17 September 2020
EMA/CHMP/603684/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Nyvepria

International non-proprietary name: pegfilgrastim

Procedure No. EMEA/H/C/005085/0000

Note

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

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List of abbreviations

Abbreviation	Definition
ACF	animal component-free
ADA	anti-drug antibody
AE	adverse event
AEI	adverse event of interest
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANC_Cmax	maximum observed value for absolute neutrophil count
ANC_Tmax	time of maximum value for ANC
ANC0-inf (or AUCinf)	absolute neutrophil count from time zero to infinite time
ARDS	acute respiratory distress syndrome
AS	active substance (also named PF-06881894 in the report)
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC0-inf	area under the serum pegfilgrastim versus time curve from the time of
dose	administration (time zero) extrapolated to infinity
AUC0-t	area under the serum pegfilgrastim versus time curve from the time of
dose	administration (time zero) to the time of the last measurable concentration
AUEC	area under the effect curve
AUECANC (or AUECANC0-t)	area under the effect versus time curve for ANC from time zero to the
last	measurable concentration (also referred to as AUECANC0-t)
AUECANC0-inf	area under the effect curve for absolute neutrophil count from time
zero	extrapolated to infinity
CCIT	container closure integrity testing
CD	circular dichroism
CEX	cation exchange chromatography
CI	confidence interval
Cmax	maximum observed serum pegfilgrastim concentration
CMC	chemistry, manufacturing, and controls
CPPs	critical process parameters
CQA	critical quality attributes
CTD	common technical document
Da	dalton
DF	diafiltration
DNA	deoxyribonucleic acid
DP	drug product
DSC	differential scanning calorimetry
E. coli	*Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
ELSD	evaporative light scattering detection
EMA	European Medicines Agency
EPAR	European public assessment report
EPC	end of production cells
EU	European Union
EVA	ethylene vinyl acetate bags
FDA	Food and Drug Administration

FI	filgrastim intermediate
FP	finished product
G-CSF	granulocyte colony-stimulating factor
GMR	geometric mean ratio
HCP	host cell protein
HDX-MS	hydrogen-deuterium exchange by mass spectrometry
HIC	hydrophobic interaction chromatography
HMWS	high-molecular-weight species
IB	inclusion bodies
ICH	The International Council for Harmonisation of Technical Requirements
for	Pharmaceuticals for Human Use
IC-HPLC	ion exchange high performance liquid chromatography
IPTG	isopropyl β-D-Thiogalacto Pyranoside
ISR	injection site reaction
KD	ratio of dissociation constant (koff)/association constant (kon)
koff	dissociation constant
kon	association constant
LFT	liver function test
LoQ	limit of quantitation
MAA	Marketing Authorisation Application
MCB	master cell bank
MedDRA	Medical Dictionary for Regulatory Activities
met-HuG-CSF	recombinant methionyl human granulocyte colony-stimulating factor
MFI	microflow imaging
MO	major objection
MOA	mechanism of action
mPEG-p	monomethoxypolyethylene glycol propionaldehyde
MW	molecular weight
N	number of subjects
n	number of subjects meeting prespecified criteria
NA	not applicable
NAb	neutralizing antibody
NHL	non-Hodgkin’s lymphoma
NMR	nuclear magnetic resonance spectroscopy
NMT	not more than
NORs	normal operating ranges
PD	pharmacodynamic(s)
PDE	permitted daily exposure
PEG	polyethylene glycol
PETG	polyethylene terephthalate glycol
PFS	prefilled syringe
pI	isoelectric point
PK	pharmacokinetic(s)
PPQ	process performance qualification
PT	preferred term
PV	process validation
QTPP	quality target product profile
RCB	research cell bank
RH	relative humidity
RNS	rigid needle shield

RP	reference medicinal product
RP-HPLC	reversed phase high performance liquid chromatography
SAE	serious adverse event
SC	subcutaneous
SD	standard deviation
SDref	standard deviation of the comparator product lot results
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC	size exclusion chromatography
SEC-HPLC	size exclusion high performance liquid chromatography
SmPC	Summary of Product Characteristics
SMQ	standardized MedDRA query
SOC	system organ class
SPR	surface plasmon resonance
SV-AUC	sedimentation velocity – analytical ultracentrifugation
t½	elimination half-life
TEAE	treatment-emergent adverse event
TK	toxicokinetics
Tm	melting temperatures
Tmax	time to maximum serum pegfilgrastim concentration
UF	ultrafiltration
UPLC-MS	ultra performance liquid chromatography with mass spectroscopy
US	United States
USP	United States Pharmacopoeia
UV	ultraviolet
vs	versus
WBC	white blood cells
WCB	working cell bank
λz	elimination rate constant

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Pfizer Europe MA EEIG submitted on 12 September 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Nyvepria, through the centralised procedure falling within the Article 3(1) and point 1 Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication: "Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes)."

The legal basis for this application refers to:

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

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

The chosen reference product is: Neulasta

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

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

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

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

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

- Product name, strength, pharmaceutical form: Neulasta, 6 mg, solution for injection in pre-filled syringe
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22 August 2002
- Marketing authorisation granted by:

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

Information on Paediatric requirements

Not applicable.

Information relating to orphan market exclusivity

Similarity

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

Scientific advice

The applicant received Scientific Advice on 09 November 2017 (EMEA/H/SA/3699/1/2017/III) for the development programme supporting the indication granted by CHMP.

The Scientific Advice pertained to the following quality, preclinical and clinical aspects of the dossier:

Quality:

- Methods for in-process release specification for the filgrastim intermediate
- The strategy to support a demonstration of biosimilarity in terms of physicochemical and biological analyses between the biosimilar candidate, pegfilgrastim-US and pegfilgrastim-EU

Preclinical:

- The appropriateness and adequacy of the non-clinical comparability studies to demonstrate similarity to the reference medicinal product.

The main clinical aspects under consideration were:

- The design of the non-inferiority immunogenicity study in healthy volunteer as part of the totality of evidences to support the demonstration of similarity of the biosimilar candidate, pegfilgrastim-EU and pegfilgrastim-US.
- Validations of the immunoassays to support the immunogenicity assessment in the clinical studies
- Alternative specific testing to detect PEG moiety
- The Pharmacovigilance plan and Risk Minimisation measures taking into consideration the already established safety profile of the Reference Medicinal product

Date	Reference	SAWP Co-ordinators
09/11/2017	EMEA/H/SA/3699/1/2017/III	*Dr Juha Kolehmainen,* Dr Andreas Kirisits

1.2. Steps taken for the assessment of the product

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

Rapporteur: Ondřej Slanař Co-Rapporteur: Koenraad Norga

The application was received by the EMA on	12 September 2019
The procedure started on	3 October 2019
The Rapporteur's first Assessment Report was circulated to all CHMP members on	20 December 2019
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	19 December 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	6 January 2020
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	30 January 2020
The applicant submitted the responses to the CHMP consolidated List of Questions on	23 April 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	3 June 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	11 June 2020
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	25 June 2020
The applicant submitted the responses to the CHMP List of Outstanding Issues on	17 August 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	2 September 2020
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 Nyvepria on	17 September 2020

2. Scientific discussion

About the product

PF-06881894 (Nyvepria) has been developed as a similar biological medicinal product to Neulasta (INN: pegfilgrastim) (6 mg solution, prefilled syringe ready to use, for manual subcutaneous injection) which was approved in the European Union (EU) in August 2002 (EMEA/H/C/000420, Amgen Europe B.V., the Netherlands). The proposed indication for PF-06881894 is the same as that for Neulasta.

The indication proposed for PF-06881894 in the EU is:

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

Neulasta is a pegylated G-CSF (ATC Code: L03AA13, immunostimulants, colony stimulating factor). G-CSF produced in Escherichia coli (E. coli) by recombinant deoxyribonucleic acid (DNA) technology, is not glycosylated and contains an N-terminal methionine. Pegylation of recombinant filgrastim does not appear to affect its binding capacity to the G-CSF receptor and functionality on granulopoiesis.

Endogenous G-CSF is the primary regulating factor for neutrophils. The G-CSF acts by binding to G-CSF receptors, resulting in stimulated proliferation, differentiation, commitment, and target cell functional activation. Endogenous G-CSF is known to stimulate proliferation of the mitotic cells to reduce the maturation time of the non-mitotic cells in the bone marrow and to prolong the life span and enhance the function of mature neutrophils. Endogenous G-CSF is produced by different cell types including macrophages, monocytes, fibroblasts, stromal cells in bone marrow and endothelial cells. Endogenous G-CSF is triggered by inflammatory signals as well as by lipopolysaccharide released from bacteria.

Pegfilgrastim has the same mechanism of action (MoA) as endogenous G-CSF and filgrastim, i.e. it acts on haematopoietic cells by binding to the specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation.

Pegylation (i.e. addition of the 20 kDa polyethylene glycol [PEG] molecule) increases both the molecular weight and the size of filgrastim. The molecular weight increases from 19 kDa (filgrastim) to 39 kDa (pegfilgrastim). This is still below the 60-70 kDa molecular weight that is considered to be required to avoid glomerular filtration and subsequent renal elimination. The hydrodynamic radius, however, increases approximately 2.5- to 3-fold (calculated using equations in Fee and Van Alstine). This increases the size of filgrastim from ~4 nm to ~6 nm in diameter. In general, proteins ≥6 nm in diameter (e.g. haemoglobin 6.4 nm and albumin 7 nm) avoid glomerular filtration. With the reduction in renal clearance, the primary means of pegfilgrastim removal from the circulation is by neutrophil-mediated clearance. Both neutrophils and neutrophil precursors express G-CSF receptor, which binds pegfilgrastim and the drug-receptor complex is internalised and degraded inside the cell.

Pegylation serves to prolong the circulating half-life of biologic agents. Because of the prolonged half-life of pegfilgrastim, pegfilgrastim does not require daily injection as with filgrastim and can be given once per chemotherapy cycle.

Type of Application and aspects on development

Legal basis

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.

Biosimilarity

The chosen reference product is:

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

- Product name, strength, pharmaceutical form: Neulasta, 6mg, Solution for injection in pre-filled syringe
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22-08-2002
- Marketing authorisation granted by:

X Union

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

The development programme for PF-06881894 was designed to demonstrate biosimilarity to the pegfilgrastim products marketed globally as Neulasta, using the licensed products sourced from both the US and EU as representative of global supply.

In general, PF-06881894 development followed a stepwise approach to demonstrate similarity across Chemistry, Manufacturing and Control (CMC), quality, and nonclinical and clinical (pharmacokinetics PK, pharmacodynamics PD, safety and immunogenicity) data consistent with feedback received from health authorities during the biosimilar product development meetings, and health authority guideline documents.

- Legal basis: Article 10(4) of Directive 2001/83/EC, as amended – relating to applications for a biosimilar medicinal product.
- CHMP guidelines
    - Guideline on similar biological medicinal products (CHMP/437/04 Rev 1).
    - Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1) (EMA/CHMP/BWP/247713/2012).
    - Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev 1).
    - Guidance on similar biological medicinal products containing recombinant GCSF (EMEA/CHMP/BMWP/31329/2005). This guideline is currently revised, see Concept paper on the revision of the guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (EMA/CHMP/BMWP/214262/2015), and the draft of the revised guideline (EMEA/CHMP/BMWP/31329/2005 Rev 1).
    - Guideline on Immunogenicity assessment of therapeutic Proteins (EMEA/CHMP/BMWP/14327/2006 Rev 1)
    - Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr)

For the quality biosimilarity analysis, the applicant performed an extensive comparability exercise including side-by-side testing by a combination of orthogonal analytical methods, which are properly qualified, and by using up to 17 batches of pegfilgrastim-EU and pegfilgrastim-US and up to 10 batches of Nyvepria DP. The quality biosimilarity testing programme included a combination of physicochemical, biochemical and biological activity tests, which covered all important quality attributes of pegfilgrastim. Also, comparative degradation studies were performed to study the degradation profile of Nyvepria and EU- and US-sourced Neulasta. Taken together, the quality biosimilarity analysis was in compliance with the applicable EMA guidance (CHMP/437/04 Rev 1 and EMA/CHMP/BWP/247713/201).

The goal of the clinical program was to demonstrate that there are no clinically meaningful differences between PF-06881894 and Neulasta.

Based on feedback received from the US FDA and EMA regulatory interactions, the clinical development program for the proposed biosimilar PF-06881894, the following 2 comparative clinical studies were conducted:

- Study ZIN-130-1505: An open-label, randomized, single-dose, comparator-controlled crossover PD/PK equivalence study in healthy volunteers to compare PF-06881894 to pegfilgrastim-US and pegfilgrastim-EU.

- Study C1221005: A randomized, open-label, multiple-dose, non-inferiority, parallel-group immunogenicity study in healthy volunteers to demonstrate the non-inferiority of PF-06881894 versus pegfilgrastim-US with respect to immunogenicity. It should be noted that the comparative immunogenicity study (C1221005) had already started at the time of the EU scientific advice (EMA/CHMP/SAWP/720012/2017). Its study design was selected based on FDA Guidance for Industry. Therefore, CHMP remarks on this study were rather for the sake of scientific discussion rather than reflecting a formal request of the CHMP. In particular, a blinded design would have been preferred, and the proposed non-inferiority design is not requested for safety data.

The CHMP has agreed that the clinical dossier for a biosimilar application for a PEG-filgrastim may comprise of healthy volunteer trials only, provided that biosimilarity can be sufficiently demonstrated based on a strong and convincing physicochemical and functional data package and comparable pharmacokinetic and pharmacodynamic profiles.

The ZIN-130-1505 study established the PD and PK equivalence of: PF-06881894 to pegfilgrastim-US; PF-06881894 to pegfilgrastim-EU; and pegfilgrastim-US to pegfilgrastim-EU.

In the comparative immunogenicity study C1221005, Pfizer proposed to compare the biosimilar and pegfilgrastim-US. According to the "Guideline on Similar Biological Medicinal Products" (CHMP/437/04 Rev 1), the reference medicinal product should be a medicinal product authorised in EEA. However, it may be possible in some cases for an applicant to compare the biosimilar in clinical studies with a non-EEA authorised comparator. In this case, the applicant needs to provide adequate data or information to scientifically justify the relevance of these comparative data and establish an acceptable bridge to the EEA-authorised reference product. The type of bridging data needed will always include data from analytical studies that compare all three products (the proposed biosimilar, the EEA-authorised reference product and the non EEA-authorised comparator), and may also include data from clinical PK and/or PD bridging studies for all three products. Given that biosimilarity has been demonstrated between EU- and US-sourced Neulasta, the quality bridge is considered adequately established and the use of US-sourced Neulasta lots in clinical studies is therefore considered acceptable. Since immunogenicity of PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU was previously assessed in a 3 arm crossover PD/PK equivalence study in healthy volunteers (ZIN-130-1505) demonstration of equivalence between PF-06881894 against a single reference product (e.g. pegfilgrastim-US) in the proposed comparative immunogenicity study is acceptable.

Overall, the comparative PD, PK, and safety findings from Study ZIN-130-1505 and the comparative immunogenicity and safety data from Study C1221005 contributed to the totality of evidence supporting a demonstration of biosimilarity of PF-06881894 to Neulasta.

Study ZIN-130-1504, which was a non-comparative, parallel-group study characterising the PD, PK, and safety (including immunogenicity) of PF-06881894 in patients with non-distantly metastatic breast cancer, is not considered to be integral to the biosimilar program and hence will not be discussed in the overview of clinical pharmacology, efficacy, or safety. An overview of biopharmaceutics and associated analytical methods, however, includes bioanalytical methods used in the 3 studies.

Interaction with EMA or Rapporteurs:

Interactions with competent authorities	**Topics mainly discussed**	**Date of final letter**
EMA/H/SA/3699/1/2017/III	The CHMP provided response to the applicant's questions related to analytical methods to measure impurities. The biosimilarity assessment on the quality level was further discussed with CHMP advice to include enough batches in order to achieve a trustworthy platform for the assessment. Further, CHMP was asked about the non-clinical repeat-dose toxicity study and the need for additional non-clinical studies. With respect to clinical part of the dossier, CHMP responded to questions related to design of clinical study, non-inferiority margin, endpoints, statistical methods and immunogenicity assessment.	09 November 2017
Pre-submission meeting with Rapp and Co-Rapp	The Rapporteurs responded to questions related to overall analytical similarity strategy, release and shelf-life specifications or raw material. Further questions were focused on non-clinical data, demonstration of biosimilarity, clinical endpoints and safety data analysis.	14 May 2019
EMA pre-submission meeting	Topics discussed during this meeting were mainly focused on administrative issues and organisation of the whole dossier and CTD.	03 June 2019

2.1. Quality aspects

2.1.1. Introduction

Nyvepria, referred to as PF-06881894 FP, is a proposed biosimilar to the Neulasta reference licensed product.

The finished product is presented as a solution for subcutaneous (SC) injection containing 6 mg/0.6 ml of pegfilgrastim as active substance. Other ingredients are sorbitol, sodium acetate trihydrate, glacial acetic acid, polysorbate 20 and water for injection.

Nyvepria is supplied in a single-dose prefilled syringe (Type I glass), with a plunger stopper and plunger rod, stainless steel needle and needle cover with an automatic needle guard. Each pre-filled syringe contains 0.6 ml of solution for injection.

2.1.2. Active Substance

General information

PF-06881894 (pegfilgrastim) is a covalent conjugate of recombinant methionyl human granulocyte-colony stimulating factor (G-CSF) (referred to as filgrastim) and a 20 kDa monomethoxypolyethylene glycol propionaldehyde (mPEG-p).

Filgrastim is expressed in *Escherichia coli* (*E. coli*) as a 175 amino acid protein with a theoretical average mass of 18,799 Da. Filgrastim contains 5 cysteines, 4 of which oxidise to form disulfide bonds Cys37-Cys43 and Cys65-Cys75. One cysteine remains reduced (free thiol, Cys18). Filgrastim expressed in bacterial cells has no post-translational modifications.

PF-06881894 is synthesised by Schiff-base reduction of a 20 kDa mPEG-p with the N-terminal amine of filgrastim at Met1. The mPEG-p used for pegylation is a heterogeneous mixture with varying numbers (approximately 412 to 536) of ethylene oxide units. As a result, PF-06881894 exhibits molecular weight dispersity with an observed distribution of approximately 37.0 to 42.5 kDa. The described general properties include the physical form, colour, clarity, pH, osmolality, isoelectric point, extinction coefficient and biological activity of the PF-06881894.

Manufacture, characterisation and process controls

Manufacture of AS and filgrastim intermediate is performed at Hospira Zagreb d.o.o., Prudnička cesta 60, 10291 Prigorje Brdovečko, Croatia and Hospira Adelaide Pty Lts, 6 Dalgliesh St, Adelaide 5031, Australia. A third party manufactures the critical intermediate, mPEG-p. During the procedure, a MO was raised to request an updated QP declaration from the manufacturer Hospira Zagreb d.o.o., Brdovečko, Croatia to include dates and confirmation of the audits for the respective manufacturing sites. This was provided therefore appropriate GMP authorisations are now available for all sites.

Description of manufacturing process and process controls

The manufacturing process for PF-06881894 active substance (AS) is a three-stage process.

- Stage 1: upstream process, manufacture of PF-06881894 Inclusion Bodies (IB)
- Stage 2: downstream process, PF-06881894 Filgrastim Intermediate (FI) purification process
- Stage 3: pegylation, purification and formulation process, resulting in PF-06881894 AS

The IB process uses a recombinant *Escherichia coli (E. coli*) cell line that contains the plasmid DNA encoding the sequence for filgrastim protein and is grown in suspension culture using animal component-free (ACF) media. Cells from the working cell bank (WCB) are thawed and expanded to produce a seed culture. The seed culture is used to inoculate a production fermenter. Production fermentate is harvested, homogenised and centrifuged to produce PF-06881894 IB containing filgrastim protein. The recovered IBs are dispensed and frozen at the proposed condition in HDPE bottles.

The IBs are thawed and undergo dissolution and refolding to produce the active filgrastim molecule. The product is then captured by a cation exchange chromatography (CEX) step and further processed by a flow through cation exchange chromatography (CEX) mixed mode chromatography step, a hydrophobic

interaction chromatography (HIC) step and an additional CEX chromatography step. Concentration and buffer exchange in an ultrafiltration/diafiltration (UF/DF) step is followed by formulation and filtration. The filtered PF-06881894 FI is filled into polyethylene terephthalate glycol (PETG) bottles, the closures sealed, labelled and frozen at the proposed storage condition. The PF-06881894 FI is tested and released according to the specifications. It is shipped frozen for further processing to Hospira Zagreb, under conditions validated for frozen shipment.

The FI is thawed and pegylated, where 20 kDa mPEG-p is covalently bound to FI. Powdered mPEG-p critical intermediate is used to prepare mPEG-p solution which is used on the same day the pegylation reaction is performed. The pegylated FI is further purified by CEX chromatography prior to concentration and buffer exchange by UF/DF. The protein concentration is adjusted to a target concentration. Polysorbate 20 is then added and the formulated bulk solution is processed by a final (0.2 µm) filtration to produce the AS which is stored in PETG bottles at the proposed storage condition until finished product (FP) manufacture. Reprocessing conditions for specific steps have been defined. The process has been sufficiently described and in-process controls are adequately set to control the process.

Control of materials

All raw materials used in the AS manufacturing process are described and are either compendial grade or are tested according to in-house standards. Composition of the different media and buffers is detailed. In house specifications for non-compendial materials are provided. Activated mPEG-p is considered as an intermediate; PEG is considered as a starting material. No human or animal-derived raw materials or excipients were used in the development of the recombinant cell line or establishment of the master cell bank (MCB) and working cell bank (WCB). No human or animal-derived raw materials or excipients are used in the manufacture of PF-06881894 IB, FI and active substance.

The information on the origin, production and composition including certificates of analysis was provided for the following raw materials: glycerol, bacto agar, yeast extract and vegetable peptone. No human or animal-derived compounds were used during production of these materials.

The construction of the expression plasmid and the transformation of the *E. coli* strain to generate the research cell bank (RCB) was described in detail. The RCB was characterised and then used to derive a master cell bank (MCB) and WCB. Demonstration of cell substrate stability was performed according to the ICH guidelines (ICH Q5B and ICH Q5D). Testing of genetic stability, identity, purity and contamination by adventitious agents was performed on end of production cells (EPC). The cell banking system is adequately described, and the cell banks have been tested and characterised (including demonstration of genetic stability) in accordance with the requirements of ICH Q5D. Stability of MCB and WCB is verified annually. Furthermore, the protocol for preparation and qualification of future WCBs was provided.

Control of critical steps and intermediates

The control of the critical steps and intermediates is sufficiently described. Detailed information was provided on the control of the filgrastim intermediate. The proposed specifications (including tests for appearance, pH, identity, protein concentration, potency, purity, impurities, bioburden, bacterial endotoxins, host cell proteins, host cell DNA) and acceptance criteria are deemed acceptable. Analytical methods were described, and method validation reports provided. More details on the control strategy is provided in the AS process validation section of this report. Batch data were provided confirming that all lots complied with the specifications. Regarding the host cell protein assay, which is a release test for FI, the applicant demonstrated that the anti-HCP polyclonal antibody has a sufficiently high coverage with regard to the HCPs and is thus deemed suitable for the intended use.

The PF-06881894 filgrastim intermediate is filled into sterile polyethylene terephthalate glycol (PETG) bottles. The stability data support the proposed shelf life and storage conditions for filgrastim intermediate.

Methoxypolyethylene glycol propionaldehyde (mPEG-p) is a protein pegylation agent used in the manufacture of PF-06881894 AS.

A separate section is provided (according to the CTD structure of the AS section) for the mPEG-p critical intermediate including information on the manufacturer, process and controls, control of materials, control of intermediates and critical steps, process validation, characterisation, impurities, specifications (including justifications), analytical methods (including their validation), batch data, reference standard, container closure and stability. The starting materials and intermediates involved in the manufacture of mPEG-p are controlled by the supplier according to their respective specifications. There are no materials of animal origin used in the manufacture of mPEG-p.

Acceptance criteria for the proposed mPEG-p commercial release specifications (performed in-house on each lot of mPEG-p received by Pfizer) are provided and include colour, clarity, pH, water content, identity, average MW, polydispersity, purity, assay, impurities, bacteria; endotoxins, bioburden. The proposed retest period is supported by stability data.

Process validation

Two separate manufacturing facilities (Hospira Adelaide, Australia and Hospira Zagreb, Croatia) are involved in the manufacturing process. A sequential approach to validation was taken in which the AS processes were validated separately to accurately model the way batches of AS will be manufactured during commercial operations. Reproducibility of each of the manufacturing stages (IB, FI and AS) was demonstrated in three, sequential manufacturing batches at the commercial scale and these are referred to as process performance qualification (PPQ) batches. Process validation (PV) studies were performed to validate other aspects of the manufacturing process such as resin lifetime, dispensing uniformity and shipping.

Three PF-06881894 IB were produced and three FI batches from these lots. These PF-06881894 FI batches were then used to produce three independent AS batches. All release results met the proposed commercial specifications. Process parameter and in-process test data from the PPQ campaigns (IB, FI and AS) were within pre-defined control limits for the commercial process. The monitored parameters are in agreement with routine process controls and the process is considered to be adequately validated. The strategy for the setting of individual process parameters was justified. The proposed shelf life is deemed acceptable based on the supportive data.

The applicant has provided more detailed information on how criticality of process parameters was assessed. It is indicated that regardless of their classification, all process parameters are adequately controlled. Any deviations from normal operating ranges are investigated. The effective and consistent removal of a wide range of potential fermentation media-derived impurities, host cell-derived impurities and purification process-derived impurities was demonstrated for the AS manufacturing process. All impurities (host cell DNA and HCP, IPTG, antifoam, EDTA and elemental impurities) are reduced to very low levels which do not pose safety concerns. Additional PV studies were performed for the reuse, sanitisation and storage of chromatography resins and manufacturing scale resin lifetime studies.

In-process hold times were validated at small scale or at manufacturing scale to demonstrate biochemical stability of in-process eluates and pools under controlled conditions. Shipment conditions for frozen PF-06881894 FI in PETG bottles at the proposed temperature of storage are qualified for shipment from the site of manufacture of PF-06881894 FI to the site of AS manufacture.

Manufacturing process development

The applicant has described the development of the manufacturing process. From the beginning the commercial scale process was used for all batches produced thus far (engineering lots, non-clinical lots, clinical lots and PPQ lots). Only minor changes (mainly optimisations of process parameters ranges) were introduced in the process variants used for non-clinical, clinical and PPQ lots.

Studies were performed to define the criticality of quality attributes and process parameters. Depending on the outcome of these studies and a risk assessment, a control strategy for the process was proposed.

Bridging studies have been conducted for analytical methods with significant technical changes during the development; this included the reversed phase high performance liquid chromatography (RP-HPLC), size exclusion chromatography (SEC), and ion exchange high performance liquid chromatography (IC-HPLC) methods. The method bridging data demonstrated that the proposed commercial methods showed improved robustness or capability.

Characterisation

The characterisation program adequately demonstrates that the primary structure, higher order structure, and functional characteristics of PF-06881894 AS/FP are consistent with the expected structure and function of the pegfilgrastim molecule.

The structural and functional characteristics of PF-06881894 have been examined for the following quality attributes: amino acid sequence, pegylation site and linker composition, mass-average molecular weight and molecular-weight dispersity, free thiols, secondary structure, disulfide linkages, structural dynamics, melting temperature, sedimentation coefficient, protein structure, extinction coefficient, isoelectric point, in vitro potency, receptor binding, receptor binding affinity and kinetics.

The product-related substances and impurities present in PF-06881894 AS and FP have been investigated in the FP lots used for the biosimilarity exercise, including aged and stressed samples. These substances/impurities include oxidation products, deamidation products, reduced species, pegylation variants, size variants, isomerisation products and truncated species. For each product-related species a rationale mainly based on the scientific literature is provided for classifying it as a product-related impurity or substance. The levels of the product-related impurities remain suitably low.

The potential residual chemicals, leachates and impurities have been identified through a risk assessment. These process-related impurities are controlled in FI and/or AS using a combination of control elements and demonstrated to be consistently removed to acceptable safety levels by the FI and/or AS purification process through process validation studies. In addition, specifications are in place for host cell protein (HCP) and residual DNA in the filgrastim intermediate. Specified impurities have been present in product studied in clinical trials and are as such clinically qualified with regard to safety.

Specification

The specifications for the PF-06881894 AS include appropriate specifications for physicochemical attributes, identity, potency and purity. Satisfactory justification has been provided for the specifications.

The AS specifications include a minimal limit for the main peak purity for each of the purity/impurities assays. In addition, the AS specifications for impurities include a separate limit for HMW species, oxidised and reduced variants. Free PEG content is also part of the AS specifications. The levels of cyanide and boron were satisfactorily low and justified. A routine control test for residual cyanide content was included in the AS release specification.

Analytical methods

The analytical procedures have been described, including the system suitability and assay acceptance criteria for each method. The non-compendial analytical procedures were properly validated according to ICH guidelines. The purpose of the cell-based bioassay analytical procedure is to measure the in vitro functional activity of PF-06881894 AS and FP.

