London, 26 May 2016
EMA/172440 /2016
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal Assessment report

Zioxtenzo

International non-proprietary name: pegfilgrastim

Procedure No. EMEA/H/C/004211/0000

Note

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

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Table of contents

List of abbreviations

ADA	Anti-drug antibodies
AE	Adverse event
ANC	Absolute neutrophil count
AUC	Area under the curve
$AUC_{0-\infty}$	Area under the curve measured from the time of dosing and extrapolated to infinity
AUC_{0-last}	Area under the curve measured from the time of dosing to the last measurable concentration
$AUEC_{0-last}$	Area under the effect curve measured from the time of dosing to the last measurable concentration
CD34+	Cluster of differentiation 34 positive
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
C_{max}	Measured maximum serum concentration after administration
CSR	Clinical study report
CV%	Coefficient of variation as percentage
ECG	Electrocardiogram
EEA	European Economic Area
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
E_{max}	Maximum effect attributable to the study drug
EOS	End of Study
FAS set	Full analysis set
FDA	Food and Drug Administration
GeoMean	Geometric mean
IMP	Investigational medicinal product
MAA	Marketing authorization application
PD	Pharmacodynamics
PFS	Pre-filled syringe
PK	Pharmacokinetics
PP	Per protocol
SAF set	Safety analysis set
s.c.	Subcutaneous(ly)
SFU	Safety follow-up
SOC	System organ class
TAC	Taxotere® (docetaxel 75 mg/m^2) in combination with Adriamycin® (doxorubicin 50 mg/m^2) and Cytoxan® (cyclophosphamide 500 mg/m^2)
TEAE	Treatment-emergent adverse event
$t_{max,E}$	Time point of E_{max}

1. Recommendations

Based on the review of the data on quality, safety and efficacy, the CHMP considers that the application for ZIOXTENZO in the treatment of

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) is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the List of Questions (see section 6).

The major objections precluding a recommendation of marketing authorisation pertain to the following principal deficiencies:

- The PK study failed to show equivalence of the biosimilar candidate and the originator
- The GMP compliance status of a manufacturing site to manufacture biological products has not been confirmed

Inspection issues

GMP inspection(s)

A request for GMP inspection has been adopted for a manufacturing site in order to verify the GMP compliance status.

The outcome of this/these inspection(s) is required for the Committee to complete its examination of the application and will be needed by Day 181.

GCP inspection(s)

There were no known Health Authority inspections conducted at sites participating in ZIOXTENZO clinical studies. Furthermore no inspections had been requested or are planned for ZIOXTENZO clinical studies.

All clinical studies were conducted in compliance with Good Clinical Practice (GCP), including the archiving of essential documents.

New active Substance status

N/A

Questions to be posed to additional experts

none

2. Executive summary

2.1. Problem statement

The active substance of ZIOXTENZO is Pegfilgrastim, a recombinant human granulocyte colony-stimulating factor (G-CSF). ZIOXTENZO has been developed as a biosimilar to the pegfilgrastim Neulasta, which was approved on August 22nd, 2002 for reduction of 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 recommended dose of ZIOXTENZO solution for injection is the same as for Neulasta: 6 mg (a single pre-filled syringe) per cycle, administered by subcutaneous (s.c.) injection into the thigh, abdomen or upper arm at least 24 hours after cytotoxic chemotherapy.

2.2. About the product

The active substance is a recombinant human granulocyte colony-stimulating factor (G-CSF) with a single 20 kDa peg-filgrastim as active substance. The Applicant intends to claim the same therapeutic indications as granted for Neulasta in the European Union (EU).

2.3. The development programme/compliance with CHMP guidance/scientific advice

The concept of biosimilar product development and the general principles to be applied are described in the EMA Guideline on similar biological medicinal products (CHMP/437/04 Rev 1). The application of these principles is designated as the “biosimilar approach”.

