients with severe chronic neutropenia a) Phase IV study b) Safety follow-up of study patients in co-operation with the SCN European registry 2) Co-operation with apheresis centres for healthy stem cell donors	**Routine (labelling)** • Allergic Reactions: Allergic-type reactions, including anaphylaxis, skin rash, urticaria, angioedema, dyspnoea and hypotension are mentioned in section 4.8 of the SPC In section 4.3 of the SPC known hypersensitivity to the active substance or to any of the excipients is mentioned as contraindication.

Osteoporosis in patients with severe chronic neutropenia	**Routine** Routine pharmacovigilance reporting **Additional** 1) Pharmacovigilance program in patients with severe chronic neutropenia a) Phase IV study b) Safety follow-up of study patients in co-operation with the SCN European registry	**Routine (labelling)** In section 4.8 osteoporosis is mentioned as an undesirable effect in patients with severe chronic neutropenia.
Transformation to leukaemia or myelodysplastic syndrome in patients with severe chronic neutropenia	**Routine** Routine pharmacovigilance reporting **Additional** 1) Pharmacovigilance program in patients with severe chronic neutropenia a) Phase IV study b) Safety follow-up of study patients in co-operation with the SCN European registry	**Routine (labelling)** Transformation to leukaemia or myelodysplastic syndrome is mentioned in section 4.4 of the SPC under special precautions in patients with severe chronic neutropenia.
Immunogenicity (Incidence and clinical implications of anti-G-CSF antibodies)	**Routine** Routine pharmacovigilance reporting **Additional** 1) Pharmacovigilance program in patients with severe chronic neutropenia a) Phase IV study	Immunogenicity effects have not been observed in the clinical development of filgrastim. So far no neutralizing antibodies have been reported for the reference product Neupogen in the literature. Therefore no risk minimization activities are deemed to be necessary at the moment.
GvHD in cancer patients	**Routine** Routine pharmacovigilance reporting	**Routine (labelling)** In section 4.4 of the SPC it is stated there have been reports of GvHD and fatalities in patients receiving G-CSF after allogeneic bone marrow transplantation.
GvHD in recipients of allogeneic PBPC mobilised with filgrastim	**Routine** Routine pharmacovigilance reporting	**Routine (labelling)** In section 4.4 of the SPC 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 GvHD when compared with bone marrow transplantation.

Haematological malignancy in normal donors	**Routine** Routine pharmacovigilance reporting **Additional** 1) Co-operation with apheresis centres for healthy stem cell donors 2) Safety follow-up of healthy subjects of phase I study EP06-103	**Routine (labelling)** In section 4.4 of the SPC it is stated that transient cytogenic 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.
Use during pregnancy and lactation	**Routine** Routine pharmacovigilance reporting	**Routine (labelling)** It is mentioned in section 4.6 of the SPC that in pregnancy the possible risk of filgrastim use to the foetus must be weighed against the expected therapeutic benefit. It is not known whether filgrastim is excreted in human milk, therefore it is not recommended for use in breast-feeding women.

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The composition of filgrastim and Neupogen is quantitatively similar except the buffer system for filgrastim is glutamate and for Neupogen it is acetate. The data presented confirm that filgrastim and Neupogen are comparable with respect to primary structure, secondary and tertiary structure, molecular mass, hydrophobicity, molecular size, charge, binding and (*in vitro*) bioactivity. Product related impurities were thoroughly investigated. Aggregates and truncated forms showed no significant differences. The data show a consistently lower level of deamidated and oxidised forms..These differences did not appear to impact on bioactivity (*in vitro* bioassay) or stability. In conclusion, the physicochemical and biological analysis of filgrastim fully supports its biosimilarity to Neupogen.

The Applicant has established the in-house reference standard in absolute terms and has also compared it to the comparator product, Neupogen and the WHO International Standard 88/502.

Non-clinical pharmacology and toxicology

The toxicity studies performed by the Applicant were in accordance with the CHMP guidance for biosimilar rhG-CSF development and CHMP scientific advice. No significant differences between the products filgrastim and Neupogen were observed.

Efficacy

In line with CHMP Scientific Advice, pharmacodynamic data in healthy volunteers (absolute neutrophil and $CD34^+$ cell counts) were presented to establish the clinical efficacy. The data submitted supported the comparability of filgrastim and Neupogen.

Safety

A direct comparison of the safety profile of the test and reference products was possible based on studies in healthy volunteers. ADRs associated with filgrastim were consistent with those reported in

normal donors as described in AMGEN Neupogen SPC. Overall, these data supported the comparability of the products. The small single-arm trial in cancer patients submitted allowed, to a certain extent, to rule out any unexpected safety issue and suggests low immunogenicity of the test product. Additional long-term safety and immunogenicity data will be collected post-marketing as described in the RMP.

- User consultation

The Patient Information Leaflet (PIL) has been tested in English in accordance with Articles 59(3) and 61(1) of Directive 2001/83/EC, as amended by Directive 2004/27/EC. It was found to contain all the necessary information in a way that is accessible and understandable to those who participated in this test. The CHMP considered that the tested PIL meets the requirements set for User Testing.

Risk-benefit assessment

This application for a recombinant human G-CSF is based on a claim of biosimilarity to an approved product (Neupogen). CHMP Guidance has been issued for biotechnology-derived proteins in general, and recently for rhG-CSF in particular (non-clinical and clinical issues). The Applicant started their development before the specific guideline was finalised and sought for CHMP Scientific Advice, which they generally followed. The primary purpose of this assessment is not the characterisation of the benefit/risk profile of the product as such but the qualitative and quantitative evaluation of the similarity of the product to the reference chosen by the Applicant. The quality, non-clinical and clinical data presented supported the comparability of the two products.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that routine pharmacovigilance was adequate to monitor the safety of the product.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Zarzio in the following indications:

- 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 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 are similar in adults and children receiving cytotoxic chemotherapy.
- Mobilisation of peripheral blood progenitor cells (PBPC).
- In children and adults 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 filgrastim is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.
- Treatment of persistent neutropenia (ANC $\leq 1.0 \times 10^9$/l) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other therapeutic options are inappropriate.

was favourable and therefore recommended the granting of the marketing authorisation.