Batch analysis

A summary of data from 15 AS batches used for nonclinical and clinical studies, stability, engineering and development studies and process performance qualification (PPQ) was provided. All AS batches were manufactured at the intended commercial AS manufacturing site using the intended commercial-scale process. All AS batches met the specifications applicable at the time of AS release. The results demonstrate consistent performance of the AS manufacturing process at commercial scale.

Reference materials

The applicant has provided an overview of all reference standards for filgrastim intermediate and active substance that have been used during development. All standards were properly qualified. Potency was qualified against the NIBSC 12/188 pegfilgrastim International Standard (either directly or indirectly). Also, qualification programs were proposed for future FI and AS reference standards. The approach how new primary reference standards will be qualified is considered satisfactory.

Container closure

The PF-06881894 filgrastim intermediate (FI) and PF-06881894 active substance are filled into sterile polyethylene terephthalate glycol (PETG) bottles sealed with high-density polyethylene (HDPE) closures. PETG and HDPE resins are compliant with 21 CFR 177.1315(b)(1) and 174.5 and 21 CFR 176.170(c), 177.1520(c)3.2a, respectively. Both materials met the requirements for USP Class VI designation and the USP Cytotoxicity Test. Both materials met the specifications for Physicochemical Tests in accordance with USP <661>. PETG bottles with HDPE closure are leak tested. Extractables and leachables testing has been performed. From a toxicology point of view, no safety concerns are raised. Container closure system suppliers were provided.

Stability

Shelf-life and storage conditions for the AS have been proposed and found acceptable.

The stability program for PF-06881894 AS is conducted in accordance with ICH guidelines for stability of AS (ICH Q1A and Q5C) and includes the following studies: long-term at 2 to 8°C (5ºC); accelerated at 25ºC/60% RH; stressed at 40°C/75% RH; 3 cycles of temperature cycling at 25°C/5°C; 3 cycles of temperature cycling at -20°C/5ºC; cold stress at -20°C and photostability, under ICH Q1B Option 2 light exposure.

The protocols for the stability studies are provided. The AS shelf-life specification covers a sufficient range of stability-indicating tests including tests for specific impurities (high-molecular-weight species-HMWs, oxidised species, reduced species, total related proteins) and purity (main peak). Polysorbate 20, bacterial endotoxins test, and bioburden are part of the AS shelf-life specification too. Data from these stability studies support the proposed AS shelf-life. All AS stability batches were manufactured using the intended commercial scale AS manufacturing process at the commercial manufacturing site.

Long-term, real time stability data has been provided. All results comply with the specifications. The available long-term stability data demonstrate that PF-06881894 AS is stable for the proposed shelf life and storage conditions.

Accelerated stability data has been provided. One batch of PF-06881894 AS is included in the temperature cycling (25°C/5°C) study, in the temperature cycling (-20°C/5°C) study and in the cold stress (-20°C) study. Temperature cycling between recommended storage and 25°C (3 cycles) did not impact the AS quality. Temperature cycling between recommended storage and -20°C (3 cycles) did not impact the AS quality. Cold stress of -20°C did not impact the AS quality.

The data from the photostability studies demonstrate that unprotected AS is susceptible to photo-degradation under ICH Q1B (option 2) photostability conditions, in combination with accelerated temperature conditions.

The proposed shelf life at the proposed storage conditions are accepted for the AS.

2.1.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The PF-06881894 finished product (FP) is developed as a proposed biosimilar to the authorised Neulasta reference medicinal product. All excipients (sodium acetate trihydrate, glacial acetic acid, sorbitol (E420), polysorbate 20, water for injections) are of compendial grade and controlled according to the Ph. Eur. No excipients of human or animal origin nor novel excipients are used for the manufacturing process of PF-06881894. To deliver a dosage of 6 mg in 0.6 mL, a target fill volume of 0.627 mL was determined to be appropriate. There are no overages.

The PF-06881894 FP presentation dose strength is developed to match the reference product's configuration of 6 mg/0.6 mL, utilising the same protein concentration of 10 mg/mL. Supportive formulation development studies and compatibility studies were performed.

The manufacturing site, final formulation composition and manufacturing process utilised throughout the development, clinical campaign and validation are representative of the proposed commercial process and therefore data from these are relevant for the commercial process without requiring comparability data. Up to date, 10 different batches have been manufactured.

The quality target product profile (QTPP) was developed based on the PF-06881894 FP development studies, including the product attributes relevant to similarity and expectations for pharmaceutical acceptability. The strategy used for process control and categorisation of process parameters was explained by the applicant. It is indicated that within the overall approach to control strategy both critical process parameters (CPPs) and non-critical process parameters (non-CPPs) have a high degree of control within site quality systems, irrespective of the criticality designation. Normal operating ranges (NORs) are detailed in the batch record and any excursions from the NOR for both critical and non-critical process parameters trigger an investigation. Individual steps of the manufacturing process are sufficiently controlled. Process development included studies designed to understand the pooling and mixing of AS, hold time in the compounding tank pre-bioburden filtration, the hold time in transfer tank post bioburden filtration, sterile filtration, the hold time in ethylene vinyl acetate bags (EVA), aseptic filling and stoppering and visual inspection. Summary results of these studies support the proposed process design. The compatibility of materials used and the container closure system has been confirmed.

The comprehensive risk assessment to identify potential risk factors for nitrosamine formation in the active substance, finished product and primary packaging processes, identified no risk for small molecule nitrosamine (cohort of concern) formation. Additionally, from a toxicological perspective, there is no risk of the pegfilgrastim molecule itself forming a nitrosamine, requiring cohort of concern control.

Container closure

The container closure system was described and meets the requirements of Ph. Eur. The PF-06881894 finished product (FP) is supplied in 6 mg/0.6 mL single-use prefilled syringe (PFS) for manual subcutaneous injection. The PF-06881894 PFS is comprised of a glass syringe barrel with a 27-gauge ½-inch staked needle and a rigid needle shield (RNS, or needle cover), a plunger stopper, a plunger rod with a thumb pad, and a passive needle guard (safety device) with finger grips and an inspection window allowing for visual inspection of the syringe.

Extractables studies were performed with no safety concern observed. The results for potential leachables were below the LOQ for all compounds identified. Information on the sterilisation of the container closure material was submitted as requested by EMA/CHMP/CVMP/QWP/850374/2015. The applicant provided information that the UltraSafe Plunger Rod and Needle Guard (safety device) are no longer CE-marked due to BD's decision to withdraw the CE mark for their UltraSafe products and therefore these medical device components are required to comply with the Essential Requirements as outlined in Annex I of the Medical Devices Directive (93/42/EEC). The applicant provided an overview (e.g. checklist) of the applicable requirements, accompanied by relevant data reports demonstrating their compliance.

Manufacture of the product and process controls

Hospira Zagreb d.o.o (a Pfizer Company) is the site that is responsible for batch release. Adequate GMP authorisation is provided for this site.

The nominal formulation strength for the FP is 10 mg/mL. Unless otherwise specified, the manufacturing process steps occur at controlled room temperature (15 to 25°C) during batch manufacture. Preparation of formulation buffer (Step 1): The formulation buffer is compounded with the same excipients and excipient concentrations as the finished product, to use for flushing the filters prior to introducing the FP to the filter membrane for both the bioburden reduction and sterile filtration steps.

Preparation of PF-06881894 FP solution (Step 2): PF-06881894 AS, stored in polyethylene terephthalate glycol (PETG) bottles at 2 to 8°C, is transferred to the compounding area. Up to two different lots of AS may be included in the manufacture of one FP lot. The PF-06881894 AS is pooled and mixed to prepare the FP solution which can be held in a stainless-steel vessel for up to 24 hours at room temperature.

Bioburden reduction filtration (Step 3): The PF-06881894 FP solution is transferred through a sterile 0.2 µm bioburden reduction filter into a stainless-steel transfer vessel.

Sterile filtration (Step 4): The PF-06881894 transfer-filtered FP solution is sterile-filtered using two 0.2 µm single-use filters.

Aseptic filling and plunger stoppering (Step 5): The containers and closures used for filling and stoppering are received from the supplier as sterile, ready to use and this process is performed under class 100 conditions. Visual inspection (Step 6) and secondary packaging (Step 7) follow. The PF-06881894 FP units are labelled, assembled with the plunger rod and safety device and packaged into cartons. The shelf cartons are then packed and transferred to the warehouse for storage at 2 to 8°C (Step 8). The final finished product is shipped under validated qualified conditions.

There are no reprocessing steps for the manufacture of PF-06881894 FP. In-process tests with associated control limits for the PF-06881894 FP manufacturing process are provided.

A total of 3 PPQ lots of PF-06881894 FP were manufactured as part of the FP process validation program. All lots met the pre-defined acceptance criteria for the critical process parameters (CPPs), non-critical process parameters (non-CPPs), in-process testing, and critical quality attributes (CQAs) identified for

the manufacturing process. These results demonstrate the capability to reproducibly manufacture FP and show that the manufacturing process is under control.

The applicant provided justification with regards to the process control strategy including categorisation of individual process parameters and in-process controls. There is no criticality categorisation of in-process controls but their acceptance criteria are defined and a decision of potential batch rejection is based on evaluation of any deviation. The only rejection parameter is filter integrity.

In-process hold times for PF-06881894 FP manufacturing have been justified through development activities and additional qualification studies. Fill uniformity studies (3 lots) demonstrate consistency of product quality of the filled syringes throughout the duration of the aseptic filling operations. All test results passed pre-determined acceptance criteria. Aseptic process simulations (media fills) are performed twice a year to (re-) validate the filling process. Shipping of FP has been validated.

Product specification

The specification includes appropriate tests for physico-chemical attributes, identity, purity and potency. Suitable justification was provided for the specifications. The specifications are deemed sufficient to control the quality of the FP.

A few 'other concerns' were raised regarding the proposed acceptance criteria during the procedure and the proposed finished product specifications were amended. As regards purity, individual types of product-related impurities (such as HMWS or oxidised and reduced specifies) are monitored separately and main peak representation is now included. Furthermore, the specification range for osmolality was tightened. Due to the specificity of the finished product process (only blending), no new impurities are introduced in the finished product compared to active substance. Elemental impurities in the FP have been addressed. All elements for five PPQ batches were below the 30% of the permitted daily exposure (PDE) concentration limits as specified in ICH Q3D.

Analytical methods

Analytical methods, their evaluation and acceptance criteria have been changed during the development. These changes are supported by submitted validation data. Non-compendial methods are similar for AS and FP (see active substance section), except for container closure integrity testing (CCIT), and syringe function. The latter two methods were described and properly validated in accordance with ICH guidelines.

Batch analysis

All FP lots were produced at the intended commercial FP manufacturing site using the intended commercial-scale process and in the intended product-contact commercial container closure system with the exception of final syringe assembly (i.e., non-product contact components such as the plunger rod and needle guard).

The batch analysis results for FP lots released using the clinical and process performance qualification (PPQ) specification test methods in place at the time are presented and show compliance with the specifications.

Reference materials

The same reference standard is used for the FP and the AS (See AS section).

Stability of the product

A shelf-life of 36 months at 5°C +/- 3°C is proposed for the FP.

The stability program for PF-06881894 FP is conducted in accordance with ICH guidelines for stability (ICH Q1A and Q5C) and includes the following studies: long-term at 2 to 8°C (5°C); accelerated at 25°C/60% relative humidity (RH); stressed at 40°C/75% RH; in-use at 25°C/60% RH post long-term storage at 5°C; 3 cycles of temperature cycling at 25°C/5°C; 3 cycles of temperature cycling at -20°C/5°C; cold stress at -20°C; photostability under ICH Q1B Option 2 light exposure and photostability under manufacturing light exposure.

The stability data provide justification for the proposed shelf-life of 36 months for PF-06881894 FP stored at the long-term storage condition of 5°C. PF-06881894 FP lots were placed on the long-term 5°C stability program and there is a minimum of 36 months of stability data available. Suitable stability-indicating tests were used. There was no substantial change observed for any quality attribute. All PF-06881894 FP stability lots were manufactured using the commercial-scale FP manufacturing process, and all stability data generated to date at the long-term 5°C storage condition meet the proposed commercial PF-06881894 FP specifications. Changes introduced in the finished product specifications are reflected in the stability studies adequately.

In addition, stability data demonstrated that PF-06881894 FP is stable for the proposed in-use storage. Exposure of PF-06881894 FP placed in the representative secondary packaging demonstrated that the packaged PFS is stable to light exposure. However, the approved conditions as stated in the SmPC are:

'Store in a refrigerator (2°C – 8°C). Nyvepria may be exposed to room temperature (not above 25°C) for a maximum single period of up to 15 days. Nyvepria left at room temperature for more than 15 days should be discarded. Do not freeze. Accidental exposure to freezing temperatures for a single period of less than 24 hours does not adversely affect the stability of Nyvepria. Keep the container in the outer carton in order to protect from light. '

Based on the available stability data, the shelf-life and storage conditions as stated in the SmPC are acceptable.

Adventitious agents

No raw materials or excipients of biological origin are employed in the manufacture of AS and FP. The active substance is expressed in *E. coli* which does not support replication of mammalian viruses. The *E. coli* MCB and WCB were tested for the presence of non-lysogenic and lysogenic bacteriophages which may replicate in *E. coli*. Controls at various process stages provide for an adequate control of potential bacterial and fungal contaminations. In summary, the information provided on adventitious agents is considered acceptable. FP is tested for sterility and bacterial endotoxins.

2.1.4. Biosimilarity

A comprehensive bioanalytical approach was used to assess the similarity of PF-06881894 to the US-licensed Neulasta Reference Medicinal Product (RP; referred to as pegfilgrastim-US) and the EU-approved Neulasta RP (referred to as pegfilgrastim-EU) as well as the similarity between pegfilgrastim-US and pegfilgrastim-EU.

The scope of the analytical similarity assessment included comparative testing of 17 lots of pegfilgrastim-US and 17 lots of pegfilgrastim-EU procured over time and 10 lots of PF-06881894 FP. Details of the US and EU reference product lots are given in **Table 1**. All 10 PF-06881894 lots are independent FP lots

manufactured from different AS batches and unique filgrastim intermediate (FI) batches using the proposed commercial-scale manufacturing process at the intended commercial FP manufacturing site.

Table 1 Summary of pegfilgrastim-US and pegfilgrastim-EU lots used in the analytical similarity assessment

Product	Lot Number	Expiration Date	Lot Use (In Addition to Similarity Testing)	Country of Origin
Pegfilgrastim-US	1035686	Sep-15	--	US
	1036285	Oct-15	--	US
	1057096	Nov-17	Stability	US
	1057097	Feb-18	--	US
	1057133	Oct-17	Clinical (C1221001), Stability	US
	1057373	Jan-18	Clinical (C1221001), Nonclinical	US
	1057416	Mar-18	--	US
	1060058	Jun-18	--	US
	1064191	Sep-18	--	US
	1071087	Jan-19	Clinical (C1221005)	US
	1072044	Jul-19	Clinical (C1221005)	US
	1078875	May-19	--	US
	1083446	Apr-20	--	US
	1084476	Nov-19	--	US
	1085896	Apr-20	--	US
	1089511	Nov-19	--	US
	1094104	Jun-20	--	US
Pegfilgrastim-EU	1039830D	Oct-15	--	EU (Germany)
	1041021D	Nov-15	--	EU (Germany)
	1058436B	Aug-17	Nonclinical, Stability	EU (Poland)
	1060064C	Oct-17	Clinical (C1221001), Stability	EU (Germany)
	1061466C	Oct-17	Clinical (C1221001), Stability	EU (Germany)
	1061815D	Nov-17	--	EU (Poland)
	1065041B	Apr-18	--	EU (Germany)
	1066011C	Sep-18	--	EU (Croatia)
	1069490C	Dec-18	--	EU (Germany)
	1079877A	Oct-19	--	EU (Germany)
	1085288A	Jan-20	--	EU (Romania)
	1087927A	Jun-20	--	EU (Romania)
	1088493A	Jun-20	--	EU (Denmark/Sweden /Finland)
	1088500D	Nov-20	--	EU (Germany)
	1090139B	Nov-20	--	EU (Germany)
	1093686K	Dec-20	--	EU (Latvia/Hungary /Lithuania)
	1094582	Feb-21	--	EU (Croatia)

The criteria for assessing the similarity between PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU were developed based on the pegfilgrastim-US and pegfilgrastim-EU product information, both qualitative and quantitative. Pegfilgrastim-US and pegfilgrastim-EU lots acquired on the open market during PF-06881894 product development provide visibility to the quality attributes and their ranges known to be safe and efficacious. This information was used to develop criteria for assessing the analytical similarity of PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU for each quality attribute.

Multiple orthogonal analytical characterization and routine methods were used to assess eachof these quality attributes. For quantitative attributes critical to similarity assessment, the quality range and acceptable criterion was established. (i.e. melting temperature, total related proteins, total charge variants, protein concentration, deliverable content, in-vitro potency, receptor binding assay, Receptor Binding Affinity Relative K_D). For other attributes, a graphical comparison approach was introduced. The described strategy is acceptable. The proposed acceptance criterion (90% of tested values need to fall within the quality range) is not justified in context of the wide quality range based on mean plus/minus three times standard deviation (3-sigma interval). However, the provided data overall, support the analytical similarity among PF-06881894, pegfilgrastim-US and pegfilgrastim-EU and therefore no further discussion was requested regarding the proposed statistical acceptance criterion.
A summary of the findings of the biosimilarity analysis is given in **Table 2**.

Table 2 Summary of biosimilarity analysis

Molecular Parameter	**Attribute**	**Methods for Control and Characterization**	**Key Findings**
Primary structure	Amino acid sequence	Glu-C Peptide Mapping (RP-UPLC-MS)	Identical primary sequence, sequence of principal peptides was resolved, 100% sequence coverage was obtained.
Primary structure	Pegylation Site and Linker Composition	Modified Glu-C Peptide Mapping (RP-UPLC-MS)	Identical S1 peptide sequence, the same pegylation site and the same linker composition.
Primary structure	Molecular Weight by Intact Mass	RP-UPLC-MS intact mass method	A slight shift in mass distribution is attributable to small differences in the distribution of EO units in the mPEG-p moiety. Molecular-weight dispersity (Mw/Mn) was consistent between Nyvepria and reference product lots.
Primary structure	Free Thiol content	Ellman's Assay	The results are consistent with the presence of 1 free thiol in pegfilgrastim molecule.
Primary structure	Isoelectric Point	Capillary Isoelectric Focusing	The range of isoelectric point was consistent between Nyvepria and reference product lots.
Higher order structure	Secondary and tertiary structure	CD spectroscopy	Comparable far- ultra-violet -UV CD spectra and relative theoretical secondary structure content was demonstrated.

Molecular Parameter	Attribute	Methods for Control and Characterization	Key Findings
Higher order structure	Disulfide Linkages	Non-reduced peptide mapping method	Results were consistent, all samples have identical disulfide linkages. No peptides corresponding to mismatched disulfide bonds are observed.
Higher order structure	Structure Dynamics (orthogonal method for secondary and tertiary structure analysis)	Hydrogen-Deuterium Exchange (HDX)	Relative difference in fractional uptake of deuterium for amino acids or peptides was negligible between samples for the sequence covered at all time points.
Higher order structure	Sedimentation Coefficient (orthogonal method for tertiary structure and HMW analysis)	Sedimentation Velocity Analytical Ultracentrifugation (SV-AUC)	Monomer sedimentation coefficient was in narrow range for all tested samples and aligned with reference product.
Higher order structure	Melting Temperature (Tm) (tertiary structure – thermal stability)	Differential Scanning Calorimetry, DSC	The thermogram for pegfilgrastim exhibits a broad thermal transition which is consistent with a multi-step unfolding process. All Tm values were within the quality range based on reference product.
Higher order structure	Secondary and tertiary Protein Structure	Nuclear Magnetic Resonance Spectroscopy	^{1}H 1D NMR spectra and ^{1}H-^{15}N HMQC 2D NMR spectra were highly similar between Nyvepria and reference product lots.
Product related variants and impurities	Total related proteins (oxidised pegfilgrastim, oxidation Met127, des-pegylated species, reduced pegfilgrastim, Gln108 deamidation)	Reverse phase chromatography (RP-HPLC)	The post-main peak species are apparent in both reference product samples but not as visible in Nyvepria samples. Total Related Proteins reference products were similar but Total Related Proteins in examined Nyvepria lots were consistently lower. Provided chromatograms were highly similar.
Product related variants	Total charge variants	Ion chromatography (IC-HPLC)	Levels of total charge variants are generally low for all tested samples. A shift towards absence of charged variants was reported for some Nyvepria lots.

Molecular Parameter	Attribute	Methods for Control and Characterization	Key Findings
Product related variants and impurities	Size variants (aggregates)	Size-exclusion chromatography (SEC)	Overall, the total size variants in Nyvepria lots were reported to be lower than in reference product lots.
Product related variants and impurities	Size Variants (orthogonal methods)	SDS-PAGE	Nyvepria lots showed similar band patterns as reference product lots with the reference product samples showing additional low-level bands all with very low intensities.
Product related impurity	Residual PEG	RP-HPLC-ELSD (Evaporative light scattering detection)	Provided data demonstrates that the level of Residual PEG and rate of increase over time is similar.
Product related impurity	Met127 oxidation	Reverse phase chromatography (RP-HPLC)	Met127 oxidation in Nyvepria lots examined is consistently lower compared to reference lots.
Product related variants and impurities	Oxidation variants, deamidation variants and N-term-des-PEG species	RP-UPLC-MS RP-HPLC IC-HPLC Glu-C Peptide Mapping	Results indicate that the primary site of oxidation is at Trp59 but levels of oxidised Trp59 species remain low throughout the product shelf-life. Pegfilgrastim also undergoes low levels of oxidation at Met residues. These variants were at very low levels. Levels of deamination at Gln108 in Nyvepria lots were similar to reference products. Levels of deamidated species tested by IC-HPLC are considered slightly lower compared to reference products. Majority of deamidation variants identified by Glu-C peptide mapping were under the reportable limit. Deamidation variants slightly increase over time however, the trends and rate are similar between Nyvepria and reference products.

Molecular Parameter	Attribute	Methods for Control and Characterization	Key Findings
Product related impurities	Reduced Species	Reverse phase chromatography (RP-HPLC)	The measured percent reduced species was at or below the LOQ for all tested samples.
Product related impurities	Des-Pegylated Species	Reverse phase chromatography (RP-HPLC) and Size-exclusion chromatography (SEC)	Whereas the des-pegylated species reference products are similar, the level of des-pegylated species in Nyvepria is below the method LOQ for all examined lots.
Product related impurities	N-terminal Des-Pegylated Species	Glu-C Peptide Mapping	Levels of pegylation at alternative site are low. Low levels of N-terminal S1 peptide lacking pegylation are detected by Glu-C peptide mapping but all levels are below reportable limit.
Finished product attribute	Protein Concentration	UV-Vis spectrophotometry	All tested samples were within the quality range based on reference product.
Finished product attribute	Extractable volume and deliverable content	Ph. Eur. <2.9.17>	Extractable volume was tested and was found to be similar between reference product and Nyvepria. Deliverable content was consistent and similar for all tested samples.
Finished product attribute	Subvisible particles across the size ranges of ≥ 2 um, ≥ 5 um, ≥ 10 um, and ≥ 25 um	MicroFlow Imaging (MFI)	The data demonstrated that the subvisible particle concentrations of Nyvepria are in general lower than those of reference products in each size group.
Finished product attribute	pH	Potentiometric determination Ph. Eur. <2.2.3>	In general, pH values of Nyvepria and reference product are considered similar.
Finished product attribute	Osmolality	Ph. Eur. <2.2.35>	All measured osmolality values are within the appropriate range ensuring an isotonic product for parenteral administration.

Molecular Parameter	Attribute	Methods for Control and Characterization	Key Findings
Finished product attribute	Polysorbate 20	RP-HPLC coupled with evaporative light scattering detection (ELSD)	All measured Polysorbate 20 concentration values are within a narrow range.
Finished product attribute	Appearance, Colour, and Clarity	Ph. Eur. <2.2.2> and Ph. Eur. <2.2.1>	All Nyvepria reference product lots tested are "Clear, colorless solution"
Finished product attribute	Visible Particles	Ph. Eur. <2.9.20>	All Nyvepria reference product lots tested are "Practically free of visible particles".
Functional characterization	In Vitro Potency	M-NFS-60 bioassay	The average and range of in-vitro potency are comparable to those observed for reference product.
Functional characterization	Receptor binding affinity to the G-CSF receptor	Competitive receptor binding assay (CRBA)	The average and range of binding affinity to the immobilised G-CSF receptor for Nyvepria are comparable to those observed for reference products.
Functional characterization	Receptor Binding Affinity and Kinetics	Surface Plasmon Resonance (SPR)	k_{on}, k_{off} and K_D values for Nyvepria and US sourced reference product are within the quality range.

A comprehensive analysis of the primary structure was performed. The results provided demonstrate that the amino acid sequence is identical between PF-06881894 and the EU- and US-sourced RP and consistent with the theoretical pegfilgrastim amino acid sequence. In addition, analysis of the pegylation site, linker composition, intact mass, free thiol and isoelectric point (pI) also confirm consistency between all lots analysed. A missing qualification report for non-reduced peptide mapping method for disulfide mapping analysis was provided by the applicant upon request.

It is noted that a minor shift to higher molecular weight (MW) is observed in PF-06881894 (i.e. ~0.3 to 0.4 kDa), which is attributable to small differences in the mPEG moiety. This is not considered to have a clinical impact. In addition, molecular-weight dispersity and mPEG mass distribution were similar. Based on these data, the observed small differences in MW are considered sufficiently justified.

Analysis of the higher order structure showed that the pegfilgrastim secondary structure by far-UV circular dichroism (CD) is consistent between PF-06881894 and the EU- and US-sourced RP, which is predominantly α-helical. The expected intramolecular disulfide bonds are identical in PF-06881894, pegfilgrastim-EU, and pegfilgrastim-US. The tertiary structures are demonstrated to be similar based on similarity of melting temperature, deuterium uptake, and sedimentation coefficient of pegfilgrastim monomer. The overlays of the 1D and 2D NMR spectra of all PF-06881894, pegfilgrastim-US, and

pegfilgrastim-EU lots tested were also similar. These results confirm that the pegfilgrastim protein in PF-06881894, pegfilgrastim-EU, and pegfilgrastim-US have a high degree of similarity in higher order structure.

Analysis of product-related substances and impurities showed that the levels of total related proteins, total charge variants, total size variants, Met127 oxidation, and deamidated species are slightly lower in PF-06881894 as compared to the levels observed in pegfilgrastim-EU and pegfilgrastim-US, which were similar. On the other hand, levels of other HMWS, des-pegylated species, total size variants, residual PEG, Trp59 oxidation, Gln108 deamidation, reduced species, N-terminal des-pegylated species and other low abundant pegfilgrastim-related species were comparable between PF-06881894 and both EU- and US-sourced RP. In addition, apart from determination of total related proteins, total charge variants and total size variants, the applicant also measured the levels of individual product-related substances and impurities. However, evaluation of individual size variants was requested, to further support the biosimilarity claim. The applicant differentiated the individual size variants and performed similarity assessment for individual variants. Based on the provided summary, other HMWS and Des-pegylated species were similarly low for all PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU lots. dimer in pegfilgrastim-US and pegfilgrastim-EU are similar, PF-06881894 lots have consistently lower levels of dimer.

Early PF-06881894 FP lots had a slightly higher protein concentration and slightly lower deliverable volume than pegfilgrastim-EU and pegfilgrastim-US lots. The manufacturing process was therefore slightly adjusted with regards to target protein concentration and fill weights to better match the strength of the RP. Hence, PF-06881894 FP lots produced after these adjustments are more similar to the EU- and US-sourced RP. Importantly, all lots of PF-06881894 showed consistent deliverable content similar to the lots of EU- and US-sourced RP. All other FP attributes (i.e. appearance, colour, clarity, pH, osmolality, polysorbate 20 content, and visible particles) are demonstrated to be similar between PF-06881894 and pegfilgrastim-EU and pegfilgrastim-US, with the exception of subvisible particles content, which appears to be lower in PF-06881894. Although there are some very small differences, the results confirm that the pegfilgrastim FP in PF-06881894, pegfilgrastim-EU, and pegfilgrastim-US have a similar quality profile regarding the FP attributes tested.

The functional activity of pegfilgrastim in PF-06881894 is similar to that of pegfilgrastim-EU and pegfilgrastim-US, as demonstrated by generally comparable means and ranges for *in vitro* potency, relative (receptor binding) potency, and receptor binding affinity and kinetics. In addition, overlays of receptor binding kinetic curves of PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU were also similar.

In support of the analytical similarity study between PF-06881894 and the RP, the applicant also performed comparative stability studies at long-term (5 °C), accelerated (25 °C/60% RH), and stressed (40 °C/75% RH) storage conditions, as well as comparative forced degradation studies induced by peroxide, heat, light or high pH. Based on the results presented, it can be concluded that the stability data obtained at long-term, accelerated and stressed conditions are comparable with regards to the rates and routes of degradation between PF-06881894, pegfilgrastim-US and pegfilgrastim-EU.