According to the guideline a biosimilar is a “biological medicinal product that contains a version of the active substance of an already authorized original biological medicinal product (reference medicinal product) in the EEA”. A company may develop a biological medicinal product claiming to be “similar” to a reference product, which has already been granted a marketing authorization within the EEA, on the basis of complete dossier in accordance with the provisions of Article 8 of Directive 2001/83/EC, as amended. For this scenario, the legal basis of Article 10(4) of Directive 2001/83/EC and Section 4, Part II, Annex I of said Directive lays down the requirements for the MAA based on the demonstration of the similar nature of the two biological medicinal products.

CHMP guidelines/Scientific Advice

- European Medicines Agency (EMA)/Committee for Medicinal Products for Human Use (CHMP) (2015) Similar biological medicinal products. CHMP/437/04 Rev 1, 30 April 2015. London, United Kingdom.
- European Medicines Agency (EMA)/Committee for Medicinal Products for Human Use (CHMP) (2015) Similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues.
- EMEA/CHMP/BMWP/42832/2005 Rev1, 01 July 2015. London, United Kingdom. European Medicines Agency (EMA)/Committee for Medicinal Product for Human Use (CHMP) (2012) Similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues. EMEA/CHMP/BMWP/24773/2012, 01 December 2014. London, United Kingdom.
- European Medicines Agency (EMA)/Committee for Medicinal Products for Human Use (CHMP) (2006) Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: nonclinical and clinical issues. Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor. EMEA/CHMP/BMWP/31329/2005, 1 June 2006. London, United Kingdom. The development program for ZIOXTENZO was discussed and presented, also retrospectively, to the following authorities:

  EMA:

  – Initial Scientific Advice in Nov 2009 (EMEA/H/SA/1419/1/2009/III, 19-Nov-2009)

- Pre-submission Meeting in Sep 2015 (Minutes of the Pre-submission Meeting, 7.2-Sep-2015)

European National Authorities:

- Medicines and Healthcare Products Regulatory Agency (MHRA) Scientific Advice Meeting in Sep 2014 (Minutes of the MHRA Scientific Advice Meeting, 7.2-Sep-2014)
- Federal Institute for Drugs and Medical Devices (BfArM) Scientific Advice Meeting in Sep 2014 (Minutes of the BfArM Scientific Advice Meeting, 7.2-Sep-2014)
- Austrian Medicines and Medical Devices Agency (AGES) Scientific Advice Meeting in Dec 2014 (Minutes of the AGES Scientific Advice Meeting, 7.2-Dec-2014)

2.4. General comments on compliance with GMP, GLP, GCP

The GMP compliance status of a manufacturing site to manufacture biological products has not been confirmed. A pre-approval inspection is requested with inspection outcome to be submitted with the responses at D181 of the procedure.

All toxicity studies were conducted in compliance with Good Laboratory Practice (GLP). However, the studies on dose formulation analytics and toxicokinetics were not performed under GLP and are therefore excluded from the statement of compliance.