Upon request, the applicant submitted analytical reports and raw data package for representative lots tested in the analytical similarity assessment and comparative stability studies. Analytical data for SEC, IC-HPLC, RP-HPLC methods and response curves for potency and binding assays were provided and the data support the overall conclusion on biosimilarity as discussed in the quality assessment report.

The provided data were highly consistent between Nyvepria and reference products, with the exception of total charge variants analysis. The recently produced Nyvepria lots have reduced content of total charged variants (predominantly deamidated species recognised as impurities) compared to the reference product and other Nyvepria lots. These differences were considered minor and without

practical influence on safety or efficacy. In conclusion, biosimilarity can be considered demonstrated, all remaining other concerns on biosimilarity have been solved.

2.1.5. Discussion on chemical, pharmaceutical and biological aspects

One major objection related to the QP declaration / GMP compliance of the active substance was satisfactorily solved. An updated QP declaration was provided.

All other concerns were also satisfactory solved. However, the applicant is asked to provide the validation data of the pooling of up to two PF-06881894 filgrastim intermediate batches upon availability (see recommendation). The AS and FP manufacturing process and process controls are described in detail. Control of raw materials is adequately performed. A cell bank system consisting of MCB and WCB was established and tested and qualified. As regards the critical intermediate mPEG-p, the applicant has provided detailed information on the process and controls. The manufacturing process was appropriately validated. The AS and FP specifications proposed by the applicant are deemed suitable to control the quality of AS and FP. Analytical methods were described in detail. AS and FP specifications are properly justified.

The available long-term stability data demonstrate that PF-06881894 AS is stable for the proposed shelf life at the proposed storage condition, and that PF-06881894 FP is stable for up to 36 months when stored at 5°C. Accordingly, the proposed shelf lives are deemed acceptable.

Biosimilarity analysis

For the biosimilarity analysis, the applicant performed an extensive comparability exercise including side-by-side testing using a combination of orthogonal analytical methods, which were properly qualified, and by using up to 17 batches of pegfilgrastim-EU and pegfilgrastim-US and 10 batches of Nyvepria FP.

In general, all quality attributes analysed proved to be highly similar between Nyvepria and both EU- and US-sourced Neulasta. A minor shift to higher molecular weight is observed for the PEG moiety, which is attributable to mPEG lot-to-lot variability. However, molecular weight dispersity and mPEG mass distribution were similar between Nyvepria and the RP, confirming that the minor shift is unlikely to have any clinical impact. Furthermore, secondary and tertiary structures are demonstrated to be consistent and highly similar between Nyvepria and both EU- and US-sourced Neulasta. Product-related substances and impurities appeared to be slightly higher in both EU- and US-sourced Neulasta compared to Nyvepria, especially with regards to total related proteins (due to lower levels of Met127 oxidation and deamidation), total charge variants (due to lower levels of deamidation) and total size variants (due to lower levels of dimers). Based on the data presented, the lower levels of product-related substances and impurities rather suggest that Nyvepria has a higher purity profile. HMWS B (dimers) level was found to be consistently lower for Nyvepria compared to pegfilgrastim-US and pegfilgrastim-EU and as a result, total size variants were also consistently lower in Nyvepria samples. In general, the differences observed were small and not considered clinically relevant. In addition, comparative stability and forced degradation studies, comparison of other finished product attributes and in vitro potency, relative potency, and receptor binding affinity and kinetics were highly similar for Nyvepria and both EU- and US-sourced Neulasta. These data further confirm that the pegfilgrastim protein in Nyvepria, EU- and US-sourced Neulasta have similar higher order structure and functional conformation, which is required for biological activity. In conclusion, the data derived from these studies demonstrated similarity to the reference medicinal products.

2.1.6. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. The data derived from the biosimilarity studies demonstrated similarity of Nyvepria to the reference medicinal products at the quality level.

2.1.7. Recommendation(s) for future quality development

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

Area	Number	Description	Classification*
Quality	001	The applicant is asked to provide the validation data of the pooling of up to two PF-06881894 filgrastim intermediate batches upon availability.	REC

REC-recommendation

2.2. Non-clinical aspects

2.2.1. Introduction

The nonclinical programme of PF-06881894 also referred to as HSP-130, Hospira Pegylated G-CSF, PegG-CSF HSP, and Pegfilgrastim included a series of *in vitro* comparative studies. The assays used comprised of an *in vitro* cell-based proliferation assay, a competitive receptor binding assay, and a Surface Plasmon Resonance (Biacore) assay for determination of receptor binding affinity (K D and Relative K D) and the binding rate kinetics (k on and k off). Please see the Quality part above with regards to the details on the assessment.

In vivo pharmacological activity of PF-06881894 was assessed as part of the 4-week comparative toxicity study in CD [Crl:CD (SD)] rats (Study 1550-064).

The nonclinical toxicology program consisted of a GLP-compliant comparative 4-week, repeat-dose subcutaneous (SC) toxicity study of PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU.

Rats were selected as an appropriate test species for providing a meaningful toxicological and similarity comparison between PF-06881894 and the pegfilgrastim-US or pegfilgrastim-EU reference products based on the pharmacological relevance established from the Originator's nonclinical experience. Pfizer conducted a Good Laboratory Practice (GLP)-compliant, 4-week repeat-dose subcutaneous (SC) comparative toxicity study with a 6-week recovery period in Sprague-Dawley (SD) rats, with PF-06881894, pegfilgrastim-US and pegfilgrastim-EU, along with a concurrent control. The subcutaneous route was selected as it is the approved clinical route of administration for Neulasta 9, and consistent with the route of administration evaluated in the nonclinical toxicology program with pegfilgrastim. This study used PF-06881894 manufactured at the same scale and process used to manufacture clinical lots

of PF-06881894, thereby maximising the relevance of the nonclinical study to the clinical development program. In this nonclinical study, the potential toxicity, toxicokinetics (TK), pharmacodynamics (PD), including measurement of absolute neutrophil count [ANC], a well-established biomarker for G-CSF treatment), local tolerance, and immunogenicity (ADA, anti-pegfilgrastim antibodies) of PF-06881894, pegfilgrastim-US or pegfilgrastim-EU were characterised for an evaluation of comparability.

2.2.2. Pharmacology

Several complementary functional assays were utilised to assess pegfilgrastim biological activity as part of the PF-06881894 analytical similarity assessment. The assays used included an *in vitro* cell-based proliferation assay, a competitive receptor binding assay, and a Surface Plasmon Resonance (Biacore) assay for determination of receptor binding affinity (K D and Relative K D) and the binding rate kinetics (k on and k off).

In vivo pharmacological activity of PF-06881894 was assessed as part of the 4-week comparative toxicity study in CD [Crl:CD (SD)] rats (Study 1550-064). In rats, the magnitude of the changes in total leukocyte and neutrophil counts were similar at comparable doses of PF-06881894, pegfilgrastim-US or pegfilgrastim-EU indicating expected pharmacological effect.

No secondary pharmacodynamic, safety and pharmacodynamic drug interactions studies were conducted.

2.2.3. Pharmacokinetics

Toxicokinetic and anti-drug antibody (ADA, anti-pegfilgrastim antibodies) evaluations were conducted as part of a 4-week repeat-dose comparative toxicity study in SD rats with PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU (Study 1550-064).

The bioanalytical method used to determine the serum concentrations of pegfilgrastim in rat was a commercially available enzyme-linked immunosorbent assay (ELISA) kit designed for the quantitative determination of G-CSF. Overall, the ELISA method for the quantitation of pegylated G-CSF in rat serum is considered as successfully validated and suitable for intended use.

The qualitative immunoassay method used to screen rat serum for the presence of anti-pegfilgrastim antibodies (ADA) was validated at ICON Development Solutions in accordance with 21 CFR 58 GLP as it applies to bioanalysis. The method of cut point assessment is considered adequate. Overall, the method for the screening of anti-human PEG G-CSF in rat serum is considered as properly set up and validated.

Two apparent outliers were excluded from pharmacokinetic comparison of biosimilarity ratio between products. Rationale for exclusion and calculation of the ratio with and without concerning animal data was provided in the study report and this approach is agreed. The group mean AUC 0-120hr exposure ratios between pegfilgrastim-US or pegfilgrastim-EU and PF-06881894 on Day 1 ranged from 0.921 to 1.17 and on Day 29, from 0.869 to 1.23. The pegfilgrastim exposure was independent of sex, AUC 0-120hr and C max values increased with increasing dose in a greater than dose-proportional manner on Days 1 and 29 and was similar between treatments groups at the same dose level treated with PF-06881894, pegfilgrastim-US or pegfilgrastim-EU. From non-clinical perspective similarity in PK profile has been sufficiently demonstrated.

The presence of anti-pegfilgrastim ADA increased over time following repeated administration and appeared to impact systemic exposure to PF-06881894, US-licensed Neulasta, and EU-licensed Neulasta for several animals on Day 29. However, there was no apparent correlation between dose and the

incidence of animals with anti-pegfilgrastim ADA or between test article (PF-06881894, US-licensed Neulasta and EU-licensed Neulasta) and the incidence of animals with anti-pegfilgrastim ADA.

End point titer (EPT) values in several animals suggests that the presence of ADA at a sufficient titre may impact systemic exposure to pegfilgrastim. However, no significant differences in total incidence of anti-pegfilgrastim ADA has been observed between PF-06881894, pegfilgrastim-US and pegfilgrastim-EU and calculated PK biosimilar ratio has not been influenced.

There are no distribution, metabolism, excretion or additional studies provided by the applicant and those studies are not needed for testing of biosimilars.

2.2.4. Toxicology

Single-dose toxicity studies with PF-06881894 have not been conducted. This is in alignment with relevant biosimilar guidelines.

The nonclinical toxicology program consisted of a GLP-compliant comparative 4-week, repeat-dose subcutaneous (SC) toxicity study of PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU.

The comparative repeat-dose toxicity study was conducted with PF-06881894 drug substance Batch PFS01P/14. This lot was manufactured at the same scale and using the same process as the clinical PF-06881894 Drug Substance lots.

The doses of 200 and 1800 µg/kg for this comparative toxicity study were selected by the applicant to match previous nonclinical toxicity studies (sub-chronic) of PF-06881894 in the rodent. PF-06881894 is a recombinant human granulocyte colony stimulating factor analogue of filgrastim, which increases the body's production of neutrophils. PF-06881894 was expected to produce dose-responsive pharmacologic effects to the haematopoietic system as detailed below without causing adverse effects. In MPI Research Study Number 1550-051, PF-06881894 was administered to rats weekly by subcutaneous injection for 4 weeks at 200, 600, or 1800 µg/kg/dose followed by a 4-week recovery. All dose levels were well tolerated with no definitive adverse findings. The primary treatment related findings were associated with expected pharmacology of granulocyte colony stimulating factor (G-CSF), which included a dose-responsive increase in leukocytes (mainly neutrophils), an increase in M:E ratio, and increased haematopoiesis in bone marrow, spleen, and liver.

In rats, GLP study included assessment of toxicity based on mortality, cageside, clinical, dermal, and ophthalmoscopic observations, body weight and food consumption, and clinical and anatomic pathology. Toxicokinetic (TK) assessment was conducted for the test articles. Immunogenicity (anti-pegfilgrastim antibody) assessment was also conducted.

In general, study design, followed parameters, doses, route of administration, species and duration of treatment was adequately chosen to address the potential differences between concerned products.

Overall, there were no differences noted in the severity or incidence of microscopic changes among groups given PF-06881894, US-licensed Neulasta, or EU-licensed Neulasta once weekly (for a total of five doses) at each respective dose. All test article-related microscopic effects were reversible, considered to be related to the pharmacological activity of the test articles, and were not considered to be adverse.

Reproduction toxicology, genotoxicity and carcinogenicity studies were not conducted as these are not routine requirements to demonstrate similarity of biological medicinal products containing recombinant G-CSF as active substance.

No stand-alone studies have been conducted to evaluate the local tolerance in line with the guideline on local tolerance. There were no differences noted in the severity or incidences of microscopic changes

between treatment groups at the same dose level treated with PF 06881894, pegfilgrastim-US or pegfilgrastim EU.

PF-06881894 was previously developed as a new biologic entity with prior supportive noncomparative nonclinical studies completed to enable the conduct of a clinical development program. The noncomparative data provided do not indicate different toxicity profile in comparison to reference product Neulasta (EPAR) or those in the comparative four-week toxicity study in rats.

End point titre (EPT) values in several animals suggests that the presence of ADA at a sufficient titre may impact systemic exposure to pegfilgrastim. However, no significant differences in total incidence of anti-pegfilgrastim ADA has been observed between PF-06881894, pegfilgrastim-US and pegfilgrastim-EU and calculated PK biosimilar ratio has not been influenced.

2.2.5. Ecotoxicity/environmental risk assessment

No ERA studies were provided with reference to the guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 Corr 2).

Conjugated PEG component is commonly used, considered safe and represents no additional environmental risk. Regarding the fact that the active substance pegfilgrastim is a polypeptide which is expected to be largely metabolised after administration and easily biodegraded in the environment, the omission of ERA studies indeed can be accepted as described in above mentioned guideline. A biosimilar, pegfilgrastim is already used in existing marketed products and no significant increase in environmental exposure is anticipated. Therefore, pegfilgrastim is not expected to raise a risk to the environment. The justification provided is adequate.

2.2.6. Discussion on non-clinical aspects

Non-clinical scientific advice was requested from CHMP regarding to comparative 4-week repeat-dose study (1550-064) in rats in 2017. The study was considered by CHMP as adequate and sufficient as the only *in vivo* comparative study for the nonclinical data package according to EMEA/CHMP/BMWP/31329/2005: Biosimilar medicinal products containing recombinant granulocyte-colony stimulating factor (Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues). Specifically, no reproductive/development /carcinogenicity studies or to demonstrate the pharmacodynamic (PD) effect of PF-06881894 in separate non-neutropenic and neutropenic *in vivo* animal models were proposed and conducted. This is adequate.

The pharmacological activity of PF-06881894 was consequently assessed as part of the 4-week comparative toxicity study in CD [Crl:CD (SD)] rats (Study 1550-064). In rats, the magnitude of the changes in total leukocyte and neutrophil counts were similar at comparable doses of PF-06881894, pegfilgrastim-US or pegfilgrastim-EU indicating expected pharmacological effect.

Toxicokinetic and anti-drug antibody (ADA, anti-pegfilgrastim antibodies) evaluations were conducted as part of a 4-week repeat-dose comparative toxicity study in SD rats with PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU (Study 1550-064). From non-clinical perspective similarity in PK profile has been sufficiently demonstrated.

Reproduction toxicology, genotoxicity and carcinogenicity studies were not conducted as these are not routine requirements to demonstrate similarity of biological medicinal products containing recombinant G-CSF as active substance.

No stand-alone studies have been conducted to evaluate the local tolerance. This is considered acceptable as it is in line with the guideline on local tolerance. There were no differences noted in the severity or incidences of microscopic changes between treatment groups at the same dose level treated with PF 06881894, pegfilgrastim-US or pegfilgrastim EU.

With regards to the environmental risk assessment, pegfilgrastim is not expected to raise a risk to the environment.

2.2.7. Conclusion on the non-clinical aspects

From a non-clinical point of view, this marketing authorisation application is approvable.

2.3. Clinical aspects

2.3.1. Introduction

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.

The applicant's investigational drug, PF-06881894 (historically referred to as HSP-130), is a pegylated recombinant human granulocyte colony-stimulating factor (G-CSF) that is currently being developed as a proposed biosimilar to Neulasta (pegfilgrastim).

PF-06881894 is a covalent conjugate of recombinant human methionyl G-CSF (met-HuG-CSF, or filgrastim) and a single methoxypolyethylene glycol moiety of approximate 20,000 Daltons. The Filgrastim Intermediate is produced in Escherichia coli (E. coli) transformed with a genetically engineered plasmid containing the gene sequence encoding the met-HuG-CSF protein product. Commercial forms of recombinant human G-CSF (rh-G-CSF) include E. coli-derived G-CSF, which is non-glycosylated, e.g. filgrastim (Neupogen, Amgen) and Chinese hamster ovary cell-derived G-CSF, which is glycosylated, e.g. lenograstim (Chugai Pharma).

Throughout this report, Neulasta sourced from the US will be referred to as pegfilgrastim-US, and Neulasta sourced from the EU will be referred to as pegfilgrastim-EU. Neulasta is used as a collective term to indicate both pegfilgrastim-US and pegfilgrastim-EU and pegfilgrastim is used as a collective term to indicate both PF-06881894 and Neulasta.

The comparative exercise for clinical similarity assessment included 2 studies in healthy volunteers: a randomised single-dose comparative PD/PK study (ZIN-130-1505 [C1221001]; hereafter referred to as ZIN-130-1505) comparing PF-06881894 to pegfilgrastim-US and pegfilgrastim-EU (

Figure 1); and a comparative immunogenicity study (C1221005) comparing PF-06881894 to pegfilgrastim-US (**Figure 2**). The clinical studies and their endpoints for similarity assessments are listed in the table below. Both studies were completed at the time of the submission.

Additionally, as the clinical development program for PF-06881894 was started as a new entity licensure pathway, an ascending single- and multiple-dose study in women with non-distantly metastatic breast

cancer (ZIN-130-1504 [C1221002]; hereafter, referred to as ZIN-130-1504) was conducted. As this study was non-comparative, it was not considered integral to the biosimilar clinical program (only supportive) and its results will not be described in this report.

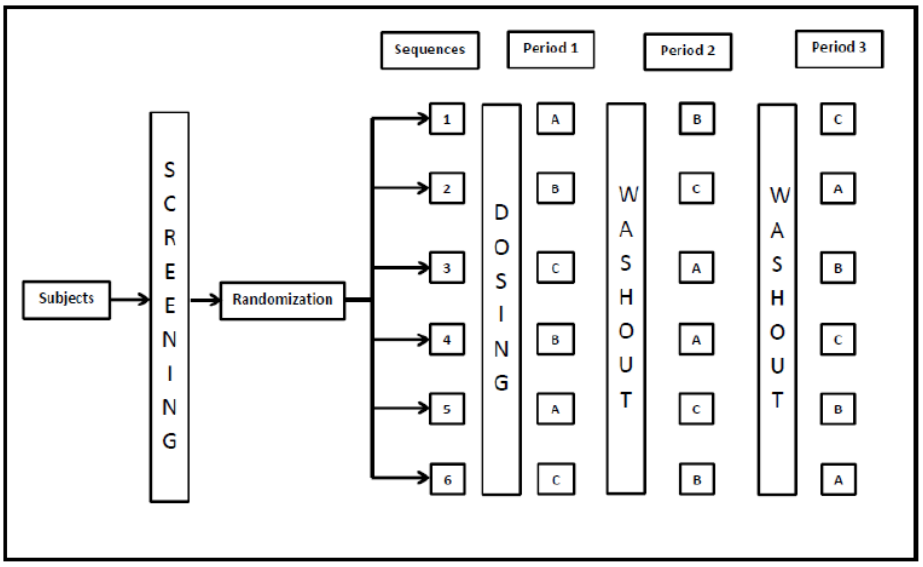
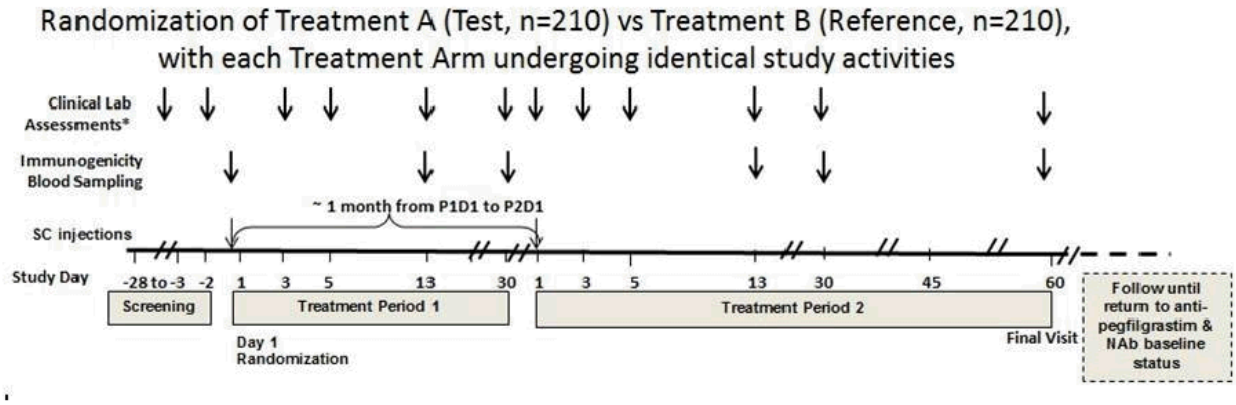


Figure 1: Study design ZIN-130-1505 (C1221001) (Treatment A: PF-06881894, 6 mg, single SC injection in the deltoid region; Treatment B: pegfilgrastim-US, 6 mg, single SC injection in the deltoid region; Treatment C: pegfilgrastim-EU, 6 mg, single SC injection in the deltoid region; 25 or 26 subjects enrolled in each sequences) (source: CSR zin-130-1505)



Source: Module 5.3.5.4 C1221005 Study Report Body Figure 1

*Clinical Lab Assessments included the following:

- Screening: including chemistry, hematology (included ANC, platelets) and UA with PCR if 1+ to 4+ protein; serum β-hCG; urine drug/alcohol/cotinine screen;
- Day -2: urine β-hCG; Chemistry Panel; hematology (included ANC, platelets); urine drug/alcohol screen;
- P1D3, P1D5, and P1D13: Enzyme Panel; hematology (included ANC, platelets);
- P1D30 (±2): Chemistry Panel; hematology (included ANC, platelets);
- P2D1: Urine β-hCG; Chemistry Panel; hematology (included ANC, platelets);
- P2D3, P2D5, and P2D13: Enzyme Panel; hematology (included ANC, platelets);
- P2D30 (±2): Chemistry Panel; hematology (included ANC, platelets); UA with PCR if 1+ to 4+ protein and urine β-hCG;
- Early Termination: Chemistry Panel; hematology (included ANC, platelets); UA with PCR if 1+ to 4+ protein and urine β-hCG;
- P2D60 (±5): Urine β-hCG; Chemistry Panel; hematology (included ANC, platelets).

Abbreviations: ANC = absolute neutrophil count; β-hCG = beta-human chorionic gonadotropin; D = day; n = number of subjects; NAb = neutralizing antibody; P = Period; PCR = protein: creatinine ratio; SC = subcutaneous; UA = urinalysis.

Figure 2: Study design C1221005. Each randomised subject was to receive a total of 2 doses of 6 mg assigned treatment (PF-06881894 or pegfilgrastim-US), which was administered as 1 SC injection each on Period 1 Day 1 (P1D1) and Period 2 Day 1 (P2D1). In Treatment Period 2, each subject was to receive 1 SC dose of the same regimen received in Treatment Period 1. There was an interval of approximately 1 month between P1D1 and P2D1, which was consistent with clinical use of pegfilgrastim-US and its half-life. The duration of Treatment Period 1 was 30 (± 2) days and the duration of Treatment Period 2 was 60 (± 5) days.) (source: CSR C1221005)

- Tabular overview of clinical studies

Protocol No. Country	Study Design and Objective	Treatment Groups	No. of Subjects (by Treatment Group)	Demographics	Duration of Treatment	Study Start/Status
COMPARATIVE PD/PK STUDY REPORT						
C1221001 (ZIN-130-1505) Comparative PD/PK Study (non-IND Study) - Integral to biosimilar pathway Australia	Open-label, randomized, single-dose, comparator-controlled, 3-treatment, 3-period, 6-sequence, crossover, study to assess the PD and PK of PF-06881894, and pegfilgrastim-US and pegfilgrastim-EU in healthy subjects. **Primary Objective:** • To assess the PD equivalence of PF-06881894 with pegfilgrastim-US and pegfilgrastim-EU administered as a single SC dose. **Secondary Objectives:** • To assess the PK equivalence of PF-06881894 with pegfilgrastim-US and pegfilgrastim-EU administered as a single SC dose • To assess the PD and PK equivalence of pegfilgrastim-US and pegfilgrastim-EU when administered as a single SC dose • To assess the safety of PF-06881894.	A single SC dose of: • Treatment A: PF-06881894 6 mg • Treatment B: pegfilgrastim-US 6 mg • Treatment C: pegfilgrastim-EU 6 mg There were 3 treatment periods and 6 sequences within the study as follows with a target of at least 56 days of washout between each study drug: • Sequence 1: A/B/C • Sequence 2: B/C/A • Sequence 3: C/A/B • Sequence 4: B/A/C • Sequence 5: A/C/B • Sequence 6: C/B/A	Total Treated 153: 26 in Sequence 1, 4, and 6 25 in Sequence 2, 3, and 5 Completed: 142: 24 in Sequence 1 23 in Sequence 2 21 in Sequence 3 24 in Sequence 4 25 each in Sequence 5 and 6	Sex: 81 M/72 F Mean (SD) Age: 30.4 (12.48) years Range: 18.0, 65.0 years Race: W/B/A/O: 132/4/8/9	Subjects received a single 6 mg SC dose of study drug (PF-06881894 or pegfilgrastim-US or pegfilgrastim-EU) in each of 3 treatment periods, and were enrolled in the study (through the follow-up visit in Period 3) for a total study duration of approximately 143 days.	21 Aug 2015/03 Jun 2016 (Study Completion Date)
COMPARATIVE IMMUNOGENICITY STUDY REPORT						
C1221005 - Integral to biosimilar pathway United States	Randomized, open-label, multiple-dose, and parallel design non-inferiority study to assess the immunogenicity of multiple doses of PF-06881894 and pegfilgrastim-US in healthy subjects **Primary Objective:** • To assess the non-inferiority of PF-06881894 versus pegfilgrastim-US with respect to immunogenicity (anti-pegfilgrastim	Each randomized subject received a total of 2 doses of assigned treatment:[b] • PF-06881894, 6 mg, SC injection – Regimen A (Test Product) • Pegfilgrastim-US, 6 mg, SC injection – Regimen B (Reference Product)	Randomized: 422 (212 in PF-06881894 and 210 in pegfilgrastim-US) Treated: 210 each in PF-06881894 and pegfilgrastim-US Completed: 376 (186 in PF-06881894 and 190 in pegfilgrastim-US)	Sex: 183 M/237 F Mean (SD) Age: 37.17 (11.65) years for PF-06881894 group 36.05 (11.34) years for Pegfilgrastim group Range: 18, 65 years Race: W/B/A/O: 150/54/2/4 for PF-06881894 group	The duration of Treatment Period 1 was 30 (± 2) days and the duration of Treatment Period 2 was 60 (± 5) days.	27 Oct 2017/ 25 Jul 2018 (Study Completion Date)

	antibodies). **Secondary Objectives** • To assess the safety of PF-06881894 and pegfilgrastim-US[a]			141/62/5/2 for Pegfilgrastim group		
NON-COMPARATIVE PD/PK STUDY						
C1221002 (ZIN-130-1504) Not integral to biosimilar pathway Hungary and Spain	Open-label, non-comparative, parallel-group Phase 1-2 study characterizing the PD, PK, and safety (including immunogenicity) of PF-06881894 in patients with non-distantly metastatic (non-Stage IV) breast cancer who had not received chemotherapy prior to enrollment in this study. **PHASE 1 (CYCLE 0)** **Primary Objective:** • To characterize the PD response of ANC and $CD34^+$ count to PF-06881894 at doses of 3 mg and 6 mg when administered as a single SC dose without chemotherapy to determine whether it would be appropriate to study multiple doses of 3 mg in the context of background chemotherapy. **Secondary Objectives:** • To characterize the PK of PF-06881894 at doses of 3 mg and 6 mg when administered as a single SC dose without background chemotherapy • To characterize the safety of PF-06881894 at doses of 3 mg and 6 mg when administered as a single SC dose without background chemotherapy.	**Phase 1 (Cycle 0)**[c] • Regimen A: PF-06881894, 3 mg, single SC injection in the deltoid region (n = 6) • Regimen B: PF-06881894, 6 mg, single SC injection in the deltoid region (n = 6). **Phase 2 (Cycles 1-4)**[d] • Regimen B: PF-06881894, 6 mg, single SC injection in the deltoid region, at least 24 hours after administration of chemotherapy (Day 2) in Cycle 1, Cycle 2, Cycle 3, and Cycle 4 (n = 13).	**Phase 1 (Cycle 0)** Treated: 12 (6 subjects each in 3-mg and 6-mg PF-06881894) Completed: 12 (6 subjects each in 3-mg and 6-mg PF-06881894) **Phase 2 (Cycles 1-4):** Treated: 13 Completed: 13	Sex: 25 F Mean (SD) Age: 59.3 years (10.9) Range: 39.0-78.0 years Race: W/B/A/O: 24/0/1/0	**Phase 1 (Cycle 0)** PF-06881894 3 mg, Single dose PF-06881894 6 mg, Single dose The duration of Cycle 0 was for approximately 30 (± 2) days . **Phase 2 (Cycles 1-4)** PF-06881894 6 mg: one dose for each of the 4 cycles The duration of Cycles 1 to 4 was for approximately 94 days (including Follow-up Visit)	21 Dec 2015/ 05 Oct 2017 (Study Completion Date)

	PHASE 2 **(CYCLES 1-4)** **Primary Objective:** • To characterize the PD response of DSN in Cycle 1 to PF-06881894 over a range of doses when administered as single and multiple SC doses. **Secondary Objectives:** • To characterize the PD response of ANC to PF-06881894 in Cycles 1 and 4 over a range of doses when administered as single and multiple SC doses • To characterize the PK of PF-06881894 in Cycles 1 and 4 over a range of doses when administered as single and multiple SC doses • To characterize the safety, including immunogenicity, of					
	PF-06881894 over a range of doses when administered as single and multiple SC doses.					

Abbreviations: A = Asian; AE = adverse event; AESI = adverse events of special interest; ANC = absolute neutrophil count; B = Black; CD34+ = haematopoietic progenitor cell antigen; DSN = duration of severe neutropenia; ECG = electrocardiogram; EU = Europe; F = female; IND = Investigational New Drug; No. =

Number; O = other; PD = pharmacodynamics; PK = pharmacokinetics; SC = subcutaneous; SD = standard deviation; US = United States; W = white a. Assessed by clinical AEs, including AEs of Special Interest (AESIs), laboratory values, vital signs, physical examination, 12-lead electrocardiograms (ECGs), concomitant medication, and local injection site reactions (ISRs).

[b]. One injection was administered on Period 1 Day 1 (P1D1) and on Period 2 Day 1 (P2D1) for a total of 2 doses. In Treatment Period 2, each subject received 1 SC dose of the same regimen received in Treatment Period 1.

[c]. In Phase 1 (Cycle 0), subjects were sequentially enrolled to receive PF-06881894 treatment (3 mg [Regimen A] or 6 mg [Regimen B]) without concomitant or background chemotherapy. Dose-escalation from Regimen A to B was based on safety assessments (vital signs, concomitant medications, laboratory assessments, electrocardiogram [ECG], physical examination, and any AEs occurring post dose administration through Day 30) in the 6 evaluable subjects receiving Regimen A. Based on these assessments, there was no contraindication for dose-escalation and screening for the subsequent dose level (Regimen B) which was subsequently initiated (Module 5.3.4.2 ZIN 130 1504 Study Report Body Section 9.1.1)

[d]. In Phase 2, a second group of subjects received up to 4 cycles of PF-06881894 (6 mg by SC injection) with concomitant background chemotherapy. The 3 mg dose was not included in Phase 2 based on results of Cycle 0 where, as expected, the PD and PK results for the 3 mg dose tended to be lower than the results for the 6 mg (Module 5.3.4.2 ZIN 130 1504 Study Report Body Section 9.1.2).

2.3.2. Pharmacokinetics

The clinical pharmacology of PF-06881894 was characterised based on the ZIN-130-1505 PD/PK study in healthy subjects.

The primary objective of the single-dose comparative PD/PK study was to assess the PD and PK equivalence of PF-06881894 with pegfilgrastim-US and pegfilgrastim-EU administered as a single 6 mg SC dose. In addition, the assessment of safety (including immunogenicity) of PF-06881894 and pegfilgrastim-US and pegfilgrastim-EU was a secondary objective for this study.

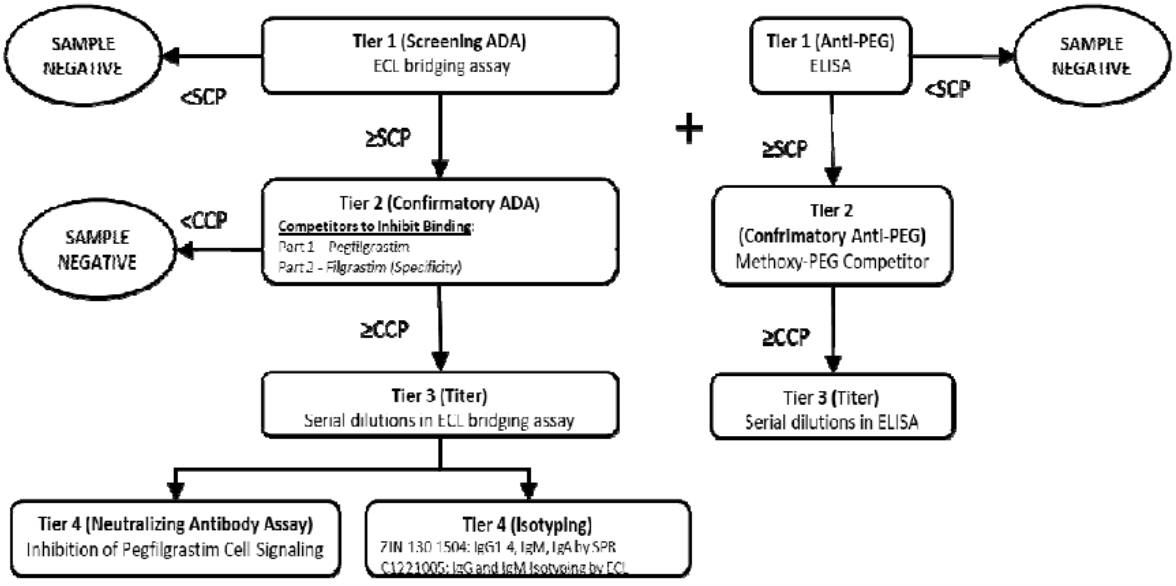
Bioanalytical methods

Pegfilgrastim concentrations in serum were determined using an electrochemiluminescence (ECL) immunoassay validated over a calibration range of 100 - 5000 pg/mL at Inventiv Health Clinical Lab, US. Samples of subjects dosed with PF-06881894, pegfilgrastim-US or pegfilgrastim-EU were all analysed using a calibration curve produced by PF-06881894, which is acceptable as precision and accuracy results were similar for the different compounds.

It was shown that an anti-pegfilgrastim antibody interfered with the measurement of PF-06881894 and pegfilgrastim-US. The magnitude of interference was dependent on the concentration of the antibody and pegfilgrastim. PK equivalence was shown previously that it was not influenced by the ADA status, and the impact of ADA on PK bioequivalence has been further discussed using the ADA confirmatory cut point set at 1% false positive rate. Following clarification provided by the applicant regarding the false positive rate, it is considered as an acceptable one. The low immunogenicity potential of the product is acknowledged. The previously provided results for the Study C1221005 have been updated during the evaluation using a confirmatory CP of 9.33% instead of 8.41% (which was the CP for filgrastim specificity). This change resulted in reclassification of data for 3 subjects, with ultimately 20 (10.0%) and 24 (12.1%) subjects in the PF 06881894 and pegfilgrastim US groups, respectively, having samples that tested positive for anti-pegfilgrastim antibody using a confirmatory CP of 9.33%

Anti-drug antibody assays

A multi-tiered test strategy was applied to evaluate relative immunogenicity of Pegfilgrastim (PF-06881894 or PF-06881894). Anti-Pegfilgrastim and anti-PEG antibodies were assessed in two parallel assays. Identical bioanalytical methods were applied to studies ZIN-130-1504, ZIN-130-1505 and C1221005, except for the isotypes determination assay.



Abbreviations: CCP = confirmatory cut-point; ECL = electrochemiluminescence; SCP = screening cut-point; SPR = Surface Plasmon Resonance assay, ELISA = enzyme-linked immunosorbent assay; ADA = anti-drug antibody; PEG = polyethylene glycol

Figure 3: Schematic for multitier immunogenicity sample testing strategy

Initially, samples were subject to a screening assay. The bridging assay format used Sulfo-TAG-Pegfilgrastim and biotinylated Pegfilgrastim as the labelled antigens, and electrochemiluminescence (ECL) detection of complexes of ADA with bridged labelled antigen on streptavidin-coated MSD plates.

All screened positive samples were tested in a confirmatory assay in the same assay format using excess Pegfilgrastim as the competing agent. Confirmed antibody positive samples were then further evaluated for Filgrastim specificity before proceeding to measurement of Ab titre using the same ECL assay format.

The ability of this one-assay approach, using the biosimilar as binding and detection reagents, to detect ADA against either Neulasta-US, Neulasta-EU or Pegfilgrastim was confirmed using an affinity-purified rabbit anti-PEG-GCSF polyclonal Ab selected as positive control Ab. Pre-study cut-points (CP) were established for studies ZIN-130-1504 and ZIN-130-1505 whereas in-study CP were established for study C1221005. The reliability of the CP for the study samples has been discussed and is considered reliable for the study ZIN-130-1505. For studies ZIN-130-1505 and C1221005, the confirmatory CP was set at a 0.1% false-positive rate. As required, the applicant has submitted the immunogenicity results observed using the confirmatory CP with a false-positive rate of 1% for the studies (instead of 0.1%). For study C1221005, although the number of subjects with negative baseline anti-pegfilgrastim antibody and confirmed post-dose positive anti-pegfilgrastim antibody at any visit increase, the number is similar between PF-06881894 and Pegfilgrastim-US. For study ZIN-130-1505, the numbers of patients with positive anti-pegfilgrastim antibody remain close to the numbers observed with the confirmatory CP with a false-positive rate of 0.1%.

Confirmed anti-Pegfilgrastim antibody positive samples were further assessed for neutralizing capability using a cell-based assay and antibody isotypes were determined using two different approaches.

The presence of NAb in serum samples was evidenced by the G-CSF mediated luminescence inhibition in transfected U937 cell line. NAb against Pegfilgrastim were assessed in study C1221005 and against both Pegfilgrastim and Filgrastim in study ZIN-130-1505. For the Pegfilgrastim-NAb assay validation, an affinity-purified goat anti-hG-CSF polyclonal IgG Ab was selected as the positive control. Reliability of the pre-study CP used for the study ZIN-130-1505 was confirmed. Environmental influences on the assay performance were assessed.

Lastly, an ECL-based assay allowing the measurement of anti-Pegfilgrastim IgG and IgM concentrations and a surface plasmon resonance (SPR)-based assay that allow the identification of Ig subtypes (IgA, IgG, IgM) and IgG subclasses were used on positive samples from study C1221005 and study ZIN-130-1505, respectively. Isotypes assessment was exploratory.

Detection and characterization of anti-PEG antibodies

Anti-PEG Ab were measured by ELISA. According to the applicant, this method was found to provide the highest sensitivity and specificity to all forms of anti-PEG antibodies tested (IgG, IgM, and pooled human anti-sera). The ELISA was validated using a human serum positive and negative control pools. The choice of the coating (mono-pegylated BSA) and the competitive (methoxy PEG) agents were deemed appropriate. Regarding the design of the assay, rabbit anti-Hu IgG/A/M and goat anti-mouse IgG as detector Ab were used. The selection of the positive control originating from human samples is supported. In-study CP was appropriately used in study C1221005. In the study ZIN-130-1505, the parametric confirmatory cut point (CCP) has been used (72.1% inhibition).

Study ZIN-130-1505 (C1221001)

A Phase 1 Study assessing the pharmacodynamic and pharmacokinetic equivalence of PF-06881894 with pegfilgrastim-US and pegfilgrastim-EU administered as a single subcutaneous dose to healthy volunteers.

Study design

This was a Phase 1 open-label, randomised, single-dose, comparator-controlled, 3-treatment, 3-period, 6-sequence, crossover study to assess the PD and PK of sponsor's pegylated filgrastim, PF-06881894, and pegfilgrastim-US and pegfilgrastim-EU in healthy volunteers. Eligible subjects were to be randomly

assigned to 1 of 6 sequence groups to receive each of the following study drugs over 3 study periods. A total of 150 subjects were to be randomised to the 6 sequences, such that 25 subjects would be randomised to each of the sequences.

The primary objective of the study was to assess the PD equivalence of PF-06881894 with pegfilgrastim-US and pegfilgrastim-EU administered as a single SC dose.

The secondary objectives of this study were:

- To assess the PK equivalence of PF-06881894 with pegfilgrastim-US and pegfilgrastim-EU administered as a single SC dose;
- To assess the PD and PK equivalence of pegfilgrastim-US and pegfilgrastim-EU when administered as a single SC dose;
- To assess the safety of PF-06881894.

Dose and mode of administration

- Treatment A: PF-06881894, 6 mg, single SC injection in the deltoid region;
- Treatment B: pegfilgrastim-US, 6 mg, single SC injection in the deltoid region;
- Treatment C: pegfilgrastim-EU, 6 mg, single SC injection in the deltoid region.

There were a 3 treatment periods and 6 sequences within the study.

Study drug was administered in the morning on Day 1 in each period. Subjects were provided with a snack approximately 10 hours before dosing and then fasted until breakfast was served following collection of the 2 hour PK sample.

Sampling schedule

Blood samples (5.0 mL) for pegylated filgrastim assay were collected by either IV catheter or venipuncture into evacuated collection tubes within 1 hour prior to dose and at 1, 2, 4, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, and 288 hours postdose.

Wash-out

56 days

Test and reference products

PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU are sterile, clear solutions stabilised with polysorbate 20 and sorbitol. Study drug was packaged in prefilled syringes (PFS) containing specific concentration per amount of fill volume.

Table 3: Study Drug Description

Investigational Product Description	Vendor Lot Number	Pfizer Lot Number	Strength/Potency	Dosage Form
PF-06881894	N/A	2051124	6 mg / 0.6 mL	PFS
Pegfilgrastim-US	1054726	N/A	6 mg / 0.6 mL	PFS
	1057095	N/A	6 mg / 0.6 mL	PFS
	1057133	N/A	6 mg / 0.6 mL	PFS
	1057373	N/A	6 mg / 0.6 mL	PFS
Pegfilgrastim-EU	1056658B	N/A	6 mg / 0.6 mL	PFS
	1057625B	N/A	6 mg / 0.6 mL	PFS
	1060064C	N/A	6 mg / 0.6 mL	PFS
	1061466C	N/A	6 mg / 0.6 mL	PFS

During the course of the study, more than one batch of the reference product was used. The applicant showed similar protein content and potency in the used batches.

Population(s) studied

A total of 153 subjects were enrolled in the study. Of the enrolled subjects, 142 (92.8%) completed the study and 11 (7.2%) prematurely discontinued from the study. Subject disposition is summarised in Table below.

Table 4: Subject Disposition – Enrolled Population

	Screened N = 300 n (%)	PF/US/EU N = 26 n (%)	US/EU/PF N = 25 n (%)	EU/PF/US N = 25 n (%)	US/PF/EU N = 26 n (%)	PF/EU/US N = 25 n (%)	EU/US/PF N = 26 n (%)	Total N = 153 n (%)
Screened	300							
Screen Failed	147 (49.0)	0	0	0	0	0	0	0
Prematurely Discontinued Study		2 (7.7)	2 (8.0)	4 (16.0)	2 (7.7)	0	1 (3.8)	11 (7.2)
Completed Study		24 (92.3)	23 (92.0)	21 (84.0)	24 (92.3)	25 (100.0)	25 (96.2)	142 (92.8)
Safety Population		26 (100.0)	25 (100.0)	25 (100.0)	26 (100.0)	25 (100.0)	26 (100.0)	153 (100.0)
PK Population		24 (92.3)	24 (96.0)	24 (96.0)	25 (96.2)	20 (80.0)	26 (100.0)	143 (93.5)
PK Population Plus Subjects with Positive Anti-pegylated filgrastim Results		26 (100.0)	25 (100.0)	25 (100.0)	26 (100.0)	25 (100.0)	26 (100.0)	153 (100.0)
PD Population		24 (92.3)	24 (96.0)	24 (96.0)	25 (96.2)	20 (80.0)	26 (100.0)	143 (93.5)
PD Population Plus Subjects with Positive Anti-pegylated filgrastim Results		26 (100.0)	25 (100.0)	25 (100.0)	26 (100.0)	25 (100.0)	26 (100.0)	153 (100.0)
Prematurely Discontinued Study		2 (7.7)	2 (8.0)	4 (16.0)	2 (7.7)	0	1 (3.8)	11 (7.2)
Adverse event		0	1 (4.0)	0	1 (3.8)	0	0	2 (1.3)
Other		0	0	0	1 (3.8)	0	0	1 (0.7)
Physician decision		0	1 (4.0)	0	0	0	0	1 (0.7)
Withdrawal by subject		2 (7.7)	0	4 (16.0)	0	0	1 (3.8)	7 (4.6)

Overall, demographic characteristics were similar among sequence groups. For all subjects randomised, 52.9% were male and 47.1% were female subjects; the majority of subjects were white (86.3%). The age of subjects ranged from 18 to 65 years, with a mean age of 30.4 years. The overall range for subject weight was 48.80 to 100.00 kg and BMI was 19.00 to 29.80 kg/m^2.

Analysis Populations:

Safety Population: All subjects who receive at least one dose of study drug (= 153 subjects). All safety analyses will be conducted on the safety population.

Pharmacodynamic Population: Subjects who receive at least one treatment and have sufficient data to calculate the primary PD endpoints of $AUEC_{ANC}$ and ANC_C_{max}. Sufficient data is defined by meeting the following criteria: having at least 11 samples for evaluation of $AUEC_{ANC}$ (must include the pre-dose and 288 hours post-dose samples) and ANC_C_{max} can be reliably determined. Subjects who have confirmed positive anti-peg filgrastim (antidrug) antibodies at any time will not be included in the PD Population. Sensitivity analyses regarding impact of antidrug antibody including subjects with confirmed positive anti-peg filgrastim antibodies will be performed. Pharmacodynamic equivalence determination will be made on the PD population.

Pharmacokinetic Population: Subjects who receive at least one treatment and have sufficient data to calculate the primary pharmacokinetic parameters of C_{max} and $AUC_{0\text{-}inf}$ · Sufficient data is defined by meeting the following criteria: having at least 11 samples for evaluation of $AUC_{0\text{-}inf}$ (must include the pre-dose and 288 hour post-dose samples) and C_{max} can be reliably determined. Subjects who have confirmed positive anti-pegylated filgrastim (antidrug) antibodies at any time will not be included in the PK Population. Sensitivity analyses regarding impact of antidrug antibody including subjects with confirmed positive anti-pegylated filgrastim antibodies will be performed. The PK population will be the primary analysis set for the primary PK analyses.

A total of 143 subjects, assigned to 1 of the 6 sequence groups, were included in the PK population and PD population.

Of the 153 enrolled subjects, 10 subjects confirmed positive for anti-pegfilgrastim antibodies were excluded from PD and PK analyses.

Pharmacokinetic variables

The PK variables were calculated using non-compartmental methods in Phoenix WinNonlin

version 6.4.

The following PK parameters were calculated for each treatment:

Primary PK Parameter was

- $AUC_{0\text{-}inf}$: Area under the serum pegylated filgrastim versus time curve from the time of dose administration to time infinity
- C_{max}: Maximum observed serum pegylated filgrastim concentration,

Secondary PK Parameters were

- $AUC_{0\text{-}t}$: Area under serum pegylated filgrastim versus time curve from the time of dose administration to the time of last measurable concentration,
- T_{max}: The time to maximum serum pegylated filgrastim concentration
- $t_{1/2}$: Elimination half-life,
- λ_z: Elimination rate constant

Statistical methods

The GMRs and CIs were obtained using analysis of variance (ANOVA) with sequence, period, and treatment as factors for log-transformed data. For PD parameters, baseline ANC was included in the analysis model as a covariate.

PK equivalence was assessed by constructing the 90% CIs for the GMR (test/reference) for $AUC_{0\text{-}inf}$ and C_{max}. PK equivalence was concluded if the 90% CIs for both $AUC_{0\text{-}inf}$ and C_{max} were completely contained within the acceptance limits of 80%-125%. The same step-down testing for PD variables was performed.

In addition, the sponsor performed an additional analysis (Supplemental clinical study report) for AUC0-t. This parameter was also assessed by constructing the 90% CI for the GMR (test/reference).

Protocol deviations

No clinically relevant protocol deviations were reported. The targeted 56 days of wash-out was not achieved for many subjects, however washout was still minimal 51 days and thus considered adequate.

Pharmacokinetic results

A similar PK profile was observed for PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU. The mean t½ ranged from 49.4 hours to 54.7 hours for the 3 study drugs.

The PF-US, PF-EU, and US-EU geometric mean ratios for AUC0-inf, AUC0-t and Cmax ranged from 0.95 to 0.99. The 90% CIs for AUC0-inf, AUC 0-t and Cmax were completely contained within the predefined limit of 80% to 125% for all study drug comparisons (PF-US, PF-EU, and US-EU). These results demonstrate PK equivalence of all study drug comparisons.

Table 5: Summary of Pharmacokinetic Parameters – Pharmacokinetic Population

Parameter	Statistic	PF-06881894 N = 136	Pegfilgrastim-US N = 136	Pegfilgrastim-EU N = 138
$AUC_{0\text{-}inf}$ (h•pg/mL)	Arithmetic Mean	6766153	6935945	6901482
	(SD)	(4880797)	(4772249)	(4813258)
$AUC_{0\text{-}t}$ (h•pg/mL)	Arithmetic Mean	6751996	6919694	6885928
	(SD)	(4882297)	(4772398)	(4814739)
C_{max} (pg/mL)	Arithmetic Mean	188422.1	193669.9	194869.6
	(SD)	(112698.0)	(112423.3)	(114029.3)
T_{max} (h)	Arithmetic Mean	18.61	18.49	17.84
	(SD)	(5.634)	(5.814)	(5.589)
λ_z (/h)	Arithmetic Mean	0.0150 (0.00390)	0.0143	0.0144 (0.00463)
	(SD)		(0.00514)	
$t_{½}$ (h)	Arithmetic Mean	49.3963	54.7429	54.2813
	(SD)	(13.53258)	(21.98696)	(23.65452)

Table 6: Ratio and 90% Confidence Interval of Pharmacokinetic Parameters, Natural Log Transform: $AUC_{0\text{-}inf}$ and C_{max} – Pharmacokinetic Population

Comparison	Parameter	Statistic	PF-06881894 N = 136	Pegfilgrastim-US N = 136	Pegfilgrastim-EU N = 138	Ratio[a]	90% Confidence Interval
PF-US	$AUC_{0\text{-}inf}$ (h•pg/mL)	Geometric Mean	5397629	5503224		0.98	(0.91, 1.06)
		LS Mean (SE)	15.50 (0.058)	15.52 (0.058)			
	C_{max} (pg/mL)	Geometric Mean	154512.2	159688.0		0.97	(0.90, 1.04)
		LS Mean (SE)	11.95 (0.057)	11.98 (0.057)			
PF-EU	$AUC_{0\text{-}inf}$ (h•pg/mL)	Geometric Mean	5397629		5547385	0.97	(0.90, 1.05)
		LS Mean (SE)	15.50 (0.058)		15.53 (0.058)		
	C_{max} (pg/mL)	Geometric Mean	154512.2		161388.6	0.95	(0.88, 1.03)
		LS Mean (SE)	11.95 (0.057)		11.99 (0.057)		
US-EU	$AUC_{0\text{-}inf}$ (h•pg/mL)	Geometric Mean		5503224	5547385	0.99	(0.91, 1.07)
		LS Mean (SE)		15.52 (0.058)	15.53 (0.058)		
	C_{max} (pg/mL)	Geometric Mean		159688.0	161388.6	0.98	(0.91, 1.06)
		LS Mean (SE)		11.98 (0.057)	11.99 (0.057)		

Table 7: Ratio and 90% Confidence Interval of Pharmacokinetic Parameters, Natural Log Transform: AUC_{0-t} – Pharmacokinetic Population

	Parameter	Statistic	PF-06881894 (N=136)	pegfilgrastim-US (N=136)	pegfilgrastim-EU (N=138)	Ratio[a]	90% Confidence Interval
HSP/US	AUC_{0-t} (h×pg/mL)	Geometric Mean	5379269	5482242	-	0.98	(0.91, 1.06)
		LS Mean (SE)	15.50 (0.058)	15.51 (0.058)	-		
HSP/EU	AUC_{0-t} (h×pg/mL)	Geometric Mean	5379269	-	5526987	0.97	(0.90, 1.05)
		LS Mean (SE)	15.50 (0.058)	-	15.53 (0.058)		
US/EU	AUC_{0-t} (h×pg/mL)	Geometric Mean	-	5482242	5526987	0.99	(0.91, 1.07)
		LS Mean (SE)	-	15.51 (0.058)	15.53 (0.058)		

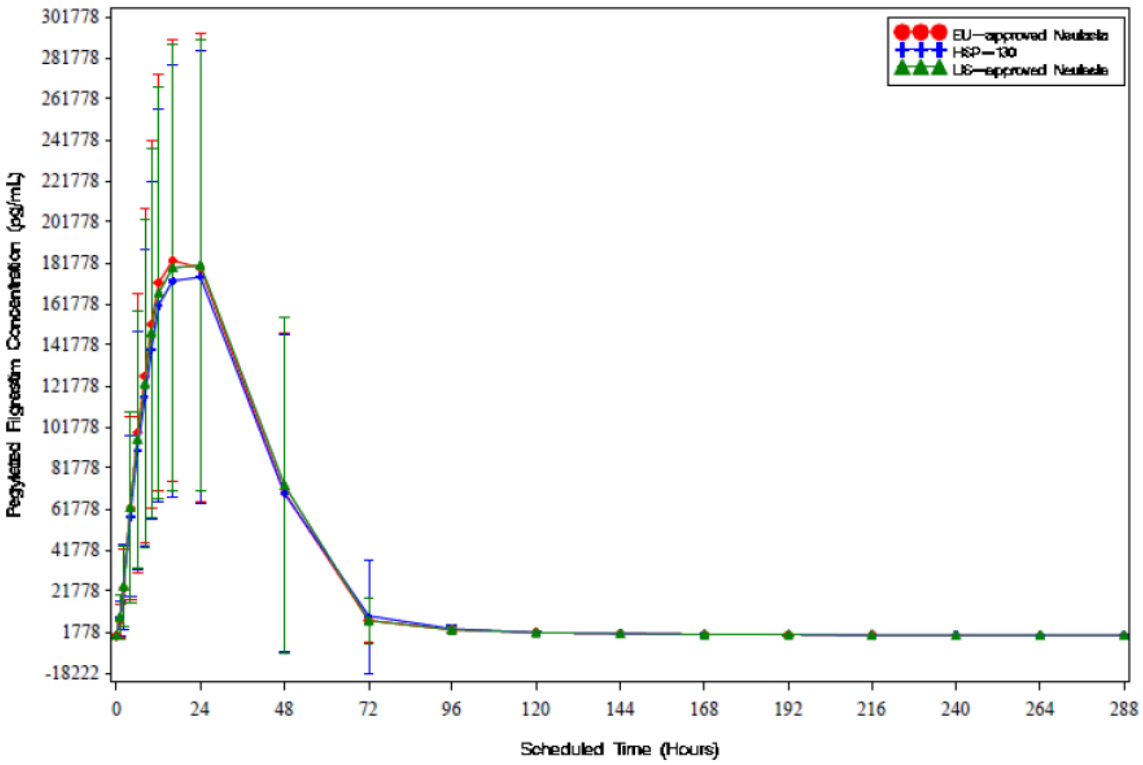


Figure 4: Mean Serum Pegylated Filgrastim Concentration Over Time. Linear Plot – Pharmacokinetic Population

Impact of ADA's on PK

The incidence of subjects with anti-pegfilgrastim antibodies was substantially lower than subjects with anti-PEG antibodies. Ten subjects (6.5%) confirmed positive for anti-pegfilgrastim antibody at least once during the study (none at baseline), whereas a total of 111 subjects (72.5%) were positive for anti-PEG antibody at least once during the study (see Immunogenicity).

The results of the sensitivity analysis that included the PK population plus the 10 subjects with confirmed positive anti-pegfilgrastim antibodies at any visit, were consistent with the results observed in the PK Population. The 90% CIs for AUC0-inf, AUC0-t, and Cmax parameters were completely contained within the predefined limit of 80% to 125% for all study drug comparisons (PF-US, PF-EU, and US-EU).