2.5. Type of application and other comments on the submitted dossier

- Biosimilar application

3. Scientific overview and discussion

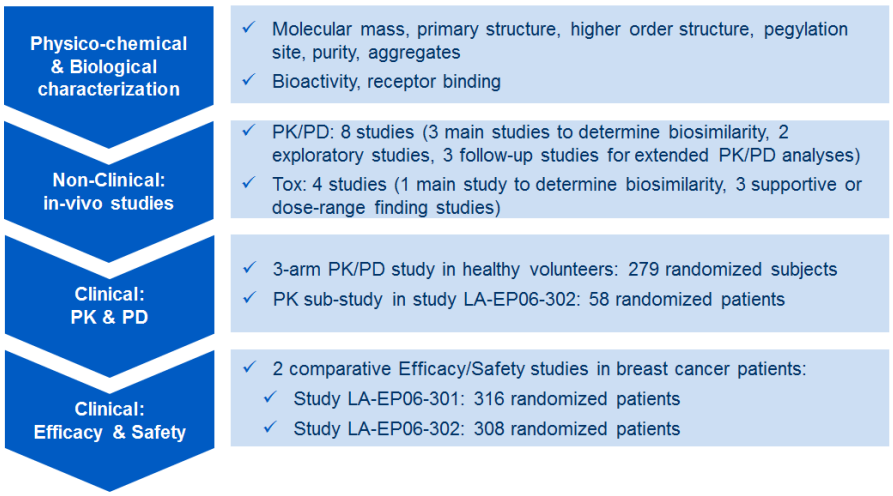
Introduction

ZIOXTENZO was developed as a similar biological medicinal product (in the following referred to as "biosimilar product") to the European Union (EU)-authorized reference product Neulasta (INN: pegfilgrastim; EMEA/H/C/000420), which is centrally authorized to Amgen Europe B.V. in the European Economic Area (EEA) (in the following Neulasta EU).

This Marketing Authorization Application (MAA) for ZIOXTENZO is submitted via the centralized procedure under Article 10(4) of Directive 2001/83/EC, as amended. Confirmation of eligibility of ZIOXTENZO to the centralized procedure under the mandatory scope according to Article 3(1) Indent 1, Biotech Medicinal Product of Regulation (EC) No. 726/2004, was received on 23-Apr-2015.

The claim of biosimilarity is based on the totality of the evidence including analytical, nonclinical and clinical data. Since Sandoz is seeking approval in the EU and in the US, both Neulasta EU and US-licensed Neulasta (in the following referred to as Neulasta US) were used in analytical studies as well as in the comparative pharmacokinetic (PK) study in the biosimilar development of ZIOXTENZO.

ZIOXTENZO Stepwise Development Program and Totality of Data:



3.1. Quality aspects

3.1.1. Introduction

The finished product is presented as a solution for subcutaneous injection containing 6 mg/0.6 mL of pegfilgrastim as active substance.

The product is available in a pre-filled syringe.

The company refers to this product as ZIOXTENZO. ZIOXTENZO was developed as a similar biological medicinal product ("biosimilar product") to the European Union (EU)-authorised reference product Neulasta (INN: pegfilgrastim; EMEA/H/C/000420). Amgen Europe B.V. is the MAH for Neulasta. EU authorised Neulasta is referred to as Neulasta EU in this report.

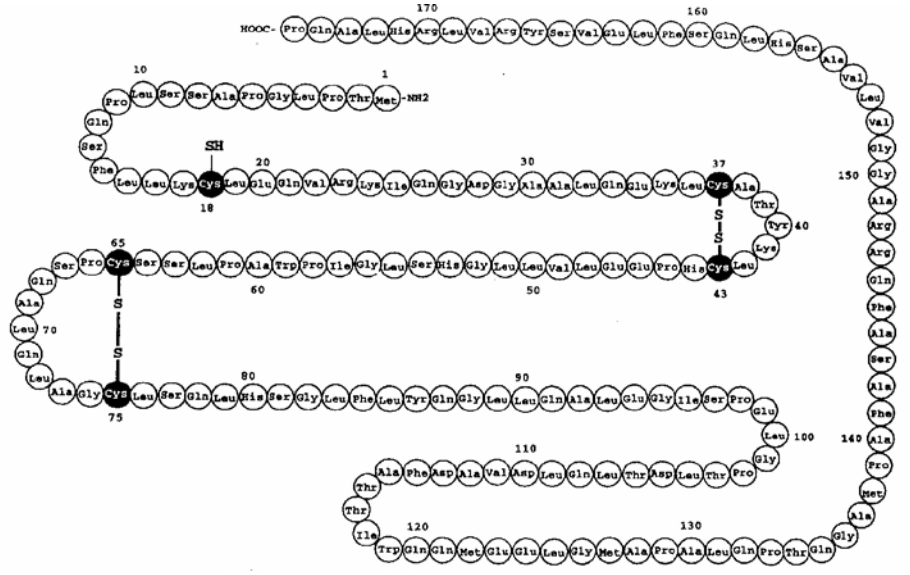
The claim of biosimilarity is based on the totality of the evidence including analytical, nonclinical and clinical data. Since Sandoz is seeking approval in the EU and in the US, both Neulasta EU and US-licensed Neulasta (referred to as Neulasta US in this report) were used in analytical studies as well as in the comparative pharmacokinetic (PK) study in the biosimilar development of ZIOXTENZO.