Table 8: Ratio and 90% Confidence Interval of Pharmacokinetic Parameters, Natural Log Transform: $AUC_{0\text{-}inf}$ and C_{max} – Pharmacokinetic Population Plus Subjects Confirmed Positive for Anti-Pegfilgrastim Antibodies

Comparison	Parameter	Statistic	PF-06881894 N = 146	Pegfilgrastim-US N = 146	Pegfilgrastim-EU N = 148	Ratio[a]	90% Confidence Interval
PF-US	$AUC_{0\text{-}inf}$ (h•pg/mL)	Geometric Mean	5397965	5396080		1.00	(0.93, 1.08)
		LS Mean (SE)	15.51 (0.057)	15.50 (0.057)			
	C_{max} (pg/mL)	Geometric Mean	154586.4	155510.6		1.00	(0.92, 1.08)
		LS Mean (SE)	11.95 (0.057)	11.98 (0.057)			
PF-EU	$AUC_{0\text{-}inf}$ (h•pg/mL)	Geometric Mean	5397965		5457434	0.99	(0.91, 1.06)
		LS Mean (SE)	15.51 (0.057)		15.52 (0.056)		
	C_{max} (pg/mL)	Geometric Mean	154586.4		158733.3	0.97	(0.90, 1.05)
		LS Mean (SE)	11.95 (0.057)		11.98 (0.057)		
US-EU	$AUC_{0\text{-}inf}$ (h•pg/mL)	Geometric Mean		5396080	5457434	0.98	(0.91, 1.06)
		LS Mean (SE)		15.50 (0.057)	15.52 (0.056)		
	C_{max} (pg/mL)	Geometric Mean		155510.6	158733.3	0.97	(0.90, 1.05)
		LS Mean (SE)		11.95 (0.057)	11.98 (0.057)		

The 90% CI for AUC(0-inf) and Cmax for anti-PEG negative subjects and subjects that, at least once during the study, were positive for anti-PEG antibodies were also contained within the standard 80% to 125% range for all study drug comparisons (PF-US, PF-EU, and US-EU).

Table 9: **Ratio and 90% Confidence Interval of AUC0-inf and Cmax - anti-PEG positive and anti-PEG negative subjects**

	Parameter	Statistic	PF-06881894 (N=105)	Pegfilgrastim-US (N=106)	Pegfilgrastim-EU (N=106)	Ratio[a]	90% Confidence Interval
Subjects with Positive Anti-PEG Antibodies							
PF/EU	$AUC_{0\text{-}inf}$ (h•pg/mL)	Geometric Mean	5242984	-	5339219	0.9818	(0.8960, 1.0759)
		LS Mean (SE)	15.48 (0.068)	-	15.50 (0.068)		
	C_{max} (pg/mL)	Geometric Mean	149186.6	-	154984.9	0.9623	(0.8764, 1.0566)
		LS Mean (SE)	11.92 (0.069)	-	11.96 (0.069)		
Subjects with Negative Anti-PEG Antibodies							
PF/EU	$AUC_{0\text{-}inf}$ (h•pg/mL)	Geometric Mean	5816077	-	5767555	0.9985	(0.8679, 1.1487)
		LS Mean (SE)	15.59 (0.111)	-	15.59 (0.110)		
	C_{max} (pg/mL)	Geometric Mean	169323.3	-	168601.6	0.9837	(0.8546, 1.1323)
		LS Mean (SE)	12.03 (0.107)	-	12.04 (0.106)		

subjects with negative anti-Peg antibodies: PF-06881894 (N=41), Peg-US (N=40), Peg-EU (N=42)

[a] Ratio is back-transformed in order to be expressed on the original scale of the measurement

AUC and Cmax values were, however, generally lower in the presence of ADAs (anti-PEG and anti-Pegfilgrastim). Since the scale of difference in PK values was comparable for Neulasta and PF-06881894 treated subjects, at least for anti-PEG, the decreased pegfilgrastim concentrations are not considered to be an issue in the context of a biosimilar application. For anti-pegfilgrastim antibodies, it is agreed with the applicant that the substantial difference in the size of the ADA positive (n=10) and negative population makes it difficult to determine if the observations represent a real difference.

Table 10: Summary of PK Parameters - Subjects with Positive Anti-PEG Antibodies (above) and Negative Anti-PEG Antibodies (below)

Parameter	Statistic	PF-06881894 (N=105)	US-approved Neulasta (N=106)	EU-approved Neulasta (N=106)
AUC 0-inf (h*pg/mL)	Arithmetic Mean	6493972	6768875	6574030
	SD	4234809	4900329	4438225
AUC 0-t (h*pg/mL)	Arithmetic Mean	6479672	6753945	6558551
	SD	4235728	4900292	4439038
Cmax (pg/mL)	Arithmetic Mean	182688.6	188495.0	186409.4
	SD	106513.3	117180.6	106241.4

Parameter	Statistic	PF-06881894 (N=41)	US-approved Neulasta (N=40)	EU-approved Neulasta (N=42)
AUC 0-inf (h*pg/mL)	Arithmetic Mean	7429159	7160543	7402429
	SD	6102711	4276234	5537410
AUC 0-t (h*pg/mL)	Arithmetic Mean	7416085	7142003	7387383
	SD	6104729	4276642	5539397
Cmax (pg/mL)	Arithmetic Mean	204914.6	203120.0	208364.3
	SD	125710.6	100107.6	130406.9

Two of the 10 anti-pegfilgrastim antibody positive subjects (subjects 0301128 and 0301164) had a single sample positive for NAb that occurred on P1 Day 13. The PK profile of subject 0301128 suggests that NAb may reduce the PK response. However, the effect of NAb is less clear for subject 0301164. Although a lower PK response was observed for this subject in period 2, the values for AUC and C_{max} were still contained in the range of values (min-max) obtained for the PK population.

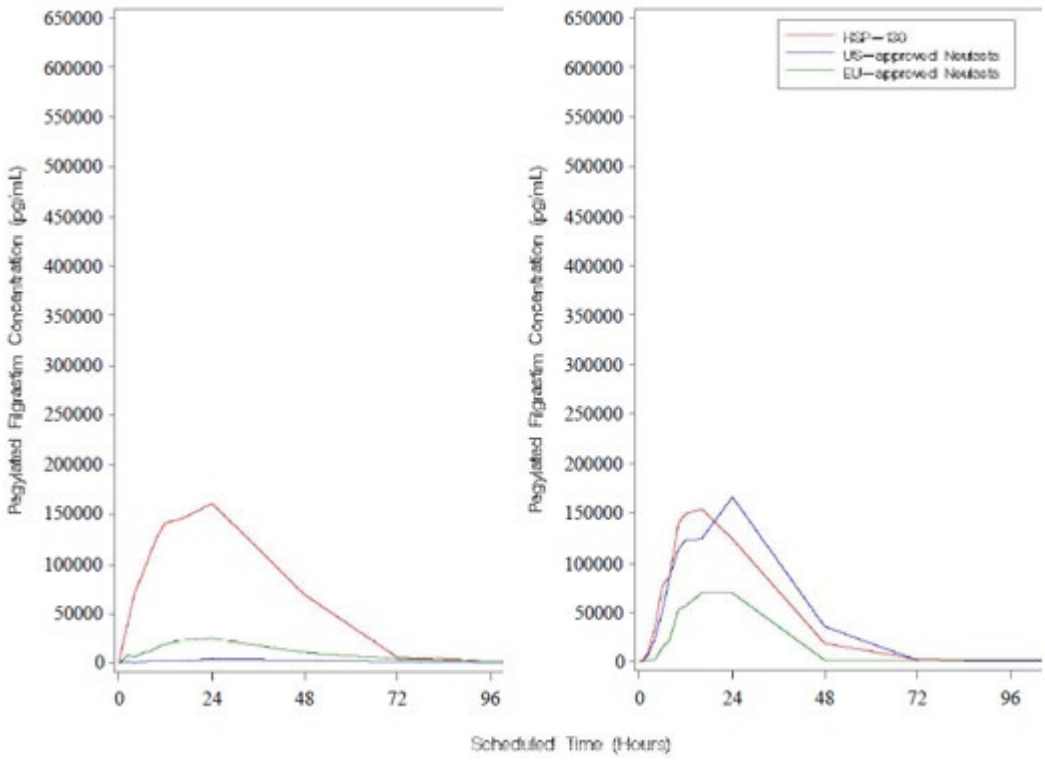


Figure 5. Pegfilgrastim concentration time profile: subject 0301128 (left) and 0301164 (right)

Based on the submitted study ZIN-130-1505 (C1221001) it can be concluded that PF-06881894 have similar PK profiles with Neulasta EU and Neulasta US administered at a dose of 6 mg.

The applicant also submitted report of Study C1221002 (ZIN-130-1504)**.** This was A Phase 1-2 Ascending Dose Study to Assess the Pharmacodynamics, Pharmacokinetics, and Safety of PF-06881894 in Subjects With Non-Metastatic Breast Cancer Following Single-Dose and Multiple-Dose Administration by Subcutaneous Injection.

The study was not an integral part of biosimilar program and is not described in detail in this part of the assessment report.

2.3.3. Pharmacodynamics

PD equivalence between PF-06881894 and Neulasta was the primary objective in the PK/PD study ZIN-130-1505. In this open-label, randomised, single-dose, 3-treatment, 3-period, 6-sequence, crossover study, ANC was assessed as a PD endpoint for similarity assessment between PF-06881894, pegfilgrastim-US and pegfilgrastim-EU administered as a single SC dose. ANC is a direct assessment of G-CSF response as it reflects the change in the number of PBPCs mobilization, drives diagnosis (e.g., grade of neutropenia), predicts prognosis (duration of severe neutropenia), and is utilised to monitor G-CSF treatment effects. Hence, ANC parameters (AUC, C_{max}) in healthy volunteers can be used as a sensitive surrogate for clinical efficacy in terms of duration of severe neutropenia after chemotherapy. Further supportive data were obtained in immunogenicity study C1221005, in which assessment of ANC was included as a safety laboratory parameter.

PK/PD Study ZIN-130-1505

Study design and data analysis

Blood samples (4.0 mL) for ANC were collected by either IV catheter or venipuncture into evacuated collection tubes within 1 hour prior to dose and at 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, and 288 hours postdose. The selected primary PD endpoints in study ZIN-130-1505 were area under the effect versus time curve for absolute neutrophil count (ANC) from the time of dose administration to 288 hours after dose administration ($AUEC_{ANC}$) and the maximum observed value for ANC (ANC_C_{max}). The secondary PD endpoint was time of maximum value for ANC (ANC_T_{max}). Supplemental analyses were performed post hoc for the additional PD endpoint, area under the effect curve for ANC from time zero to infinity ($AUEC_{ANC0\text{-}Inf}$).

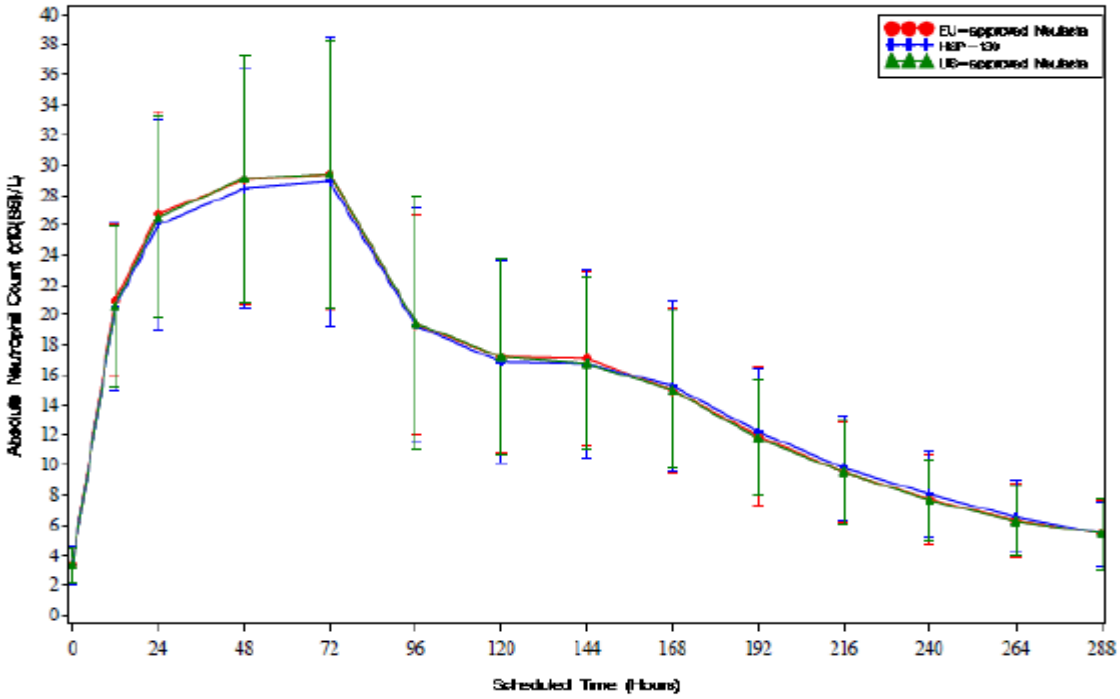
PD equivalence was assessed by constructing the 90% confidence intervals (CIs) for the GMR (test/reference) for $AUEC_{ANC}$ and ANC_C_{max}. PD equivalence was concluded if the 90% CIs for both $AUEC_{ANC}$ and ANC_C_{max} were completely contained within the acceptance limits of 80%-125%. The analysis population used to determine PD equivalence (PD population) were subjects who received at least 1 treatment and had sufficient data to calculate the primary PD endpoints. Subjects confirmed positive for anti-pegfilgrastim antibodies at any time were not included in the PD Population.

Results

Similar ANC time curves were observed for PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU. Comparable increases in mean ANC were observed after administration of PF-06881894, pegfilgrastim-EU and pegfilgrastim-US, with peak levels reached at around 3 days post-dose, which decreased thereafter. GMRs for the different parameters were close to 100% for all study drug comparisons (range 0.96 to 1.02 for primary endpoints $AUEC_{ANC}$ and ANC_C_{max}), as well as their corresponding 95% CI (range 0.94 to 1.05), indicating no difference with regard to ANC response after administration of PF-06881894, pegfilgrastim-EU and pegfilgrastim-US. When applying tighter acceptance limits (90.0-111.0%), comparability between all study drugs with regards to PD was shown as well, underlining the high degree of similarity between PF-06881894, pegfilgrastim-EU and pegfilgrastim-US. Also, the post hoc analyses for the additional PD parameter $ANEC_{ANC0\text{-}Inf}$ demonstrated 95% CIs of the GMRs for all study drug

comparisons that were fully contained within the acceptance interval of 90.0% to 111.0%, confirming the PD comparability of PF-06881894, pegfilgrastim-EU and pegfilgrastim-US.

Overall, the primary objective of this study was met and PD comparability between PF-06881894 and pegfilgrastim-EU, between PF-06881894 and pegfilgrastim-US, as well as between pegfilgrastim-EU and pegfilgrastim-US was shown.



PF 06881894 is also referred to as HSP-130, pegfilgrastim-US referred to as US-approved Neulasta, and pegfilgrastim-EU referred to as EU-approved Neulasta.
Abbreviations: EU = European Union; SD = standard deviation; US = United States.
Source: Module 5.3.4.1 Study ZIN-130-1505 Study Report Body, Figure 3

Figure 6. Mean (SD) ANC Over Time, Linear Plot - PD Population

Table 11. Primary and Supplemental Analyses: Ratio and 90% and 95% Confidence Interval of PD Parameters, Natural Log Transform: $AUEC_{ANC}$, ANC_C_{max}, and $AUEC_{ANC0\text{-}inf}$ – PD Population - Study ZIN-130-1505

Comparison	Parameter	Statistic	PF-06881894 N = 135	Pegfilgrastim-US N = 134	Pegfilgrastim-EU N = 138	Ratio[a]	90% CI	95%CI[b]
PF-US	$AUEC_{ANC}$ ($h\times10^9/L$)[c]	Geometric Mean	4541.21	4581.48		0.99	(0.97, 1.00)	(0.97, 1.00)
		LS Mean (SE)	8.42 (0.020)	8.43 (0.020)				
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	30.29	31.30		0.96	(0.94, 0.98)	(0.94, 0.99)
		LS Mean (SE)	3.41 (0.023)	3.44 (0.023)				
PF-EU	$AUEC_{ANC}$ ($h\times10^9/L$)[c]	Geometric Mean	4541.21		4588.23	0.98	(0.97, 1.00)	(0.97, 1.00)
		LS Mean (SE)	8.42 (0.020)		8.43 (0.020)			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	30.29		30.64	0.98	(0.96, 1.00)	(0.96, 1.01)
		LS Mean (SE)	3.41 (0.023)		3.42 (0.023)			
US-EU	$AUEC_{ANC}$ ($h\times10^9/L$)[c]	Geometric Mean		4581.48	4588.23	1.00	(0.98, 1.01)	(0.98, 1.02)
		LS Mean (SE)		8.43 (0.020)	8.43 (0.020)			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean		31.30	30.64	1.02	(1.00, 1.04)	(0.99, 1.05)
		LS Mean (SE)		3.44 (0.023)	3.42 (0.023)			
Supplemental Analysis: $AUEC_{ANC0\text{-}inf}$								
PF-US	$AUEC_{ANC0\text{-}inf}$ ($h\times10^9/L$)[d]	Geometric Mean	5278.19	5328.56	-	0.99	(0.96, 1.01)	(0.96, 1.01)
		LS Mean (SE)	8.57 (0.019)	8.59 (0.020)	-			
PF-EU	$AUEC_{ANC0\text{-}inf}$ ($h\times10^9/L$)[d]	Geometric Mean	5278.19	-	5296.87	0.99	(0.97, 1.02)	(0.97, 1.02)
		LS Mean (SE)	8.57 (0.019)	-	8.58 (0.019)			
US -EU	$AUEC_{ANC0\text{-}inf}$ ($h\times10^9/L$)[d]	Geometric Mean	-	5328.56	5296.87	1.01	(0.99, 1.03)	(0.98, 1.04)
		LS Mean (SE)	-	8.59 (0.020)	8.58 (0.019)			

Note: 1 The ANCOVA model is used to calculate estimates of the error variance and the least square means with baseline ANC as covariate.

2. PF = PF-06881894. EU = pegfilgrastim-EU. US = pegfilgrastim-US. PF-06881894 is also referred to as HSP-130, pegfilgrastim-US referred to as US-approved Neulasta, and pegfilgrastim-EU referred to as EU-approved Neulasta.
Abbreviations: ANC = absolute neutrophil count; ANC_C_{max} = maximum observed value for ANC; ANCOVA = analysis of covariance; $AUEC_{ANC}$ = area under the effect versus time curve for ANC from the time of dose administration to 288 hours after dose administration; $AUEC_{ANC0-inf}$ = area under the curve for ANC from time zero to infinity; CI = confidence interval; EU= European Union; LS = least square; N = number of subjects; SE = standard error; US = United States.
a. Ratio is back-transformed in order to be expressed on the original scale of the measurement.
b. 95% Confidence Interval is from the Supplemental Analysis
c. In Module 5.3.4.1 Study ZIN-130-1505 Supplemental Study Report Body, $AUEC_{ANC}$ is also referred to as $AUEC_{ANC0-t}$ in source documents
d. $AUEC_{ANC0-inf}$ is from the Supplemental Analysis
Source: Module 5.3.4.1 Study ZIN-130-1505 Study Report Body Table 9 and Module 5.3.4.1 Study ZIN-130-1505 Supplemental Study Report Body Table 2 and Table 3

Impact of ADA's on PD

Detailed analysis was provided by the applicant with regards to the impact of ADAs on PD parameters of pegfilgrastim.

With regards to the presence of anti-PEG antibodies, a total of 111 subjects (72.5%) of all subjects enrolled in the study (n = 153) were positive for anti-PEG antibody at least once during the study, occurring in similar proportions for each study drug: 36 subjects (24.3%) receiving PF-06881894, 39 subjects (26.7%) receiving pegfilgrastim-US, and 36 subjects (24.3%) receiving pegfilgrastim-EU. The first occurrence of anti-PEG antibody across all treatments occurred in Period 1 Day 1 (pre-existing), Period 1 Day 13, or Period 1 follow-up on Day 30, with exception of 1 subject that was first positive in Period 3 Day 1 prior to dosing with study drug.Similar mean values of the PD parameters could be observed for all study drugs compared to subjects negative for anti-PEG antibodies. The subgroup analyses performed by the applicant showed that the GMRs for the different PD parameters were close to 100% for all study drug comparisons in both the anti-PEG positive and anti-PEG negative subjects (range 0.96 to 1.04), as well as their corresponding 95% CI (range 0.91 to 1.10), indicating equivalence with regard to ANC response after administration of PF-06881894, pegfilgrastim-EU and pegfilgrastim-US. When applying the tighter acceptance limits (90.0-111.0%), the 95% CIs for the primary PD parameters $AUEC_{ANC}$ and ANC_C_{max} were completely contained within these acceptance limits for all study drug comparisons, underlining the high degree of similarity between PF-06881894, pegfilgrastim-EU and pegfilgrastim-US, independently of the presence of anti-PEG antibodies.

Table 12. Ratio and 90% and 95% Confidence Interval of PD Parameters, Natural Log Transform: $AUEC_{ANC}$ and ANC_C_{max} - Subjects with Positive Anti-PEG Antibodies (above) and Negative Anti-PEG Antibodies (below) - PD Population - Study ZIN-130-1505

	Parameter	Statistic	PF-06881894 (N=104)	Pegfilgrastim-US (N=105)	Pegfilgrastim-EU (N=106)	Ratio[a]	90% CI	95% CI
Subjects with Positive Anti-PEG Antibodies								
PF/US	$AUEC_{ANC}$ ($h\times10^9/L$)	Geometric Mean	4512.35	4492.04	-	0.9953	(0.9728, 1.0182)	(0.9685, 1.0227)
		LS Mean (SE)	8.41 (0.024)	8.41 (0.024)	-			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	30.24	30.81	-	0.9702	(0.9405, 1.0008)	(0.9349, 1.0069)
		LS Mean (SE)	3.40 (0.027)	3.43 (0.027)	-			
PF/EU	$AUEC_{ANC}$ ($h\times10^9/L$)	Geometric Mean	4512.35	-	4602.58	0.9732	(0.9514, 0.9954)	(0.9472, 0.9998)
		LS Mean (SE)	8.41 (0.024)	-	8.43 (0.024)			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	30.24	-	30.66	0.9763	(0.9466, 1.0068)	(0.9410, 1.0129)
		LS Mean (SE)	3.40 (0.027)	-	3.43 (0.027)			
US/EU	$AUEC_{ANC}$ ($h\times10^9/L$)	Geometric Mean	-	4492.04	4602.58	0.9778	(0.9559, 1.0002)	(0.9517, 1.0045)
		LS Mean (SE)	-	8.41 (0.024)	8.43 (0.024)			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	-	30.81	30.66	1.0063	(0.9757, 1.0377)	(0.9699, 1.0439)
		LS Mean (SE)	-	3.43 (0.027)	3.43 (0.027)			
Subjects with Negative Anti-PEG Antibodies								
PF/US	$AUEC_{ANC}$ ($h\times10^9/L$)	Geometric Mean	4590.53	4709.28	-	0.9708	(0.9478, 0.9945)	(0.9433, 0.9992)
		LS Mean (SE)	8.41 (0.036)	8.44 (0.036)	-			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	30.76	31.85	-	0.9615	(0.9201, 1.0049)	(0.9121, 1.0137)
		LS Mean (SE)	3.41 (0.039)	3.45 (0.040)	-			
PF/EU	$AUEC_{ANC}$ ($h\times10^9/L$)	Geometric Mean	4590.53	-	4550.85	1.0044	(0.9812, 1.0281)	(0.9767, 1.0328)
		LS Mean (SE)	8.41 (0.036)	-	8.41 (0.035)			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	30.76	-	30.40	1.0043	(0.9622, 1.0483)	(0.9541, 1.0572)
		LS Mean (SE)	3.41 (0.039)	-	3.40 (0.039)			
US/EU	$AUEC_{ANC}$ ($h\times10^9/L$)	Geometric Mean	-	4709.28	4550.85	1.0345	(1.0101, 1.0595)	(1.0054, 1.0645)
		LS Mean (SE)	-	8.44 (0.036)	8.41 (0.035)			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	-	31.85	30.40	1.0445	(0.9997, 1.0912)	(0.9912, 1.1006)
		LS Mean (SE)	-	3.45 (0.040)	3.40 (0.039)			

Source: Appendix Supportive Tables ISI 5.1 through 5.4
Note: $AUEC_{ANC}$ is also referred to as $AUEC_{ANC0-t}$ in source documents and as ANC_{0-t} in the supplemental source tables.
ANC_C_{max} is referred as C_{max} in the supplemental source tables.
The ANCOVA model is used to calculate estimates of the error variance and the least square means with baseline ANC as covariate

Abbreviations: ANC = absolute neutrophil count; ANCOVA = analysis of covariance; AUECANC = area under the effect versus time curve for ANC from time zero to the last measurable concentration (also referred to as AUECANC0-t); ANC_Cmax = maximum observed value for absolute neutrophil count; CI = confidence interval; EU = European Union; LS = lease square; N = number of subjects; SE = standard error; Tmax = time to maximum serum pegfilgrastim concentration; US = United States
a. Ratio is back-transformed in order to be expressed on the original scale of the measurement

With regards to the presence of anti-pegfilgrastim antibodies, a total of 10 subjects (6.5%) of all subjects enrolled in the study (n = 153) confirmed positive for anti-pegfilgrastim antibodies at least once during the study: 6 subjects (4.1%) who received PF-06881894, 2 subjects (1.4%) who received pegfilgrastim-US reference product, and 2 subjects (1.4%) who received pegfilgrastim-EU reference product. The anti-pegfilgrastim antibody response was specific for the filgrastim protein moiety for 7 out of these 10 subjects. Slightly lower mean values were reported for $AUEC_{ANC}$ for study drugs PF-06881894 and pegfilgrastim-US, while no impact on $AUEC_{ANC}$ could be observed for pegfilgrastim-EU. However, due to the small number of subjects confirmed positive for anti-pegfilgrastim antibodies (n = 10) compared to the PD-population, it is agreed with the applicant that it is difficult to determine if this represents a real difference. Subgroup analyses on subjects confirmed positive for anti-pegfilgrastim antibodies is therefore considered not reliable. Sensitivity analyses that included the PD population plus the 10 subjects with confirmed positive anti-pegfilgrastim antibodies showed consistent results with the primary analyses on the PD population, which could be expected as only 10 subjects confirmed positive for anti-pegfilgrastim.

Two of the 10 anti-pegfilgrastim antibody positive subjects had a single sample positive for NAb that occurred on P1 Day 13. Both subjects received PF-06881894 in treatment period 1. The PD profile of the first case suggests that NAbNAb may affect the PD response (in period 2, ANC_C_{max} and $AUEC_{ANC0\text{-}inf}$ values were below minimum values in the PD population). For the second subject the effect of NAb is

less clear. Although a lower PD response was observed for this subject in period 2, the values for $AUEC_{ANC0\text{-}inf}$ and C_{max} were still contained in the range of values (min-max) obtained for the PD population. These findings are consistent with the findings regarding the impact of NAb on PK.

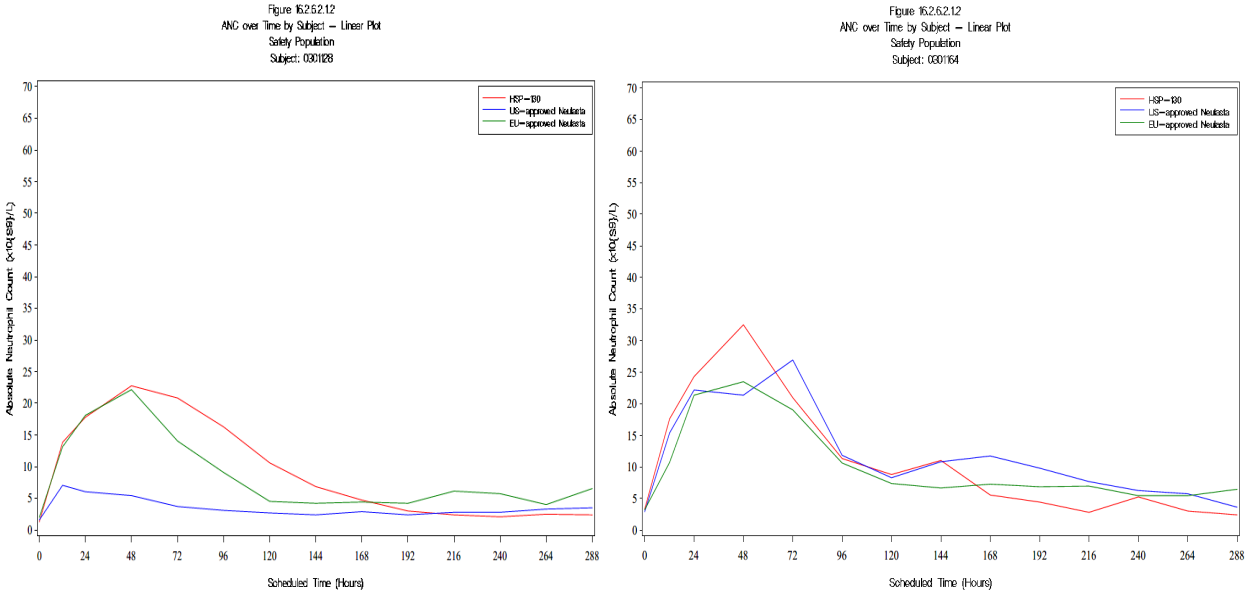


Figure 7. ANC time profile: subject 0301128 (left) and 0301164 (right) - Study ZIN-130-1505

Table 13. PD parameters

Listing of Pharmacodynamic Parameters

Subject/Age/ Race/Sex	Actual Treatment Sequence	Period/ Actual Treatment	AUC 0-inf (h*x10{S9}/L)	Cmax (x10{S9}/L)	Tmax (h)
0301128/21/W/M	HSP/US/EU	1/ HSP	2739.67	22.8	48
		2/ US	1006.265	7.1	12
		3/ EU	2463.38	22.2	48
0301164/39/W/M	HSP/EU/US	1/ HSP	3233.085	32.5	48
		2/ EU	2990.775	23.5	48
		3/ US	3532.825	26.9	72

Immunogenicity study C1221005

Although the immunogenicity study C1221005 did not include formal PD assessment, assessment of ANC was included as a safety laboratory parameter. The mean ANC time curves appeared similar for PF-06881894 and pegfilgrastim-US. Comparable increases in mean ANC were observed after administration of PF-06881894 and pegfilgrastim-US, with peak levels reached at around 3 days post-dose, consistent with the mode of action of pegfilgrastim. These data support the findings with regards to PD-equivalence between PF-06881894 and pegfilgrastim-US in study ZIN-130-1505.

This study also showed similar trends in ANC time curves between both study drugs for subjects with treatment-emergent positive anti-pegfilgrastim antibody results, as well as for subjects with positive anti-pegfilgrastim antibody results at any visit including baseline, indicating no effect on ANC by the presence of anti-pegfilgrastim antibody for both study drugs.

In this study, there was no evidence of NAb for any subject with a negative baseline anti-pegfilgrastim antibody result in either treatment group. However, one subject in the PF-06881894 group with positive baseline anti-pegfilgrastim antibody result was found to be NAb positive at 2 post-dose visits in treatment

Period 1 (Day 13 and Day 30). Although in treatment period 1 a similar ANC time profile was observed for this subject compared to the overall population, a clearly different profile was reported for treatment Period 2, with a normal ANC level on Period 2 Day 3 (4000/µL; reference range: 1700-7900/µL), which is decreasing compared to the ANC level on Period 2 Day 1 (pre-dose; 4400/µL). This observation is not consistent with the peak levels of ANC observed in the overall population at Period 2 Day 3. Moreover, this subject is the only subject in this study without an ANC result above the upper limit of the reference range (> 7900/µl) on the Period 2 Day 3 visit. As a result, it seems that for this subject ANC is affected by the presence of NAb. Therefore, based on this study, it cannot be excluded that the presence of NAb has a negative impact on PD.

Table 14: ANC laboratory data – Study C1221005 – Subject NAb positive

	Visit: Date: Study Day:	SCREENING 2018-01-17 -15	DAY-2 2018-01-30 -2	PERIOD1/DAY3 2018-02-03 3	PERIOD1/DAY5 2018-02-05 5	PERIOD1/DAY13 2018-02-13 13	PERIOD1/DAY30 2018-02-28 28	PERIOD2/DAY1 2018-03-06 34	PERIOD2/DAY3 2018-03-08 36	PERIOD2/DAY5 2018-03-10 38
Neutrophils (10^3/mm3)	1.7-7.9	4.1 NORMAL	3.3 NORMAL	27.1 HIGH *	14.1 HIGH *	1.8 NORMAL	1.7 NORMAL	4.4 NORMAL	4 NORMAL	2.1 NORMAL

HIGH/LOW indicates values above or below normal limits
CTCAE Grade is presented for lab results above or below normal limits where CTCAE criteria exist.
*: No CTCAE Grade exists

Study Day = Day relative to start of study treatment (Day 1)

2.3.4. Discussion on clinical pharmacology

Nyvepria, a biosimilar to Neulasta, is a pegylated G-CSF. The mechanism of action of G-CSF through the G-CSF receptor works by regulating the production of neutrophils within the bone marrow and effecting neutrophil progenitor proliferation, differentiation, and selected end-cell functions (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody-dependent killing, and the increased expression of some cell surface antigens). Pegylation serves to prolong the circulating half-life of biologic agents.

The clinical pharmacology of PF-06881894 was characterised based on the 3-treatment comparative PD and PK study ZIN-130-1505 PD/PK in healthy subjects. Further supportive data were obtained in immunogenicity study C1221005.

In general, the applicant's approach to demonstrate PK and PD similarity between PF-06881894 and Neulasta in a single PK/PD study (ZIN-130-1505) is considered adequate and is in line with the applicable EMA guidelines and the EMA scientific advice (EMA/CHMP/SAWP/720012/2017):

- Only healthy volunteers were included in the study which is agreed as a sensitive population to detect potential differences in PK and PD.
- Subjects were injected SC with the therapeutic dose of 6mg pegfilgrastim. This is in line with the draft guideline on similar biological products containing rG-CSF (EMEA/CHMP/BMWP/31329/2005 Rev 1), which states that a single dose in the range of 2 to 6 mg is considered suitable to detect potentially relevant differences in both PK and PD.
- Taken into account the high inter-subject variability, a cross-over design was chosen to evaluate PK and PD comparability.
- Treatments were separated by a wash-out period of minimum 51 days. This is considered long enough to avoid carry-over of pharmacological effects.

Pharmacokinetics

Pegfilgrastim concentrations in serum were determined using an electrochemiluminescence (ECL) immunoassay. It was shown that the presence of anti-pegfilgrastim antibodies interferes with the measurement of pegfilgrastim.

PK equivalence was assessed by constructing the 90% CIs for the GMR (test/reference) for the primary PK variables AUC0-inf and Cmax. Although AUC0-t was defined as a secondary PK variable, contrary to what's stated in the draft guideline, the same statistical analyses were performed for this parameter post-hoc. Therefore, this is not considered to be an issue.

PK equivalence was demonstrated for all study drug comparisons (PF-US, PF-EU, and US-EU). The geometric mean ratios for AUC0-inf, AUC0-t and Cmax ranged from 0.95 to 0.99 and the corresponding 90% CIs were completely contained within the predefined limit of 80% to 125%.

PK equivalence was also shown for the PK population plus subjects confirmed positive for anti-pegfilgrastim antibodies and for anti-PEG positive and anti-PEG negative subjects, suggesting that ADAs do not impact PK similarity.

AUC and Cmax values were generally lower in the presence of ADAs (anti-PEG and anti-Pegfilgrastim). Since the scale of difference in PK values was comparable for Neulasta and PF-06881894 treated subjects, at least for anti-PEG, these decreased pegfilgrastim concentrations are not considered to be an issue. For anti-pegfilgrastim antibodies, it is agreed with the applicant that the substantial difference in the size of the ADA positive and negative population makes it difficult to determine if the observations represent a real difference.

Two subjects tested positive for NAb and at least for one of them, the PK response (AUC and Cmax) was reduced. However, based on these data in a very low number of subjects in whom NAb could be observed, this is considered a chance finding and the presence of NAb did not appear to result in reduced clinical response or increased risk for injection site reactions.

Pharmacodynamics

The dose administered is considered adequate to establish PD similarity and is appropriate from a safety point of view. The sampling schedule seems adequate to reliably estimate the PD parameters.

ANC was determined at Australian Clinical Labs using haematology analyser. The validity of ANC results was approved by the measurement of quality control samples and by the participation in external quality control schemes.

The selection of the primary parameter, ANC as relevant pharmacodynamic marker for the activity of pegfilgrastim, is in line with the Guidance on similar medicinal products containing r-GCSF (EMEA/CHMP/BMWP/31329/2005). The primary PD endpoints $AUEC_{ANC}$ and ANC_Cmax are acceptable.

The applicant's justification for not reporting $AUEC_{0-t}$ and E_{max} of CD34+ as secondary PD endpoints (stem cell mobilisation is not an approved indication of the reference product and neither is it sought in the present marketing application, the longer washout period (8 weeks) than required gives less risk of carryover effect, etc.) is acknowledged. It is agreed that inclusion of those endpoints would have provided only supportive additional data and provided that a high biosimilarity level in quality level, PK level and in ANC results is demonstrated, there would be no evident reason to expect another effect on the stem cell mobilisation.

The open-label design of study ZIN-130-1505 could potentially contribute to higher ANC levels, as it could lead to more stressed subjects when treated with PF-06881894 (e.g. due to possible fear of receiving a new product and not an established one). However, the high degree of PD similarity

demonstrated between PF-06881894 and Neulasta does not suggest any impact of the open-label design on PD similarity.

No sound justification for the comparability range of 80%-125% and explanation for conducting supplemental PD analysis were found. However, 95% CIs (as requested in the draft GL on similar biological medicinal products containing r-GCSF, EMEA/CHMP/BMWP/31329/2005 Rev 1) for primary PD endpoints $AUEC_{ANC}$ and ANC_Cmax, calculated in the supplemental analysis, were contained within the equivalence limit of 90% to 111%, which is in line with the draft GL (EMEA/CHMP/BMWP/31329/2005 Rev 1). As such, study ZIN-130-1505 demonstrated PD equivalence between PF-06881894 and Neulasta and provided a PK/PD biosimilarity bridge between EU-sourced and US-sourced Neulasta. The high degree of PD similarity was further supported by ANC data from immunogenicity study C1221005, reporting similar mean ANC time curves for both study drugs PF-06881894 and US-sourced Neulasta.

Table 15: Primary and Supplemental Analyses: Ratio and 90% and 95% Confidence Interval of Pharmacodynamic Parameters, Natural Log Transform: $AUEC_{ANC}$, ANC_C_{max} and $AUEC_{ANC0\text{-}inf}$ – Pharmacodynamic Population – Study ZIN-130-1505

Comparison	Parameter	Statistic	PF-06881894 N = 135	Pegfilgrastim-US N = 134	Pegfilgrastim-EU N = 138	Ratio[a]	90% CI	95%CI[b]
PF-US	$AUEC_{ANC}$ ($h\times10^9/L$)[c]	Geometric Mean	4541.21	4581.48		0.99	(0.97, 1.00)	(0.97, 1.00)
		LS Mean (SE)	8.42 (0.020)	8.43 (0.020)				
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	30.29	31.30		0.96	(0.94, 0.98)	(0.94, 0.99)
		LS Mean (SE)	3.41 (0.023)	3.44 (0.023)				
PF-EU	$AUEC_{ANC}$ ($h\times10^9/L$)[c]	Geometric Mean	4541.21		4588.23	0.98	(0.97, 1.00)	(0.97, 1.00)
		LS Mean (SE)	8.42 (0.020)		8.43 (0.020)			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean	30.29		30.64	0.98	(0.96, 1.00)	(0.96, 1.01)
		LS Mean (SE)	3.41 (0.023)		3.42 (0.023)			
US-EU	$AUEC_{ANC}$ ($h\times10^9/L$)[c]	Geometric Mean		4581.48	4588.23	1.00	(0.98, 1.01)	(0.98, 1.02)
		LS Mean (SE)		8.43 (0.020)	8.43 (0.020)			
	ANC_C_{max} ($\times10^9/L$)	Geometric Mean		31.30	30.64	1.02	(1.00, 1.04)	(0.99, 1.05)
		LS Mean (SE)		3.44 (0.023)	3.42 (0.023)			
Supplemental Analysis: $AUEC_{ANC0\text{-}inf}$								
PF-US	$AUEC_{ANC0\text{-}inf}$ ($h\times10^9/L$)[d]	Geometric Mean	5278.19	5328.56	-	0.99	(0.96, 1.01)	(0.96, 1.01)
		LS Mean (SE)	8.57 (0.019)	8.59 (0.020)	-			
PF-EU	$AUEC_{ANC0\text{-}inf}$ ($h\times10^9/L$)[d]	Geometric Mean	5278.19	-	5296.87	0.99	(0.97, 1.02)	(0.97, 1.02)
		LS Mean (SE)	8.57 (0.019)	-	8.58 (0.019)			
US -EU	$AUEC_{ANC0\text{-}inf}$ ($h\times10^9/L$)[d]	Geometric Mean	-	5328.56	5296.87	1.01	(0.99, 1.03)	(0.98, 1.04)
		LS Mean (SE)	-	8.59 (0.020)	8.58 (0.019)			

Source: Module 5.3.4.1 Study ZIN-130-1505 Study Report Body Table 9 and Module 5.3.4.1 Study ZIN-130-1505 Supplemental Study Report Body Table 2 and Table 3

Note: 1 The ANCOVA model is used to calculate estimates of the error variance and the least square means with baseline ANC as covariate.

2. PF = PF-06881894. EU = pegfilgrastim-EU. US = pegfilgrastim-US. PF-06881894 is also referred to as HSP-130, pegfilgrastim-US referred to as US-approved Neulasta, and pegfilgrastim-EU referred to as EU-approved Neulasta.

Abbreviations: ANC = absolute neutrophil count; ANC_C_{max} = maximum observed value for ANC; ANCOVA = analysis of covariance; $AUEC_{ANC}$ = area under the effect versus time curve for ANC from the time of dose administration to 288 hours after dose administration; $AUEC_{ANC0\text{-}inf}$ = area under the curve for ANC from time zero to infinity; CI = confidence interval; EU= European Union; LS = least square; N = number of subjects; SE = standard error; US = United States.

a. Ratio is back-transformed in order to be expressed on the original scale of the measurement.

b. 95% Confidence Interval is from the Supplemental Analysis

c. In Module 5.3.4.1 Study ZIN-130-1505 Supplemental Study Report Body, $AUEC_{ANC}$ is also referred to as $AUEC_{ANC0\text{-}t}$ in source documents

d. $AUEC_{ANC0\text{-}inf}$ is from the Supplemental Analysis

Regarding the impact of ADAs on PD similarity, both study designs of study ZIN-130-1505 and C1221005 are considered not optimal. The crossover study design of ZIN-130-1505 is not suitable for comparing the immunogenicity of 3 treatments, therefore only exploratory immunogenicity assessment was conducted. For study C1221005, PD assessment was not a study objective, however ANC was evaluated as safety laboratory parameter. Although study designs were not optimal, the presence of anti-PEG antibodies or anti-pegfilgrastim antibody had no overall effect on PD or PK similarity.

In both studies, 3 subjects were found to have samples positive for neutralising antibodies (one case in C1221005 study, and two cases in ZIN-130-1505 study) while being treated with PF-06881894, while none of the subjects receiving pegfilgrastim-US or pegfilgrastim-EU had NAb positive samples. For 2 out of 3 subjects confirmed positive for NAb while being treated with PF-06881894, a significant abnormal PD profile was observed in the treatment period subsequent to the treatment period in which NAb were measured, which in one subject was confirmed by an abnormal PK profile (PK not measured in the other subject). However, the PD or PK profile were normal during the treatment period in which NAb were

observed, whereas during the treatment period where the PD or PK profile was abnormal, no NAb could be observed or NAb were not measured. With regards to the effect of NAb on safety, only for 1 of the 3 subjects a potential correlation with a TEAE of injection site rash was observed. In conclusion, based on data in a very low number of patients in whom NAb could be observed, the presence of NAb did not appear to result in reduced clinical response or increased risk for injection site reactions.

The study ZIN-130-1504 PD/PK, an ascending single-and multiple-dose, open-label, non-comparative, parallel-group PD/PK study in women with non-distantly metastatic breast cancer, is considered supportive.

2.3.5. Conclusions on clinical pharmacology

Based on the submitted data, PK/PD equivalence of PF-06881894 to the Neulasta Reference Products (pegfilgrastim-US and pegfilgrastim-EU) has been demonstrated.

2.4. Clinical efficacy

No dedicated efficacy studies were performed in patients. PD biosimilarity testing for a biosimilar candidate to a pegfilgrastim is a supported strategy in the draft guideline EMEA/CHMP/31329/2005 in Rev 1., which states that "pivotal evidence for similar efficacy will be derived from the similarity demonstrated in physicochemical, functional, pharmacokinetic and pharmacodynamic comparisons" and that "a dedicated comparative efficacy trial is therefore not considered necessary".

The applicant's approach to demonstrate only PD biosimilarity in healthy donors instead of a full clinical efficacy biosimilarity study is therefore supported.

2.5. Clinical safety

The safety (including immunogenicity) data were assessed in the 2 comparative clinical studies in healthy volunteers (ZIN-130-1505 and C1221005) to ensure that there are no clinically meaningful differences between PF-06881894 and Neulasta. Additionally, safety data from the non-comparative study in patients with non-distantly metastatic breast cancer (ZIN-130-1504) is presented.

Patient exposure

A total of 358 subjects were exposed to at least 1 dose of PF-06881984 (148 subjects in Study ZIN-130 1505 and 210 subjects in Study C1221005); 356 subjects received at least 1 dose of pegfilgrastim-US (146 subjects in Study ZIN-130-1505 and 210 subjects in Study C1221005).

Study ZIN-130-1505

A total of 153 subjects were enrolled in the study and randomly assigned to 1 of all 6 sequence groups to receive each of the 3 study drugs (A: PF-06881894; B: pegfilgrastim-US; C: pegfilgrastim-EU) over 3 treatment periods for a total study duration of approximately 143 days. Overall, 148 subjects were administered PF-06881894, 146 subjects were administered pegfilgrastim-US and 148 subjects were administered pegfilgrastim-EU.

A total of 142 subjects (92.8%) completed the study and 11 subjects (7.2%) were prematurely discontinued from the study.

This study was conducted in Australia.

Study C1221005

A total of 422 subjects were randomised into the study, out of which 210 subjects were administered PF-06881894 and 210 subjects were administered pegfilgrastim-US. Two subjects from the PF-06881894 group were randomised but did not receive any study drug.

Therefore, a total of 420 subjects were treated. The duration of Treatment Period 1 was 30 (± 2) days and the duration of Treatment Period 2 was 60 (± 5) days, with a total of 2 doses of either PF-06881894 or the pegfilgrastim-US during the study duration.

A total of 376 subjects (89.1%) completed the study and 46 subjects (10.9%) were prematurely discontinued from the study.

This study was conducted in the US.

Overall, in ZIN-130-1505 and C1221005 clinical studies, no major difference is observed in the disposition of the subjects depending of the treatment.

Healthy male or female subjects were enrolled into the studies. Regarding the demographic and baseline characteristics of enrolled population, the mean age, ethnicity, or mean BMI were overall comparable between the treatment groups in both studies. In ZIN-130-1505, only the sex ratio can be slightly different depending of the sequence. And, in C1221005, it can be noted that they were 15 more female subjects treated in the PF-06881984 arm compare to pegfilgrastim-US arm.

Adverse events

The applicant provided data related to adverse events (AEs) based on the 2 main clinical studies in which the safety was settled as a secondary objective. A description of analysis of AEs, manner of summarization of AEs, definition of AEs and other relevant specifications were outlined accordingly.

Table 16: Summary of Treatment-Emergent Adverse Events (Treatment-Related) by System Organ Class and Preferred Term in ≥5% of Healthy Volunteers in Any Treatment Group - Safety Population (source: 2.7.4 Summary of clinical safety)

System Organ Class/Preferred Term*	Study ZIN-130-1505			Study C1221005	
	PF-06881894 6 mg N = 148 n (%)	Pegfilgrastim-US 6 mg N = 146 n (%)	Pegfilgrastim-EU 6 mg N = 148 n (%)	PF-06881894 6 mg N = 210 n (%)	Pegfilgrastim-US 6 mg N = 210 n (%)
Number of subjects with at least 1 Treatment-Related TEAE	143 (96.6)	134 (91.8)	138 (93.2)	191 (91.0)	198 (94.3)
Gastrointestinal disorders	40 (27.0)	38 (26.0)	40 (27.0)	26 (12.4)	27 (12.9)
Abdominal pain	7 (4.7)	8 (5.5)	9 (6.1)	8 (3.8)	11 (5.2)
Nausea	26 (17.6)	22 (15.1)	23 (15.5)	<5%	<5%
General disorders and administration site conditions	49 (33.1)	50 (34.2)	47 (31.8)	43 (20.5)	32 (15.2)
Injection site bruising	5 (3.4)	9 (6.2)	10 (6.8)	0	0
Injection site erythema	9 (6.1))	9 (6.2)	8 (5.4)	<5%	0
Injection site pain	22 (14.9)	22 (15.1)	18 (12.2)	<5%	<5%
Fatigue	5 (3.4)	6 (4.1)	10 (6.8)	<5%	<5%
Non-cardiac chest pain	0	0	0	11 (5.2)	6 (2.9)
Musculoskeletal and connective tissue disorders	127 (85.8)	123 (84.2)	119 (80.4)	175 (83.3)	177 (84.3)
Arthralgia	<5%	<5%	<5%	14 (6.7)	8 (3.8)
Back pain	10 (6.8)	7 (4.8)	7 (4.7)	123 (58.6)	125 (59.5)
Musculoskeletal pain	117 (79.1)	116 (79.5)	110 (74.3)	46 (21.9)	37 (17.6)
Myalgia	<5%	<5%	<5%	24 (11.4)	35 (16.7)
Neck pain	<5%	<5%	<5%	7 (3.3)	14 (6.7)
Pain in extremity	<5%	<5%	<5%	37 (17.6)	31 (14.8)
Nervous system disorders	101 (68.2)	94 (64.4)	103 (69.6)	114 (54.3)	108 (51.4)
Dizziness	13 (8.8)	2 (1.4)	6 (4.1)	<5%	<5%
Headache	96 (64.9)	94 (64.4)	103 (69.6)	110 (52.4)	106 (50.5)

Source: Module 5.3.4.1 ZIN-130-1505 Study Report Body Table 14.3.1.2.4 and Module 5.3.5.4 C1221005 Study Report Body Table 14.3.1.4.2.
* AE terms were coded using MedDRA dictionary, Version 19.1 or higher.
Note: Subjects are counted once within each System Organ Class and for each Preferred Term and may have had more than 1 TEAE.
Abbreviations: EU = European Union; MedDRA = Medical Dictionary for Regulatory Activities; N = number of subjects in group; n = number of subjects with TEAE; TEAE = treatment-emergent adverse event; US = United States

Study ZIN-130-1505

There were 485 treatment-related TEAEs reported in 143 subjects (96.6%) who received PF-06881894. In comparison to the pegfilgrastim-US group (n = 461 treatment-related TEAEs in 134 subjects; 91.8%) and pefilgrastim-EU group (n = 460 treatment-related TEAEs in 138 subjects; 93.2%), the percentage for PF-06881894 was of 4.8% and 3.4% higher, respectively. In view of the reported treatment related TEAEs in study C1221005 higher in PF-06881894 compared to pegfilgrastim-US group (please see below), these minor numerical differences are considered as not clinically meaningful.

The SOCs for the most commonly reported all-causality TEAEs (in >50% of subjects) across the 3 study drug groups were Musculoskeletal and Connective Tissue Disorders, Nervous System Disorders, General Disorders and Administration Site Conditions, and Gastrointestinal Disorders (**Table 16**; source: table 15 CSR ZIN-130-1505). The most frequently reported treatment-related TEAEs (in ≥50% of subjects) were musculoskeletal pain and headache which corresponds to the known safety profile of Neulasta as a reference medicinal product. Also reported in ≥25% of subjects were nausea and injection site pain (all-causality).

Overall, there were 4 TEAEs considered severe which were reported in 3 subjects (2 subjects who received PF-06881894 and 1 subject who received pegfilgrastim-EU).

Further, 2 subjects discontinued the study due to TEAEs (1 who received PF-06881894 and 1 subject who received pegfilgrastim-US).

Study C1221005

Overall, in 194 subjects (92.4%) in the PF-06881894 group (616 TEAEs) and 202 subjects (96.2%) in pegfilgrastim-US group (567 TEAEs) at least one TEAE was reported. A total of 1,012 TEAEs reported in 389 subjects were considered treatment-related (91% of subjects in the PF-06881894 group and

94,3% of subjects in the pegfilgrastim-US group). The most commonly reported adverse events were assigned to the SOC Musculoskeletal and Connective Tissue Disorders and SOC Nervous System Disorders (**Table 16**). The most frequently reported treatment-related adverse events per both groups (PF-06881894 and pegfilgrastim-US) were back pain, headache and musculoskeletal pain.

In this study, an imbalance in the direction of higher incidence per PF-06881894 group could be found for the TEAEs of all causalities assigned to the SOC Respiratory, thoracic and mediastinal disorders. In case of the PF-06881894 group, in 34 subjects (16.2%) any TEAE under this SOC was reported in contrast to the pegfilgrastim-US group which included only 14 subjects (6.7%) relevant for this condition. Even though the respiratory, thoracic and mediastinal disorders, such as acute respiratory distress syndrome, pulmonary adverse reactions (interstitial pneumonia, pulmonary oedema, pulmonary infiltrates and pulmonary fibrosis), haemoptysis and pulmonary haemorrhage, are summarised in the list of adverse reactions in the section 4.8 of SmPC for Neulasta, ordered under frequency "uncommon" and in case of pulmonary haemorrhage under frequency "rare", the observed counts of related TEAEs in the PF-06881894 group seem to be significantly higher. The applicant was therefore requested to provide a thorough discussion on the identified difference.

The most frequently reported PTs for this SOC were PT Oropharyngeal pain (6 cases of treatment-related AEs in the PF-06881894 group) and PT Dyspnoea (6 cases of treatment-related AEs in the PF-06881894 group). Most of the cases of oropharyngeal pain were mild in severity (5 of 6 cases). In the remaining one case, the severity was assessed as moderate. All the events resolved without treatment except in one subject that was treated with ibuprofen. In general, the events of oropharyngeal pain do not suggest a pulmonary disorder as a cause for these events. The observed cases were accompanied by other confounding factors such as headache or other types of musculoskeletal pain. The appropriate justification including the causality assessment was provided.

In terms of PT Dyspnoea, 6 events of dyspnoea (in 6 subjects) for PF-06881894 and no events (in 0 subjects) for US-Neulasta assessed as treatment-related were reported. All these events resolved without treatment and were considered mild. The review of these cases was provided by applicant accordingly. No significant issue was identified.

Pooled data from Studies ZIN-130-1505 and C1221005

A total of 1,271 TEAEs were reported in 341 subjects (95.3%) in the PF-06881894 group and 1,167 TEAEs were reported in 341 subjects (95.8%) in the pegfilgrastim-US group.

Based on the overall summary of pooled data the AEs seems to be consistent with the known safety profile of reference medicinal product.

There was one additional study, i.e. open-label, non-comparative study Phase I-II ascending single- and multiple- dose Study ZIN-130-1504. In this study a total of 25 subjects were exposed to at least 1 dose of PF-06881894. This study included female subjects with non-distantly metastatic cancer with a mean age of 59.3 years. This study was conducted in the EU. Presented adverse events were consistent with the known safety profile of Neulasta. No deaths were reported and no subjects were discontinued from the study due to adverse events.

Adverse events of special interest

Clinically important adverse events of special interest were pre-specified in the study protocols based on the safety profile of the reference product. Additional adverse events of interest, including musculoskeletal disorders and injection site reactions, as recommended by FDA, were evaluated separately.

In study ZIN-130-1505, a total of 33 treatment-emergent AESIs were reported in 26 subjects (17.0%), with comparable distribution among the 3 study drugs. All AESIs were mild (30 AESIs) or

moderate (3 AESIs) in severity. AESIs were only reported under the categories of potential allergic reactions and splenomegaly in this study. Of the 33 treatment emergent AESIs, 18 were reported as related to the study drug (3 events in subjects who received PF-06881894, 9 events in subjects who received pegfilgrastim-US, and 6 events in subjects who received pegfilgrastim-EU).

In study C1221005, 11 subjects (5.2%) in the PF-06881894 group and 9 subjects (4.3%) in the pegfilgrastim-US group experienced at least 1 treatment emergent AESI in the study. AESIs were only reported under the categories of potential allergic reactions and thrombocytopenia in this study. The 12 events under the AESI of potential allergic reaction were mild or moderate in severity and the 10 events under the AESI of thrombocytopenia were all mild in severity. The majority of these AESIs (14 of 21 events) were considered related to the study drug by the investigator.

In the pooled data from Studies ZIN-130-1505 and C1221005, there were no cases reported for the following categories of AEs: splenic rupture, acute respiratory distress syndrome, alveolar haemorrhage, haemoptysis, leukocystosis, capillary leak syndrome, cytokine release syndrome, cutaneous vasculitis and glomerulonephritis.

In a category of potential allergic reactions, overall, 40 adverse events were reported of which most them were assigned to the PT Injection site rash per both studies. The percentage of treatment emergent adverse events of special interest of potential allergic reactions was comparable between PF-016881894 and pegfilgrastim-US groups.

However, in the Study C1221005 the incidence of TEAEs of all causalities assigned to the SOC General disorders and administration site conditions observed for PF-06881894 (44; 21.0%) was higher in contrast to pegfilgrastim-US (34; 16.2%). Based on the tabulated summary of the treatment-related TEAEs the difference of 5.3% per this SOC is present, concretely 20.5% and 15.2%, respectively. The applicant was asked to provide a detailed analysis on the identified differences with inclusion of relevant subject related circumstances (i.e. causality assessment in regard of the underlying conditions and confounding factors) and in regard of the relevant PTs reported. The most frequently reported PTs from this SOC were PT Non-cardiac chest pain, PT Injection site pain and PT Fatigue. The appropriate comparison of these results to the known safety profile of reference medicinal product was provided. Of note, none of the treatment-related AEs assigned to this SOC were assessed as serious. There was 1 event of fatigue and influenza like illness in the PF-06881894 group which was considered moderate in severity, plus 1 event of non-cardiac chest pain in the US-Neulasta group which was considered moderate in severity, too. All the remaining events were of mild severity. In the Study ZIN-130-1505, no similar difference between the PF-06881894 group and Neulasta group in terms of both number of subjects and number of events for this SOC was observed. Based on the reassessment of respective data, it was agreed that the existing differences between the treatment groups in the Study C1221005 do not indicate any significant safety findings. Due to all the circumstances surrounding the observed cases such as concurrently reported treatment-related adverse events, the known safety profile of Neulasta (i.e. frequency of relevant ADRs listed in the EU SmPC) or presence of other underlying conditions, the submitted response is considered fully accepted and no further action is warranted.