3.1.2. Active Substance

General Information

ZIOXTENZO (pegfilgrastim) has been developed and is manufactured by Sandoz. Pegfilgrastim is a covalent conjugate of recombinant human Granulocyte-Colony Stimulating Factor (r-met- HuG-CSF, filgrastim) with a single 20 kDa polyethylene glycol (PEG).

Filgrastim is an *E. coli*-derived rhG-CSF with an additional N-terminal methionine and compared to the native human form with the lack of an O-glycosylation at Thr133. It consists of 175 amino acids, with the N-terminus covalently linked to a single 20 kDa PEG (overall relative molecular mass of approx. 40 kDa). It contains five Cys-residues, with two intra-molecular disulphide bonds.



Manufacture, characterisation and process controls

Manufacturer(s): The intermediate is manufactured and tested by Manufacturer 1. The ZIOXTENZO drug substance (pegylated filgrastim) is manufactured, tested and released by Manufacturer 2. Additionally, external contract partners are involved in quality control testing of the intermediate and ZIOXTENZO drug substance bulk solution. Copies of valid GMP certificates and Manufacturing Authorisations are included in the dossier except where stated below. Drug Product (DP) manufactures are described under the DP section.

Description of manufacturing process and process controls

Filgrastim is produced in transformed *E.coli* bacteria, and purified using established biotechnology procedures. After fermentation and harvesting the target protein is isolated and purified in a sequence of downstream processing steps including several dilution, filtration and chromatography steps. During this process, a single 20 kDa polyethylene glycol (PEG) is attached to a target protein in a pegylation reaction. A pegylated and purified product solution is concentrated to the desired bulk concentration and diafiltered into the final formulation buffer. The final drug substance solution is filtered, filled into the storage containers and stored.

Control of materials

Raw materials are controlled by appropriate specifications and obtained from established suppliers. Upon receipt, these products are tested according to pharmacopoeia monographs or internal test procedures. No human or animal derived raw materials are used and acceptable documents have been provided for raw materials used in the establishment of cell substrate. However, a few minor points regarding PEG and buffers are raised.

The applicant has provided sufficient information regarding the expression construct and host cell substrate.

A two-tiered cell bank system has been established. The working cell bank (WCB) is prepared from a master cell bank (MCB). The MCB and WCB were subjected to extensive testing and characterisation to verify identity, purity and stability of the cell substrate. Plasmid integrity was investigated for end of production cells at the end of five production scale fermentation runs in the main fermentation step. In addition, genetic stability of the strain up to the post production stage was demonstrated. The

applicant has therefore provided sufficient information regarding the generation, characterisation and testing of the MCB and MWCB. A protocol for the establishment of future WCB has been provided and is acceptable.

Control of critical steps and intermediates

Process controls performed during manufacture of ZIOXTENZO drug substance have been categorised in two main groups: Operational parameters = input (process) parameters and performance parameters = output parameters (in-process controls). Process parameters as well as in-process controls are further divided into critical, key and non-key parameters. Operating ranges and acceptance ranges are defined for process parameters, acceptance, action and alert limits are established for in-process controls.

Classification of the process parameters was performed taking into account the existing product and process knowledge and experimental data. All adaptations of ranges for process parameters (PP) and in-process controls (IPC) are subject to internal change control management according to cGMP guidelines. Operational ranges (OR), acceptable ranges (AR), acceptance criteria (AC), action limits (AL) and alert limits (ALL) are defined. The critical PP and IPC for each manufacturing step are defined and do include appropriate control of sterility/bioburden and endotoxin.

If acceptance criteria are not met, the batch will be rejected.