Two cases of splenomegaly were reported only in the Study ZIN-130-1505, i.e. one each in study group (PF-06881894 and pegfilgrastim-US). Both cases required no intervention and both subjects completed the study.

Overall, 9 subjects reported thrombocytopenia. All of them were enrolled in the Study C1221005 and these events were reported in both PF-06881894 and pegfilgrastim-US groups. No statistically significant differences between study groups were observed.

Musculoskeletal pain was the most common event reported under SOC Musculoskeletal and Connective Tissue Disorders, no significant differences in Musculoskeletal disorders were reported between the

treatment groups. No significant differences in Injection site reactions were identified between the treatment groups.

The AESI incidence profiles were overall balanced between the study drug groups in the 2 comparative clinical studies, without any clinically meaningful difference identified, and no new significant safety information to the known safety profile of pegfilgrastim in the context of adverse events of special interest could be identified.

Serious adverse event/deaths/other significant events

The data reflecting serious adverse events (SAEs) and deaths were provided in a structured form with the inclusion of case narratives describing basic characteristic of the events, such as PT and SOC MedDRA term of the reaction observed, start date/end date of AE, severity, outcome, relationship to study treatment/chemotherapy and actions taken with study treatment.

In both comparative clinical studies, there were very few SAEs, and none of them was assessed as related to study drug.

Of note, no deaths were reported in the concerned clinical studies.

Study ZIN-130-1505

Overall, there were 4 TEAEs considered severe which were reported in 3 subjects (2 subjects who received PF-06881894, i.e. 1.4%, and 1 subject who received pegfilgrastim-EU, i.e. 0.7%). One event of arthralgia (left hip pain) was considered possibly related to PF-06881894 and the events of skin abrasion and laceration were reported in one subject and considered unrelated to PF-06881894. One event of renal colic was considered unrelated to pegfilgrastim-EU. Arthralgia as a type of musculoskeletal pain is listed in the tabulated summary of adverse reactions in the section 4.8 of SmPC for reference medicinal product.

Based on the analysis of serious adverse events which occurred in this study, 3 treatment-emergent SAEs were reported in 3 subjects. Each subject was enrolled in another study group. Of them, 2 subjects experienced a spontaneous abortion (1 subject from PF-06881894 group and 1 subject from pegfilgrastim-US) and in 1 subject from pegfilgrastim-EU a renal colic occurred. All the treatment-emergent SAEs were assessed as unrelated to treatment.

In regard of the cases of spontaneous abortion, as defined per protocol, all subjects had to use an adequate method of contraception to prevent pregnancy throughout the course of the study. In the section 4.6 of SmPC, the information related to pregnancy indicates that pegfilgrastim is not recommended during pregnancy and in women of childbearing potential not using contraception.

Study C1221005

A total of 5 SAEs were reported in 4 subjects (urinary tract infection, multiple injuries, 2 events of spontaneous abortion and physical assault).

One subject from the PF-06881894 group had to discontinue due to urinary tract infection. This event was reported as SAE, moderate in severity and was considered not related to the study drug by investigator.

Further, 3 subjects were from the pegfilgrastim-US group. In 1 of them, a spontaneous abortion was reported. This subject completed study. In this case, a relation of the event of spontaneous abortion to study drug pegfilgrastim-US and/or concomitant drug medroxyprogesterone acetate was assessed as reasonably possible. Due to the relevant recommendation on pregnancy in the product information as reflected above this explanation is considered adequate.

Another female from the pegfilgrastim-US group had spontaneous abortion, too. The birth control method of this subject was a spermicide condom. In line with the known safety profile of Neulasta, there are no or limited available data regarding the use of pegfilgrastim in pregnant women. Pegfilgrastim is not recommended during pregnancy and in women of childbearing potential not using contraception.

In 1 subject from the pegfilgrastim-US group, multiple injuries and physical assault were reported. These events were considered not related to study drug, which is accepted.

The submitted data and related evaluation do not reveal any safety issues which would indicate a significant difference between the biosimilar and reference medicinal product.

Laboratory findings

The relevant listings of individual laboratory parameters and other values of vital signs, physical findings and other observations related together with a targeted analysis were provided accordingly.

Per both comparative studies (Study ZIN-130-1505 and Study C1221005), in regard of the haematology parameters, a decrease in platelet count and decrease or increase in lymphocyte count following the administration of each study drug were observed.

Regarding the clinical chemistry, according to the SmPC for Neulasta, the elevations in lactate dehydrogenase and alkaline phosphatase occurred in each study drug. The levels returned to the baseline by the follow-up visit. Further, ALT, ALP and creatinine increased were reported in all groups. These findings were fully comparable between given study groups.

In the product information for Neulasta, glomerulonephritis is listed as an adverse reaction with a frequency "uncommon" and relevant warning with recommendation on urinalysis monitoring is also present. Glomerulonephritis is also identified as an important identified risk in the context of risk management planning. The urinalysis and PCR (protein-creatinine ratio) were conducted for all subjects as defined by protocol. No evidence of glomerulonephritis for any subject was reported.

The laboratory findings were consistent with the known therapeutic response and the safety profile of Neulasta in general. No relevant difference was observed between the PF-06881894 and reference medicinal products. There were no unexplained or clinically meaningful unexpected mean changes from baseline in any laboratory parameter following study drug administration (haematology and clinical chemistry parameters). In line with the pharmacological effect of pegfilgrastim, increases in leukocytes, in particular neutrophils, were observed after administration of study drugs in both clinical studies.

However, to support the biosimilarity of PF-06881894 with Neulasta, the applicant was asked to submit a table with the number of patients (%) with laboratory adverse events (as, for instance, hypoglycaemia, ALP/ALT/CRP/GGT/AST, creatinine or blood creatine phosphokinase increase…), per treatment groups for both clinical studies (ZIN-130-1505 and C1221005) and for the pooled data. It was accepted that no clinically meaningful differences were observed in the incidence of laboratory AEs in the different treatment groups when reviewing the data independently for Studies ZIN-130-1505 and C1221005 or in a pooled fashion.

Safety in special populations

No specific safety analysis based on age, sex, height, weight or body mass were carried out. Neither were extrinsic factors such as medical environment, use of other drugs, use of tobacco, use of alcohol,

and food habits subjected to a safety analysis. The effect of these factors on the safety profile of PF-06881894 is anticipated to be similar to that of Neulasta.

There were 2 pregnancies (1 subject each for PF-06881894 and pegfilgrastim-US) reported in study ZIN-130-1505. Both subjects reported use of contraception during intercourse.

In study C1221005, there were a total of 7 AEs of pregnancy reported in 7 subjects (2 subjects in the PF-06881894 group and 5 subjects in the pegfilgrastim-US group) for the study. Three subjects reported non-compliance with the protocol required highly-effective contraceptive method and a protocol deviation was documented for each subject. The other 4 subjects reported use of contraception during intercourse.

Immunological events

In this clinical biosimilar program, the immunogenicity potential and impact of PF-06881894 and Neulasta (sourced from the EU and the US) were assessed in healthy subjects. As the applicant listed, it was evaluated in clinical studies through collection of blood samples and their testing for the presence of anti-drug antibodies (ADA) and neutralizing antibodies (NAb) and by assessment of immune-based adverse effects, both acute and delayed. The appropriate tabulated summaries of immunogenicity test results were also provided.

The steps outlined are in accordance with the standard immunogenicity testing in regard of the assays for comparative immunogenicity as described in the EMA document "Guideline on immunogenicity assessment of therapeutic proteins (EMEA/CHMP/BMWP/14327/2006 Rev 1)" dated 18 May 2017. The immunogenicity assays included an ECL assay validated for testing of ADA against pegfilgrastim with a component to assess assay specificity for the filgrastim moiety, an ELISA assay validated to detect antibodies to the PEG moiety, a cell-based assay to determine NAb, an assay to determinate antibody isotype and measurement of antibody titer.

Study C1221005

Anti-pegfilgrastim Antibody and NAb

Among the subjects who were negative for anti-pegfilgrastim antibody at baseline, a total of 12 subjects in the PF-06881894 group (5.9%) and 15 subjects in the pegfilgrastim-US group (7.5%) were confirmed as positive for anti-pegfilgrastim antibody (i.e. had at least 1 positive antibody) post-dose.

According to the applicant, results from the comparative immunogenicity study C1221005 met the statistical criteria for non-inferiority of PF-06881894 vs pegfilgrastim-US with respect to anti-pegfilgrastim antibody, which was the primary endpoint in this study. The 90% CI for the risk difference in percentage of subjects with a negative baseline anti pegfilgrastim antibody test result and confirmed post-dose positive anti-pegfilgrastim antibody test result at any visit during the study was (-5.915, 2.675), with the upper bound less than the non-inferiority margin of ≤0.10 (equivalent to 10 in percentage). However, as discussed in the scientific advice (EMA/CHMP/SAWP/720012/2017), in the EU, a formal non-inferiority study is not requested for safety data. For comparative immunogenicity assessment, the parallel group design of study C1221005 is adequate.

Overall, no meaningful differences were observed between the percentages of subjects with positive anti-pegfilgrastim antibody test in the 2 treatment groups.

In regard of the presence of NAb, it was negative for all subjects with the negative baseline of anti-pegfilgrastim antibodies.

However, 1 subject (subject 10011282) from the PF-06881894 group with a positive baseline of anti-pegfilgrastim antibodies was found to be NAb positive at 2 postdose visits: baseline positive for anti-pegfilgrastim antibody (titer of 80), and positive at P1D13 (titer of 40) and at P1D30 (titer of 80); anti-pegfilgrastim antibody response were not specific for the filgrastim protein moiety; baseline negative for anti-PEG antibody, positive at P1D13 (titer of 12800) and negative at P1D28; 2 NAb positive samples at P1D13 (titer of 16) and P1D28 (titer of 8) without demonstrable clinical impact of the NAb in treatment period 1 (absolute neutrophil count consistent with those seen in other subjects; no related associated adverse events) (no PK data available). However, in the subsequent treatment period 2, the ANC level at Period 2 Day 3 was decreased compared to ANC level on Period 2 Day 1 (pre-dose) and is further decreasing at Period 2 Day 5, which is not consistent with the expected PD profile reaching ANC peak levels at Day 3 post-dose. As no NAb assessments were planned at the Period 2 Day 3 and Period 2 Day 5 visits and the patient discontinued from the study after the Period 2 Day 5 visit, no data are available regarding the presence of NAb in treatment Period 2.

A total of 39 subjects (16 subjects in the PF-06881894 group and 23 subjects in the pegfilgrastim-US group) had samples that were confirmed positive for anti-pegfilgrastim antibody at baseline and/or postdose. In these instances, the anti-pegfilgrastim titres were generally higher with the first dose (majority P1D13) and waned over time in the second study period. The higher titres observed for some subjects (3 cases) did not have any negative impact on the ANC profile when compared to other subjects that were anti-pegfilgrastim antibody positive.

Anti-PEG Antibody

Overall, the number of subjects with anti-pegfilgrastim antibodies (16 subjects in the PF-06881894 group and 23 subjects in the pegfilgrastim-US group) was substantially lower than the percentage of subjects with anti-PEG antibodies (126 subjects in the PF-06881894 group and 131 subjects in the pegfilgrastim-US group). No meaningful differences were observed between the 2 treatment groups in the percentage of subjects with confirmed positive anti-PEG antibody and in the mean of anti-PEG antibody titres.

As recommended during the (Co) Rapporteur pre-submission meeting, the applicant was requested to clarify if the difference in distribution of anti-PEG antibody titre quartile has been studied and discussed. It was confirmed that this difference has been studied in this study. Overall, the distribution of the anti-PEG antibody titers across the visits was similar in the PF-06881894 and pegfilgrastim-US treatment groups (with a maximum titer reached on P1D13).

Effect of Immunogenicity on Safety

All of the 39 subjects who had samples that were confirmed positive for anti-pegfilgrastim antibody sample at any visit including baseline and/or postdose, except 1 each in the PF-06881894 group and pegfilgrastim-US group, experienced at least 1 TEAE during the study. Overall, no meaningful differences were observed between the 2 treatment groups for subjects with positive anti-pegfilgrastim antibody test results. Review of the TEAEs in subjects with confirmed positive anti-pegfilgrastim antibody at any visit including baseline revealed no effect on TEAEs by the presence of anti pegfilgrastim antibody.

For 6 of the 7 subjects in the PF-06881894 group with more than 1 positive anti-pegfilgrastim antibody at any visit including baseline, there was no evidence of immunogenicity-associated adverse events due to the presence of anti-pegfilgrastim antibodies.

For 1 subject in the PF-06881894 group (with a positive baseline of anti-pegfilgrastim antibodies who had 2 anti pegfilgrastim antibody samples tested positive for NAb) however, immunogenicity-associated adverse events were reported (injection site rash -ISR- at P1D7 and injection site pain at P1D9; both were considered mild in severity and resolved rapidly).

As required, the applicant has submitted the immunogenicity results observed using the confirmatory CP with a false-positive rate of 1% for the studies (instead of 0.1%). For this study, although the number of subjects with negative baseline anti-pegfilgrastim antibody and confirmed post-dose positive anti-pegfilgrastim antibody at any visit increase, the number is similar between PF-06881894 and pegfilgrastim-US.

Among the subjects with negative anti-pegfilgrastim antibody at baseline, there were 12 subjects in the PF-06881894 group and 15 subjects in the pegfilgrastim-US group who had at least 1 postdose positive anti-pegfilgrastim antibody sample. All of these 27 subjects, except 1 in the PF-06881894 group, experienced at least 1 TEAE during the study. No meaningful differences were observed between the 2 treatment groups.

Of the 420 subjects enrolled in the study who received at least 1 dose of study drug, 11 subjects reported TEAEs in SMQs (narrow) Hypersensitivity, Anaphylactic Reactions, and Angioedema in study C1221005. Three of these 11 subjects were confirmed positive for anti-pegfilgrastim antibody at least once during the study including baseline: one subject in the PF-06881894 group (see before) and 2 subjects in the pegfilgrastim-US group (who reported hypersensitivity not related to the study drug).

Nineteen subjects reported ISRs in study C1221005. But only the subject in the PF-06881894 group was confirmed positive for anti-pegfilgrastim antibody at least once during the study including baseline.

General review of the immunogenicity-associated TEAEs (including hypersensitivity, anaphylactic reactions, angioedema and injection site reactions) across the treatment groups in subjects with confirmed positive anti-pegfilgrastim antibody at any visit including baseline revealed no impact on safety by the presence of anti-pegfilgrastim antibody. No meaningful differences between the treatment groups was identified.

Study ZIN-130-1505

Since the crossover study design of ZIN-130-1505 is not suitable for comparing the immunogenicity of 3 treatments, only exploratory immunogenicity assessment was conducted.

Compared to the number of subjects with positive anti-pegfilgrastim antibodies (n = 10 subjects, i.e. 6.5%), there were 111 subjects (i.e. 72.5%) positive for anti-PEG antibodies. The applicant summarised the data based on the first onset of positive result for each subject during study in regard of the concrete immunogenicity test, study drug (PF-06881894, pegfilgrastim-US and pegfilgrastim-EU) and the respective percentage based on the number of relevant subjects which received the study drug in given period (visit at Day 1, Day 13 or follow-up).

Anti-pegfilgrastim Antibody and NAb

No subjects were anti-pegfilgrastim positive at baseline. Out of the 153 enrolled subjects, 10 subjects (6.5%) confirmed positive for anti-pegfilgrastim antibody results, at least once in the study: 6 subjects (4.1%) from the PF-06881894 group, 2 subjects (1.4%) from the pegfilgrastim-US and 2 subjects (1.4%) from the pegfilgrastim-EU. The anti-pegfilgrastim antibody response was specific for the filgrastim protein moiety for 7 subjects: 4 in PF-06881894 group, 1 in pegfilgrastim-US group, and 2 subjects in pegfilgrastim-EU group.

Overall, there was a slightly higher percentage of subjects with anti-pegfilgrastim positive samples for PF-06881894 (6 subjects in PF-06881894 group vs. 2 in pegfilgrastim-US and 2 in pegfilgrastim-EU). However, 5 of the 10 anti-pegfilgrastim positive subjects had only 1 positive sample during the study: 4 subjects who received PF-06881894 and 1 subject who received pegfilgrastim-US. Each of these samples was low titre (<20 to 40) and there was no evidence of NAb. As suggested by the applicant, results could be consistent with a false positive antibody test. As required, the antibody titres, isotypes

and clinical outcomes (PK/PD and safety) were also discussed for the subjects who had 1 anti-pegfilgrastim antibody positive sample in study ZIN-130-1505 (4 subjects in PF-06881894 group and 1 subject in pegfilgrastim-US group).

However, the applicant has submitted the immunogenicity results observed using the confirmatory CP with a false-positive rate of 1% for the studies (instead of 0.1%). For this study, the numbers of patients with positive anti-pegfilgrastim antibody remain close to the numbers observed with the confirmatory CP with a false-positive rate of 0.1%.

The percentage of subjects with anti-pegfilgrastim antibody positive samples was balanced across study drugs when considering only the subjects with more than 1 positive sample: 2 subjects in PF-06881894 group (subjects 0301128 and 0301164) vs. 1 in pegfilgrastim-US and 2 in pegfilgrastim-EU.

This slightly higher overall percentage of subjects with positive anti-pegfilgrastim antibody results for PF-06881894 could be considered as acceptable due to the results at final visit (i.e. follow-up visit) when only 2 subjects from the PF-06881894 group were positive for anti-pegfilgrastim antibody. The lasting of positive anti-pegfilgrastim antibody results per given study groups did not reveal any difference that would indicate a new trend.

Out of the 10 anti-pegfilgrastim antibody positive subjects, none of the subjects receiving pegfilgrastim-US or pegfilgrastim-EU had sample positive for NAb. However, in 2 subjects from the PF-06881894 group, a positivity for NAb which occurred at day 13 in both of them was confirmed. This response was assessed as related to the PEG moiety, not to the filgrastim protein which is accepted.

The case narrative has been submitted for these 2 subjects (subjects 0301128 and 0301164). In summary:

- Subject 1 (PF-06881894 group): baseline negative for anti-pegfilgrastim antibody, and positive at P1D13 (titer of 40) and P1FU (below the titer cut point); anti-pegfilgrastim antibody response were not specific for the filgrastim protein moiety; baseline negative for anti-PEG antibody but positive in all subsequent tests (peak at P1D13 and P2D1: titer of 6400); 1 NAb positive samples at P1D13 (titer of 4*, might be related to the PEG moiety*). NAb did affect PK/PD with greater effect in period 2 and lesser effect in period 3, and potential correlation with a TEAE of injection site rash.
- Subject 2 (PF-06881894 group): baseline negative for anti-pegfilgrastim antibody, positive at P1D13 (titer of 320) and continue to be positive but declining (to be negative at P3FU); anti-pegfilgrastim antibody response were not specific for the filgrastim protein moiety; baseline positive for anti-PEG antibody and remained positive in all subsequent tests (peak at P1D13, P1FU and P2D1: titer of 3200); 1 NAb positive samples at P1D13 (titer of 8*, might be related to the PEG moiety*). NAb did not affect PK/PD significantly, no evidence of immunogenicity associated adverse event or loss of response.

Anti-PEG Antibody

The 111 subjects (72.5%) who were positive for anti-PEG antibodies (i.e. at least once it was confirmed during the study) were as follows: 36 subjects in the PF-06881894 group (24.3%), 39 subjects in the pegfilgrastim-US group (26.7%) and 36 subjects in the pegfilgrastim-EU group (24.3%).

The first occurrence of anti-PEG antibody across all treatments occurred in P1D1 (pre-existing), P1D13, or P1D30 (follow-up) with exception of 1 subject that was first positive in P3D1 prior to dosing with study drug. The anti-PEG antibody response was balanced by titre across study drugs. The data demonstrated a consistent and prominent anti-PEG antibody response across all study drugs, with a majority of subjects with pre-existing or early onset (P1D13) anti PEG antibody.

As recommended during the (Co) Rapporteur pre-submission meeting, the applicant was asked to clarify if the difference in distribution of anti-PEG antibody titre quartile has been studied and discussed. Based on the applicant's response, it is accepted that in this study, the distribution of the anti-PEG antibody titers across the visits was similar in the PF 06881894, pegfilgrastim-US, and pegfilgrastim-EU treatment groups (with a maximum titer reached on P1D13).

Effect of Immunogenicity on Safety

TEAEs which occurred in the 10 subjects who had at least 1 positive anti-pegfilgrastim antibody sample at any visit including baseline were described by the applicant by treatment period. The SOCs for the most commonly reported TEAEs across the 3 study drugs were Musculoskeletal and Connective Tissue Disorders (10 subjects), Nervous System Disorders (9 subjects), and General Disorders and Administration Site Conditions (8 subjects). The most common TEAEs across the 3 study drugs were musculoskeletal pain (10 subjects) and headache (9 subjects). All 10 subjects reported at least 1 drug-related TEAE.

Overall, no meaningful differences were observed between the 3 treatment groups for the reported TEAEs in subjects who had at least 1 positive anti-pegfilgrastim antibody sample at least once during the study (no subjects were anti-pegfilgrastim positive at baseline).

Three subjects who reported TEAEs in SMQs (narrow) Hypersensitivity, Anaphylactic Reactions and Angioedema in study ZIN-130-1505 confirmed positive treatment-emergent anti-pegfilgrastim antibody at least once during the study:

- One subject (0301190) experienced non-treatment related TEAEs of rhinitis allergic (due to concurrent illness) in pegfilgrastim-US group in Period 1. This subject was confirmed positive for anti-pegfilgrastim antibody at the P1D13 visit, and specificity testing was positive for filgrastim.
- One subject (0301128) experienced treatment related TEAE of injection site rash in PF-06881894 group in Period 1. The subject also experienced an additional treatment related Injection site rash in pegfilgrastim-US in Period 2. This subject was confirmed positive for anti pegfilgrastim antibody during the P1D13 and Period 1 Follow-up visits, and the specificity testing was negative for filgrastim; the subject was positive for NAb at the P1D13 visit (see before).
- One subject (0301164) experienced non-treatment related TEAEs of vessel puncture site rash in PF-06881894 group in Period 1. This subject was confirmed positive for anti pegfilgrastim antibody beginning at the P1D13 visit through P3D13, and the specificity testing was negative for filgrastim; the subject was positive for NAb at the P1D13 visit (see before).

The remaining subjects confirmed negative for anti-pegfilgrastim antibodies.

Seven of the subjects who reported ISRs in study ZIN-130-1505 were confirmed positive anti-pegfilgrastim antibody at least once during the study. However, only the following 2 subjects reported potential hypersensitivity-related ISRs (the remaining ISRs were injection site pain and injection site bruising):

- Subject 0301001 experienced treatment related Injection site erythema in pegfilgrastim-EU group in Period 2. This subject was confirmed positive for anti-pegfilgrastim antibody at the P1D13 visit, and specificity testing was positive for filgrastim.
- Subject 0301128 experienced treatment related Injection site rash in PF-06881894 in Period 1 (see before).

Based on the analysis of reported TEAEs from the SMQs Hypersensitivity, Anaphylactic reactions and Angioedema and Injection site reactions, the confirmation of positive anti-pegfilgrastim antibody results did not reveal any effect. The differences between given treatment groups are considered minor and not revealing any concerns.

Discontinuation due to adverse events

In both studies, there are few discontinuations due to TEAEs with no apparent difference between the PF-06881894 and Neulasta.

Study ZIN-130-1505

A total of 2 subjects discontinued the study due to TEAEs. One subject who received PF-06881894 experienced a spontaneous abortion which was assessed as unrelated to study drug. Another subject who received pegfilgrastim-US experienced a generalised rash assessed as possibly related to study drug in regard of its known potential for allergic reactions.

Three subjects were permanently discontinued from study drug administration; 1 subject who received PF-06881894 was discontinued from the study drug due to a non-drug-related abortion spontaneous, and 2 subjects who received pegfilgrastim-US were discontinued from study drug administration due to TEAEs of alanine aminotransferase (ALT) increased determined to be possibly related to study drug and rash generalised determined to be possibly related to study drug.

Plus, another subject from the pegfilgrastim-US group was erroneously reported to be permanently discontinued from the study drug by the investigator. This subject experienced an injection site pain after the final scheduled dose of study drug.

Study C1221005

A total of 46 subjects (10.9%) discontinued from the study. The reason for the discontinuation from the study included lost to follow-up (18 subjects), protocol deviations (2 subjects), withdrawal by subject (14 subjects), no longer met eligibility criteria (1 subject), other reasons (9 subjects) and 2 subjects discontinued the study due to AEs.

One subject each in the PF-06881894 group and the pegfilgrastim-US group was permanently discontinued from study drug and the study due to TEAEs. One subject from the PF-06881894 group had urinary tract infection (reported as SAE) and another one from the pegfilgrastim-US study discontinued due to nonserious angioedema. The event of urinary tract infection was moderate in severity and was considered not related to the study drug by the investigator. TEAE of angioedema was considered related to the study drug. Angioedema is listed as an adverse reaction in the SmPC for reference medicinal product.

The appropriate narratives related to the concerned cases were provided accordingly. The analysis of the cases with discontinuation due to TEAEs did not provide any new safety findings or concerns in association with PF-06881894.

2.5.1. Discussion on clinical safety

The safety assessment was focused on two comparative clinical studies, i.e. two Phase I studies (ZIN-130-1505 and C1221005) including heathy subjects, and one supportive non-comparative Phase I-II study (ZIN-130-1504) including subjects with non-metastatic breast cancer. The safety results per individual sections of the data submitted were mostly presented separately for each study and in the conclusions the data from two comparative clinical studies were pooled.

As mentioned in the scientific advice (EMA/CHMP/SAWP/720012/2017), the CHMP agrees that the clinical dossier for a biosimilar application for a PEG-filgrastim may comprise of healthy volunteer trials only, provided that biosimilarity can be sufficiently demonstrated based on a strong and convincing physicochemical and functional data package and comparable pharmacokinetic and pharmacodynamic profiles including safety/immunogenicity data. The open-label design chosen for both comparative studies is regrettable, as the reporting of adverse effects (e.g., injection site reactions) can be confounded by the taken approach since the subjects and observers are aware of the actual treatment.

Overall, the provided safety database could be considered sufficient to establish the safety profile of this medicinal product. However, as the manner of adverse event (AE) analysis, without involvement of multiple occurrence of the concrete adverse event/reaction (i.e. more than once) in individual subjects, might lead to inaccuracy and bias in the incidence proportion of adverse events in given study groups, the applicant was requested to provide an explanation regarding the conducted process and eventually add the tabulated summaries of reported TEAEs indicating the exact number of adverse events per individual subjects with appropriate discussion. Therefore, the applicant provided 4 additional tabulated summaries of reported TEAEs (2 per Study C1221005 and 2 per Study ZIN-130-1505) indicating the total number of events relevant for each PT as reported AE and reported TEAE, separately. It enables a more comprehensive assessment of potential differences between the treatment groups.
Based on the presented numbers of events, no new significant differences between the mentioned treatment groups in terms of SOC and PT MedDRA terms were identified except of SOC Respiratory, thoracic and mediastinal disorders and SOC General disorders and administration site conditions.

In the studies ZIN-130-1505 and C1221005, healthy male or female were enrolled. Regarding the demographic and baseline characteristics of enrolled population, the mean age, ethnicity, or mean BMI were comparable between the treatment groups in both studies. Only the sex ratio was slightly different between groups.

The frequencies and pattern of TEAEs were similar between PF-06881894 or pegfilgrastim-US and pegfilgrastim-EU in Studies ZIN-130-1505 and C1221005, and in line with the SmPC for Neulasta. Most TEAEs were mild or moderate in severity. No new significant safety information to the established safety profile of Neulasta was identified.

The most frequently reported treatment-related TEAEs were musculoskeletal disorders (musculoskeletal pain, back pain and headache). Of note, no deaths were reported in the concerned clinical studies. Only 4 subjects experienced treatment emergent SAEs (3 subjects in study ZIN-130-1505 and 1 subject in study C1221005) which were not related to study drugs.

A few subjects discontinued the studies due to TEAEs. Further, there were few cases of spontaneous abortion. As per study protocols, all subjects had to use an adequate method of contraception to prevent pregnancy throughout the course of the study. Among the cases which led to discontinuation of the study drug and assessed as related or possibly related to study drug they were due to known adverse reactions per pegfilgrastim, such as generalised rash, ALT increased and angioedema. Specific recommendations are provided in section 4.6 of the SmPC; Pegfilgrastim is not recommended during pregnancy and in women of childbearing potential not using contraception.