Process validation

The upstream (fermentation) process and all steps of the downstream purification process were validated.

Characterisation

The active ingredient of ZIOXTENZO was thoroughly characterised on the physicochemical level and at the level of biological activity using an array of analytical procedures to investigate all the relevant attributes of the molecule both regarding identity and purity. These included orthogonal separation methods probing hydrophobicity, charge and size, measurements of the primary, secondary and tertiary structure of the protein, and assessments of biological properties of the active substance. Potential process related impurities can be identified using established methods. These include host cell DNA and proteins, endotoxin and solvents. They are removed during the purification process or are well controlled by the control procedures. Product-related substances and impurities have also been thoroughly characterised.

Specification

The release specification for ZIOXTENZO drug substance includes appropriate tests for appearance, identity, purity, content, potency, determination of pH , microbiological attributes, selected process related substances, HCP and host cell DNA.

The shelf life specification for ZIOXTENZO drug substance is almost identical.

The biological potency of a G-CSF sample is determined by measuring its ability to stimulate proliferation of NFS-60 cells compared to the ZIOXTENZO in-house reference material.

Analytical methods

Validation of non-compendial methods is described and some points for clarification have been raised on specific tests. For example, the method description for the bioactivity assay does not cover several aspects and robustness has not been addressed. A summary of the transfer report for the bioactivity test from the validation sites to the relevant testing sites are also requested.

Batch analysis

Batch release data of pilot and commercial lots have been provided including the proposed manufacturing process at the proposed site. The results are within the specifications and confirm consistency of the manufacturing process. Data of recent commercial scale batches are also requested since these batches have been used to justify DS specifications.

Reference materials

ZIOXTENZO reference materials were prepared from ZIOXTENZO DS batches manufactured using the commercial process. Questions have been raised regarding the procedure for assignment of potency to new reference materials which may cause a drift. The Company should clarify which reference material is currently in use. Actual re-test results for the reference materials should be submitted. The current reference material should be calibrated against the WHO international standard PEGylated Granulocyte Colony Stimulating Factor and analytical data for the current reference material should be expanded.

Stability

Based on the presented results, for ZIOXTENZO DS the proposed shelf life at intended storage conditions could be acceptable subject to resolution on the minor questions raised on these data.

Comparability exercise for Active Substance

Supporting comparability studies were performed to link materials from different stages of the development program to the final manufacturing process.

There are some points for clarification although in general, comparability has been demonstrated.

3.1.3. Finished medicinal product

Description of the product and Pharmaceutical development

ZIOXTENZO 6 mg/0.6 mL solution for subcutaneous injection is a clear and colourless solution containing pegfilgrastim as active ingredient supplied as a sterile, ready-for-use product intended for a single administration. The solution is provided in pre-filled syringes. All components are made of well-established materials for the packaging of medicinal products and are in line with USP and Ph. Eur. The needle shield system constitutes a medical device. The composition of ZIOXTENZO DP is identical to the composition of the reference product Neulasta.

The excipients used comply with the respective Ph. Eur. monographs and the compendial requirements for parenteral use; they are standard excipients used for protein formulations for subcutaneous administration. However, the certificates of analysis supplied for WFI do not support the claim for compliance with Ph. Eur. as only tests for conductivity and pH are listed. No excipients of human or animal origin are used.

A quality target product profile was established to guide development of ZIOXTENZO. To develop a similar biological medicinal product comparable to Neulasta (Amgen), multiple batches of the reference product Neulasta were characterised to define the target range for ZIOXTENZO quality attributes. The

quality attributes of ZIOXTENZO were evaluated for their criticality, i.e. the impact on efficacy and safety, using a risk ranking approach as outlined in ICH Q9.

Formulation studies including stress studies (mechanical stress, temperature stress) were carried out for selection of the excipients and their concentrations. No overage is required for commercial manufacturing.