In the Study C1221005, an imbalance in the direction of higher incidence per PF-06881894 group could be found for the TEAEs of all causalities assigned to the SOC Respiratory, thoracic and mediastinal disorders. In case of the PF-06881894 group, in 34 subjects (16.2%) any TEAE under this SOC was reported in contrast to the pegfilgrastim-US group which included only 14 subjects (6.7%) relevant for this condition. The most frequently reported PTs for this SOC were PT Oropharyngeal pain (6 cases of treatment-related AEs in the PF-06881894 group) and PT Dyspnoea (6 cases of treatment-related AEs in the PF-06881894 group). Most of the cases of oropharyngeal pain were mild in severity (5 of 6 cases). In the remaining one case, the severity was assessed as moderate. In general, the events of oropharyngeal pain do not suggest a pulmonary disorder as a cause for these events. The observed cases were accompanied by other confounding factors such as headache or other types of musculoskeletal pain. The appropriate justification including the causality assessment was provided by the applicant. In terms of PT Dyspnoea, 6 events of dyspnoea (in 6 subjects) for PF-06881894 and no events (in 0 subjects) for US-Neulasta assessed as treatment-related were reported. All these events resolved without treatment and were considered mild. No significant issue was identified.

Treatment-emergent AESI were overall comparable between the treatment groups in both study ZIN-130-1505 and study C1221005. No clinically meaningful differences between the AESI reported in the study drug groups were identified (including treatment-emergent Musculoskeletal Disorders and ISRs). However, in the Study C1221005, based on the tabulated summaries the incidence of both TEAEs of all causalities and treatment-related TEAEs assigned to the SOC General disorders and administration site conditions observed for PF-06881894 was higher in contrast to pegfilgrastim-US. In case of the treatment-related TEAEs, the difference of 5.3% per this SOC is present, concretely 20.5% and 15.2%, respectively. The appropriate comparison of these results to the known safety profile of reference medicinal product was provided. The most frequently reported PTs from this SOC were PT Non-cardiac chest pain, PT Injection site pain and PT Fatigue. Of note, none of the treatment-related AEs assigned to this SOC were assessed as serious. Based on the reassessment of respective data, it was agreed that due to all the circumstances surrounding the observed cases such as concurrently reported treatment-related adverse events, the known safety profile of Neulasta (i.e. frequency of relevant ADRs listed in the EU SmPC) or presence of other underlying conditions, the existing differences between the treatment groups in the Study C1221005 do not indicate any significant safety findings.

No new significant safety information to the known safety profile of pegfilgrastim in the context of adverse events could be identified.

Laboratory parameters were analysed and no significant abnormalities were detected in general. Per both comparative studies, in regard of the haematology parameters, a decrease in platelet count and decrease or increase in lymphocyte count following the administration of each study drug were observed. Regarding the clinical chemistry, according to the SmPC for Neulasta, the elevations in lactate dehydrogenase and alkaline phosphatase occurred in each study drug. The levels returned to the baseline by the follow-up visit. Further, ALT, ALP and creatinine increased were reported in all groups. The urinalysis and PCR (protein-creatinine ratio) were conducted for all subjects as defined by protocol and also in line with the relevant warning present in the product information for Neulasta. No evidence of glomerulonephritis for any subject was reported. The laboratory findings were consistent with the known safety profile of Neulasta in general. No relevant difference was observed between the PF-06881894 and reference medicinal products.

Consistent with the individual Studies ZIN-130-1505 and C1221005 no clinically meaningful differences between PF-06881894 and pegfilgrastim-US were identified per the review of the pooled safety data from studies ZIN-130-1505 and C1221005. Overall, across the 2 comparative studies, and although some minor concerns remain, there were no clinically meaningful differences between the safety profile of the proposed biosimilar and Neulasta, and results were similar to the labels.

A specific information on establishment of immunogenicity was provided. The evaluation of submitted data and outline of respective immunogenicity evaluation demonstrated a consistent and prominent response to antibodies across all study drugs.

The immunogenicity of PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU was first assessed in a 3 arm crossover PD/PK equivalence study in healthy volunteers (ZIN-130-1505, exploratory immunogenicity assessment). Therefore, the proposed comparative immunogenicity study C1221005 is acceptable (demonstration of equivalence between PF-06881894 and pegfilgrastim-US).

Results from the comparative immunogenicity study C1221005 have been reported to meet the statistical criteria for non-inferiority of PF-06881894 vs pegfilgrastim-US with respect to immunogenicity (with a 10% non-inferiority margin). However, as discussed in the scientific advice (EMA/CHMP/SAWP/720012/2017), in the EU, a formal non-inferiority study is not requested for safety data. For comparative immunogenicity assessment, the parallel group design of study C1221005 is adequate.

In C1221005 study, no meaningful differences were observed between the percentages of subjects with negative baseline anti-pegfilgrastim antibody and confirmed postdose positive anti-pegfilgrastim antibody test in the 2 treatment groups. Among the subjects who were negative for anti-pegfilgrastim antibody at baseline, a total of 12 subjects in the PF-06881894 group and 15 subjects in the pegfilgrastim-US group were confirmed as positive for anti-pegfilgrastim antibody (i.e. had at least 1 positive antibody) postdose.

In regard of the presence of NAb, it was negative for all subjects with the negative baseline of anti-pegfilgrastim antibodies. One subject in the PF-06881894 group with positive baseline anti-pegfilgrastim antibody result was found to be NAb positive at 2 postdose visits, which may affect ANC in the second treatment period compared to ANC trends in other subjects with and without anti-pegfilgrastim antibodies. For this subject, the anti-pegfilgrastim antibody responses were not specific for the filgrastim protein moiety.

Regarding the Study ZIN-130-1505, no subjects were anti-pegfilgrastim positive at baseline. Compared to the number of subjects with positive anti-pegfilgrastim antibodies at least once postdose (n = 10 subjects, i.e. 6.5%), there were 111 subjects (i.e. 72.5%) positive for anti-PEG antibodies.

Overall, there was a slightly higher percentage of subjects with anti-pegfilgrastim positive samples for PF-06881894 (6 subjects in PF-06881894 group vs. 2 in pegfilgrastim-US and 2 in pegfilgrastim-EU). However, 5 of the 10 anti-pegfilgrastim positive subjects had only 1 positive sample during the study: 4 subjects who received PF-06881894 and 1 subject who received pegfilgrastim-US. Each of these samples was low titre (<20 to 40) and there was no evidence of NAb. These results could be consistent with a false positive antibody test.

The percentage of subjects with anti-pegfilgrastim antibody positive samples was balanced across study drugs when considering only the subjects with more than 1 positive sample: 2 subjects in PF-06881894 group vs. 1 in pegfilgrastim-US and 2 in pegfilgrastim-EU.

This slightly higher overall percentage of subjects with positive anti-pegfilgrastim antibody results for PF-06881894 could be considered as acceptable due to the results at final visit (i.e. follow-up visit) when only 2 subjects were positive for anti-pegfilgrastim antibody. The lasting of positive anti-pegfilgrastim antibody results per given study groups did not reveal any difference that would indicate a new trend.

The percentage of NAb was low in this study. Out of the 10 anti-pegfilgrastim antibody positive subjects, none of the subjects receiving pefgilgrastim-US or pegfilgrastim-EU had sample positive for NAb. However, 2 subjects from the PF-06881894 group were confirmed NAb-positive. However, the results suggested that the NAb was related to the PEG moiety, not the filgrastim protein moiety.

The 111 subjects who were positive for anti-PEG antibodies (i.e. at least once it was confirmed during the study) were as follows: 36 subjects in the PF-06881894 group, 39 subjects in the pegfilgrastim-US group and 36 subjects in the pegfilgrastim-EU group. The anti-PEG response was balanced across both study drugs with respect to percentage and titre.

Overall, in Studies ZIN-130-1505 and C1221005, no effect on ANC by the presence of anti pegfilgrastim antibody was noted. Based on the analysis of reported TEAEs from the SMQs Hypersensitivity, Anaphylactic reactions and Angioedema and Injection site reactions, the confirmation of positive anti-pegfilgrastim antibody results did not reveal any effect. In summary, overall, there were no clinically meaningful differences in the ADA assessment results or in the effect of immunogenicity on the safety results across the treatments groups.

As required, the immunogenicity results observed using the confirmatory CP with a false-positive rate of 1% for the studies (instead of 0.1%) has been submitted. For study C1221005, although the number of subjects with negative baseline anti-pegfilgrastim antibody and confirmed postdose positive anti-pegfilgrastim antibody at any visit increase, the number is similar between PF-06881894 and pegfilgrastim-US. For study ZIN-130-1505, the numbers of patients with positive anti-pegfilgrastim antibody remain close to the numbers observed with the confirmatory CP with a false-positive rate of 0.1%.

Although NAb were observed for 3 samples in the PF-06881894 group (C1221005 & ZIN-130-1505), none were positive in the reference groups (pegfilgrastim-US/EU). With regard to the effect of NAb on safety, only for 1 of the 3 subjects a potential correlation with a TEAE of injection site rash was observed. In conclusion, based on data in a very low number of patients in whom NAb could be observed, the presence of NAb did not appear to result in reduced clinical response or increased risk for injection site reactions. Although NAb were observed for 3 samples in the PF-06881894 group (C1221005 & ZIN-130-1505), it is reasonable to assume that it could be due to a more sensitive assay used here compared to the one use at the time of Neulasta approval. As the number of NAb positive samples in PF-06881894 is very low, the difference with the number of NAb positive samples in the references could be chance finding.

There was one additional study, i.e. open-label, non-comparative study Phase I-II ascending single- and multiple- dose Study ZIN-130-1504. In this study a total of 25 subject were exposed to at least 1 dose of PF-06881894. This study included female subjects with non-distantly metastatic cancer with a mean age of 59.3 years. This study was conducted in the EU. Presented adverse events were consistent with the known safety profile of Neulasta. No deaths were reported and no subjects were discontinued from the study due to adverse events. There were no new immunogenicity concerns with PF-06881894. None of the subjects were confirmed positive for anti-pegfilgrastim antibody. In this study there were no significant differences in vital sign, ECG or physical examination. The clinical laboratory findings were consistent with the known safety profile of pegfilgrastim. This study did not provide any new safety information in association with PF-06881894. As this study was non-comparative, it was not considered integral for the clinical development package. This is accepted.

2.5.2. Conclusions on the clinical safety

The safety data from the 2 comparative clinical studies conducted in healthy subjects support the biosimilarity of PF-06881894 to Neulasta. The incidence of events, their nature and severity were in general similar between Nyvepria (PF-06881894) and Neulasta groups in both studies. Overall, reported adverse drug reactions were as described in the Neulasta PI.

In the context of immunogenicity, overall, the review of percentages of subjects with positive ADA/NAb and immune-mediated AEs in subjects with positive anti-pegfilgrastim antibodies from the clinical studies

in healthy subjects does not suggest any clinically meaningful differences between PF-06881894 and Neulasta, and was consistent with the established safety and immunogenicity profile of Neulasta as reflected in the labels (low immunogenicity). Further, the results from these studies do not suggest any impact of ADA on the PD or PK profile of PF-06881894. Similar to Neulasta, and in order to minimise the risk, Healthcare Professionals are warned about the risk of immunogenicity in section 4.4 of the SmPC for Nyvepria.

The safety profile of this biosimilar containing pegfilgrastim called Nyvepria is considered comparable to the reference medicinal product.

2.6. Risk Management Plan

Safety concerns

Table 17: Summary of safety concerns

Summary of safety concerns	
Important identified risks	Capillary leak syndrome ARDS Sickle cell crisis in patients with sickle cell disease Glomerulonephritis
Important potential risks	Acute myeloid leukaemia (AML) / Myelodysplastic syndrome (MDS) Cytokine release syndrome
Missing information	None

Pharmacovigilance plan

None.

Risk minimisation measures

Table 18: Summary of Risk Minimisation Measures

Safety Concern	**Risk Minimisation Measures**
Important identified risks	
Capillary leak syndrome	Routine risk minimisation measures: SmPC sections 4.4 and 4.8; PL sections 2 and 4 Additional risk minimisation measures: None
ARDS	Routine risk minimisation measures: SmPC sections 4.4 and 4.8; PL sections 2 and 4 Additional risk minimisation measures: None
Sickle cell crisis in patients with sickle cell disease	Routine risk minimisation measures: SmPC sections 4.4 and 4.8; PL section 2 Additional risk minimisation measures: None

Glomerulonephritis	Routine risk minimisation measures: SmPC sections 4.4 and 4.8; PL sections 2 and 4 Additional risk minimisation measures: None	
Important Potential Risk:		
AML/MDS	Routine risk minimisation measures: SmPC section 4.4; PL section 2 Additional risk minimisation measures: None	
Cytokine release syndrome	Routine risk minimisation measures: None Additional risk minimisation measures: None	
Missing Information		
None	**-**	**-**

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.1 is acceptable.

2.7. Pharmacovigilance

Pharmacovigilance system

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

Periodic Safety Update Reports submission requirements

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

2.8. Product information

2.8.1. User consultation

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

2.8.2. Additional monitoring

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

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

3. Biosimilarity assessment

3.1. Comparability exercise and indications claimed

PF-06881894 (Nyvepria) has been developed as a similar biological medicinal product to Neulasta (INN: pegfilgrastim) (6 mg solution, prefilled syringe ready to use, for manual subcutaneous injection) which was approved in the European Union (EU) in August 2002 (EMEA/H/C/000420, Amgen Europe B.V., the Netherlands).

The PF-06881894 dosing regimen (frequency and duration), route of administration, the proposed indication and patient population are identical to those approved for Neulasta.

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

Quality

For the biosimilarity analysis, the applicant performed an extensive comparability exercise including side-by-side testing by a combination of orthogonal analytical methods, which are properly qualified, and by using up to 17 batches of pegfilgrastim-EU and pegfilgrastim-US and up to 10 batches of Nyvepria FP. The quality biosimilarity testing programme included a combination of physicochemical, biochemical and biological activity tests, which covered all important quality attributes of pegfilgrastim. Also, comparative degradation studies were performed to study the degradation profile of Nyvepria and EU- and US-sourced Neulasta. Taken together, the quality biosimilarity analysis was in compliance with the applicable EMA guidance (CHMP/437/04 Rev 1 and EMA/CHMP/BWP/247713/201).

Non-clinical

The nonclinical development programme for this application consisted of *in vitro* and *in vivo* studies to assess the biosimilarity between pegfilgrastim PF-06881894 (Nyvepria) and Neulasta sourced from Europe and the US.

The non-clinical programme followed the Guideline on similar biological medicinal products (CHMP/437/04 Rev 1) and the Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMA/CHMP/BMWP/42832/2005 Rev 1.). The applicant also sought Scientific Advice from the European Medicines Agency in November 2017 – in particular - on the acceptability of the proposed plan to evaluate the analytical and functional similarity of PF-06881894 to the reference product and on the supportive role of the 4-week repeated dose toxicity study performed in the rat.

The assays used included an *in vitro* cell-based proliferation assay, a competitive receptor binding assay (CRBA), and a Surface Plasmon Resonance (SPR) assay for determination of receptor binding affinity (KD and Relative KD) and the binding rate kinetics (kon and koff). These functional assays are directly related to the pegfilgrastim therapeutic mechanism of action that involves pegfilgrastim binding to receptors on the surface of hematopoietic cells in the bone marrow followed by concentration-dependent cell proliferation and differentiation. The methods used are appropriate and the *in vitro* assays have been validated or qualified.

The pharmacological activity of PF-06881894 was also assessed in the rat as part of a 4-week comparative toxicity study. This animal study is only considered as supportive for the biosimilarity assessment.

Clinical

The clinical development programme was discussed during the EMA scientific advice the applicant received for the PF-06881894 development in 2017 (EMA/CHMP/SAWP/720012/2017).

The comparative exercise for clinical similarity assessment included 2 studies in healthy volunteers: a randomised single-dose cross-over PD/PK study (ZIN-130-1505) comparing PF-06881894 to pegfilgrastim-US and pegfilgrastim-EU; and a randomised multiple dose parallel design non-inferiority immunogenicity study (C1221005) comparing PF-06881894 to pegfilgrastim-US. It should be noted that the comparative immunogenicity study (C1221005) had already started at the time of the scientific advice. Its design was selected based on FDA Guidance for Industry. A blinded design would have been ideally preferred, and the proposed non-inferiority design is not requested for safety data.

However, the CHMP has agreed that the clinical dossier for a biosimilar application for a PEG-filgrastim may comprise of healthy volunteer trials only, provided that biosimilarity can be sufficiently demonstrated based on a strong and convincing physicochemical and functional data package and comparable pharmacokinetic and pharmacodynamic profiles.

In the comparative immunogenicity study C1221005, Pfizer proposed to compare the biosimilar and pegfilgrastim-US. According to the "Guideline on Similar Biological Medicinal Products" (CHMP/437/04 Rev 1), the reference medicinal product should be a medicinal product authorised in EEA. However, it may be possible in some cases for an applicant to compare the biosimilar in clinical studies with a non-EEA authorised comparator. In this case, the applicant needs to provide adequate data or information to scientifically justify the relevance of these comparative data and establish an acceptable bridge to the EEA-authorised reference product. The type of bridging data needed will always include data from analytical studies that compare all three products (the proposed biosimilar, the EEA-authorised reference product and the non-EEA-authorised comparator), and may also include data from clinical PK and/or PD bridging studies for all three products. Since immunogenicity of PF-06881894, pegfilgrastim-US, and pegfilgrastim-EU was previously assessed in a 3 arm crossover PD/PK equivalence study in healthy volunteers (ZIN-130-1505) demonstration of equivalence between PF-06881894 against a single reference product (e.g. pegfilgrastim-US) in the proposed comparative immunogenicity study is acceptable.

3.2. Results supporting biosimilarity

Regarding the analytical similarity, the Company has conducted a robust and extensive overall biosimilarity exercise including a panel of highly sophisticated and state-of-the art methods, which characterises and compares the relevant physicochemical and biological quality attributes of the pegfilgrastim molecule.

Quality

In general, all quality attributes analysed proved to be highly similar between Nyvepria and both EU- and US-sourced Neulasta. The amino acid sequence is identical to the RMP and primary structure regarding pegylation site and linker composition, intact mass, free thiol and pI is consistent amongst all lots. A minor shift to higher molecular weight is observed for the PEG moiety, which is attributable to mPEG lot-to-lot variability. However, molecular weight dispersity and mPEG mass distribution were similar between Nyvepria and the RMP. Furthermore, secondary and tertiary structures are demonstrated to be consistent and highly similar between Nyvepria and both EU- and US-sourced Neulasta.

Product-related substances and impurities appeared to be slightly higher in both EU- and US-sourced Neulasta compared to Nyvepria, especially with regards to total related proteins, total charge variants, and total size variants. Based on the data presented, the difference in age of all Nyvepria and Neulasta

lots at the time of testing is not considered contributing to these observations. Therefore, the lower levels of product-related substances and impurities rather suggest that Nyvepria has a higher purity profile. Individual size variants were evaluated separately for the purpose of biosimilar comparability by the applicant upon request and the analysis showed consistent results between Nyvepria and Neulasta lots. In general, the differences observed were small and not considered clinically relevant. In addition, comparative stability and forced degradation studies showed that the rates, routes and profile of degradation of pegfilgrastim protein in all products (i.e. Nyvepria, pegfilgrastim-EU, and pegfilgrastim-US) are comparable (i.e. similar increase in the identified impurities over time), further supporting the similarity claim.

Furthermore, all finished product attributes concerning protein concentration, deliverable volume, deliverable content, appearance, colour, clarity, pH, osmolality, polysorbate 20 content, and visible particles are demonstrated to be similar between Nyvepria and both EU- and US-sourced Neulasta, with exception of subvisible particles content, which appears to be slightly lower in Nyvepria.

Moreover, and more importantly, *in vitro* potency, relative potency, and receptor binding affinity and kinetics were highly similar for Nyvepria and both EU- and US-sourced Neulasta. These data further confirm that the pegfilgrastim protein in Nyvepria, EU- and US-sourced Neulasta have similar higher order structure and functional conformation, which is required for biological activity.

To assess biosimilarity, the applicant used a specific statistical approach. The proposed approach using wide quality ranges and acceptance criteria was not deemed acceptable as a general principle and should be avoided. For each attribute where one or more of the data points fall out of the acceptance ranges, a scientifically sound justification should be provided if similarity is claimed. However, it is acknowledged that there is no immediate impact on the conclusion of biosimilarity in the current case since the provided data show that Nyvepria appears to be highly similar to the reference product EU Neulasta.

In conclusion, from a quality point of view, Nyvepria can be considered as biosimilar to EU Neulasta. In addition, given that biosimilarity has also been demonstrated between EU- and US-sourced Neulasta, the quality bridge is considered acceptably established and the use of US-sourced Neulasta lots in clinical studies is therefore also considered acceptable.

Non-clinical

Several complementary functional assays were utilised to assess pegfilgrastim biological activity as part of the PF-06881894 analytical similarity assessment.

The relative potency of PF-06881894 and the binding affinity to the G-CSF receptor were compared between PF-06881894 and EU- US-Pegfilgrastim reference products by Competitive Receptor Binding Assay and Surface Plasmon Resonance (SPR).

The assays used included an *in vitro* cell-based proliferation assay, a competitive receptor binding assay, and a Surface Plasmon Resonance (Biacore) assay for determination of receptor binding affinity (K D and Relative K D) and the binding rate kinetics (k on and k off). The results provided show that PF-06881894 relative potency is comprised those obtained from the reference products sourced in Europe and in the US. Similar binding affinities were also measured. Consequently, it is considered that biosimilarity of PF-06881894 (Nyvepria) and Neulasta is established through this *in vitro* comparative assessment.

In vivo pharmacological activity of PF-06881894 was assessed as part of the 4-week comparative toxicity study in rats (Study 1550-064). In rats, the magnitude of the changes in total leukocyte and neutrophil counts were similar at comparable doses of PF-06881894, pegfilgrastim-US or pegfilgrastim-EU indicating expected pharmacological effect. However, those *in vivo* studies have some limitations such

as small group size and, thus, the *in vivo* results are regarded to have only a supportive character compared to the *in vitro* results.

Clinical

Two comparative clinical studies were conducted. Study ZIN-130-1505 was a PD/PK equivalence study in healthy volunteers to compare PF-06881894 to pegfilgrastim-US and pegfilgrastim-EU and study C1221005 was a non-inferiority study in healthy volunteers to demonstrate the non-inferiority of PF-06881894 versus pegfilgrastim-US with respect to immunogenicity.

The clinical PK similarity has been shown within the conventional bioequivalence acceptance range of 80.00-125.00% for AUC0-t in ZIN-130-1505 with LS mean Ratios (%) (HSP/EU) of 97% (90%CI 90%-105%).

The clinical PD similarity between Nyvepria (PF-06881894) and Neulasta has also been shown, as for both primary PD endpoints AUECANC and ANC_Cmax in study ZIN-130-1505, as well as for secondary PD endpoints, the 95% CIs of the test/reference mean ratio were fully contained within the tighter acceptance limits of 90.0-111.0%.

Overall the safety profile in regard to AEs, SAEs, AESIs, ADRs, laboratory investigations and treatment discontinuation was, where applicable, generally well balanced between PF-06881894-treated healthy volunteers and Neulasta-treated ones in both ZIN-130-1505 and C1221005 trials.

Overall, the low proportions of subjects with positive ADA/NAb and immune-mediated AEs in subjects with positive anti-pegfilgrastim antibodies from the clinical studies in healthy subjects do not suggest clinically meaningful differences between Nyvepria (PF-06881894) and Neulasta at this point. These observations were consistent with the established safety and immunogenicity profile of Neulasta as reflected in the labels (low immunogenicity). Further, the results from these studies do not suggest any impact of ADA on the PD or PK profile of Nyvepria (PF-06881894). On a sufficiently overall high-level analysis, no unexpected safety/immunogenicity findings were uncovered and Nyvepria appears to have a similar profile compared to Neulasta (as established in the product information).

3.3. Uncertainties and limitations about biosimilarity

Quality

The analytical similarity strategy is generally acceptable. To assess biosimilarity, the applicant used a statistical approach. The acceptance criterion to pass the similarity claim was defined as: 90% of PF-06881894 analytical results should lie within the 3-sigma interval. This approach is not deemed acceptable as a general principle and should be avoided. However, it is acknowledged that there is no immediate impact on the conclusion of biosimilarity in the current case since the provided data show that Nyvepria appears to be highly similar to the reference product EU Neulasta. Provided data were highly consistent between Nyvepria and reference products with the exception of total charge variants analysis. The recently produced Nyvepria lots consist of significantly reduced content of total charged variants compared to reference product and other Nyvepria lots however, lower level of total charge variants was attributed to a lower level of deamidated impurities. Additionally, absolute percentage of these variants is very low and no impact on functional properties was observed. Therefore, no clinically meaningful impact is expected. Individual size variants were evaluated in context of analytical similarity and results were found to be similar among Nyvepria and Neulasta reference products. Analytical reports and raw data for representative lots tested in the analytical similarity assessment were provided and support the conclusion on biosimilarity. No remaining concerns were identified from the quality perspective that would contradict the biosimilarity claim.

In conclusion, there are no remaining uncertainties and limitations that have an impact on the conclusion of biosimilarity of Nyvepria and Neulasta.

Non-clinical

Regarding the non-clinical aspects of this application, there are no remaining uncertainties and limitations that have an impact on the conclusion of biosimilarity.

Clinical

From the clinical safety perspective, data are limited to studies in healthy volunteers receiving one or two doses of PF-06881894. This is acceptable in the development of a pegfilgrastim biosimilar since adverse events related to exaggerated pharmacological effects (e.g., leukocytosis, splenomegaly, bone pain) can be expected at similar frequencies if functional, PK and PD profiles can be demonstrated to be comparable.

Furthermore, a slightly higher percentage of subjects who had NAb positive samples was observed in the PF-06881894 group (1 in C1221005 & 2 in ZIN-130-1505) compared to none in the reference groups (pegfilgrastim-US/EU). However, based on these data in a very low number of patients in whom NAb could be observed, this is considered a chance finding and the presence of NAb did not appear to result in reduced clinical response or increased risk for injection site reactions.

3.4. Discussion on biosimilarity

In the development of a biosimilar product, there is no requirement to demonstrate benefit to the patient per se as this has been shown for the reference product. The benefits and risks are inferred from the similarity of the test product to the reference product in terms of the totality of evidence collected from the quality, non-clinical and clinical data.

Quality

For the biosimilarity analysis, the applicant performed an extensive comparability exercise including a combination of physicochemical, biochemical and biological activity tests. In general, the results obtained for Nyvepria, EU Neulasta and US Neulasta were highly similar for quality parameters analysed. Impurity levels were slightly lower in Nyvepria, but this is not considered to have any impact on biological safety and/or efficacy of the product. Therefore, from a quality point of view, Nyvepria can be considered as biosimilar to EU Neulasta. In addition, given that biosimilarity has also been demonstrated between EU- and US-sourced Neulasta, the quality bridge is considered acceptably established and the use of US-sourced Neulasta lots in clinical studies is therefore also considered acceptable.

Non-clinical

From a non-clinical perspective, the results of the *in vitro* assays show similarity between PF-06881894 and the reference product sourced from either Europe or the US. Those assays were performed on a sufficient number of batches, with appropriate methods and have been qualified. They are therefore deemed suitable to claim biosimilarity between PF-06881894 and the reference product Neulasta.

Pharmacokinetics

The claim of PK equivalence is supported since the 90% CIs of the test/reference ratio for both the primary and secondary PK parameters were fully contained within the acceptance interval of 80.00-125.00%.

Pharmacodynamics

Overall PD data support a high degree of similarity between PF-06881894 and Neulasta, since the 95% CIs of the test/reference ratio for both primary and secondary PD parameters were fully contained within the tighter acceptance limits of 90.0-111.0%.

Clinical safety

Safety and immunogenicity data are currently supporting similarity between PF-06881894 and pegfilgrastim-EU.

Conclusion

The analytical similarity exercise provides a comprehensive data package sufficient to demonstrate biosimilarity. All remaining quality issues on biosimilarity were solved.

Non-clinical part of the comparability has been found sufficient to demonstrate biosimilarity. All remaining issues concerning clinical part (PK, Safety and Immunogenicity profiles) have been solved, therefore Nyvepria is considered comparable to the reference medicinal product.

3.5. Extrapolation of safety and efficacy

Neulasta has a well-established efficacy and safety profile based on both clinical studies and post-marketing experiences, and is authorised for the: "reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes)".

The indication for Nyvepria is the same as per the one of Neulasta.

Extrapolation of data generated from healthy subjects to patients is possible considering the high biosimilarity demonstrated by the product in comparison to the reference medicinal product.

3.6. Conclusions on biosimilarity and benefit risk balance

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

4. Recommendations

Outcome

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

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

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

Conditions or restrictions regarding supply and use

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

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

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

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

Risk Management Plan (RMP)

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

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.