Questions have been raised on the manufacturing process development, including on comparison of the drug product manufacturing processes at the different manufacturing sites including adaptations of major manufacturing equipment and impact assessment, process changes 2) sterile filter validation and 3) leachable/ extractables.

Manufacture of the product and process controls

The manufacture of ZIOXTENZO bulk drug product (DP) includes compounding of the drug substance with the excipients to the target concentration, sterile filtration (into transport vessel and then in the filling line) and filling. Labelling, assembly and final packaging is also carried out at the DP manufacturing site.

No information on potential reprocessing/ pooling of DS batches has been provided. Further questions have also been raised on the manufacturing process.

Based on the chemical and microbiological hold times determined during small-scale studies and process validation, the storage and processing times were adequately defined. Critical and non-critical process parameters and in-process controls (with acceptance ranges) have been satisfactorily established to control the manufacturing process. Points for clarification have been raised (sterile filtration, in-process controls, hold times).

To validate the manufacturing process of ZIOXTENZO 6 mg/0.6 mL solution for injection, consecutive batches of ZIOXTENZO solution for injection were manufactured at commercial scale.

During manufacturing development, the drug product manufacturing process has been transferred. Overall, relevant parameters including stability were evaluated and the data presented in the comparability assessment demonstrate comparability of ZIOXTENZO drug product throughout the development stages. The comparability data for each process should be discussed within the DP process development section.

Product specification

The release specification and shelf-life specification for ZIOXTENZO drug product 6 mg/0.6 mL include identity, purity, potency and other general tests.

Release and shelf-life specifications for product-related substances and impurities, content and potency have been set taking into consideration the values observed for different aged batches of Neulasta reference medicinal product.

A question has been raised regarding the acceptance criteria, which should be established and justified based on data obtained from lots used in preclinical and/or clinical studies, data from lots used for demonstration of manufacturing consistency, and data from stability studies, and relevant development data. In the case that limited clinical data is available for the biosimilar to support the specification limits, the reference product ranges may also be taken into consideration.

Analytical methods

In the main part, test methods are identical to those used for control of DS. Non-compendial analytical methods are validated and are, subject to resolution of raised points for clarification (e.g., controls for container closure test), acceptable.

Batch analysis

Batch analyses results of several DP batches are presented. Analytical results conform to the respective specifications and show consistency of the process. The same product related substances and impurities are detected in drug substance and drug product – no new impurities are introduced during DP manufacture. Residual solvents are removed to below acceptable limits.

Reference materials

Reference materials are described in the Reference standards section of the drug substance part of this report.

Stability of the product

Based on the presented results, the proposed shelf life at intended storage conditions for ZIOXTENZO drug product could be acceptable - subject to the satisfactory resolution of the questions on DP stability. Since all results at the accelerated storage condition after six months and the results of the out-of-fridge stability study comply with the shelf life specification, additionally one short term storage is claimed.

Stability studies were conducted in accordance with the relevant ICH guidelines, and encompassed storage at the intended, 6 months at accelerated and 1 month at stress conditions. In addition, out-of-fridge studies were carried out. The analytical program followed the shelf life specification and included additional characterisation tests. Further, freeze/thaw studies and photo stability studies as outlined in ICH Q1B were performed.

At the intended storage condition, ZIOXTENZO drug product was found to be stable with regard to most quality attributes. The proposed shelf-life at intended storage conditions appears reasonable, however it should be reviewed and justified in relation to the questions raised regarding the proposed shelf-life specifications, which have been partly set based on Neulasta data rather than ZIOXTENZO data.

At accelerated conditions, most parameters remained unchanged. Notably, bioactivity was not reduced after storage at accelerated conditions. The proposed shelf-life claim at short-term storage conditions appears reasonable, however it should also be reviewed and justified in relation to the questions raised regarding the proposed shelf-life specifications.

Photostability studies confirmed the developmental studies showing that ZIOXTENZO drug product is sensitive to light. When ZIOXTENZO drug product was subject to 3 freeze/thaw cycles essentially no change could be observed for any parameter tested.

The post-approval stability protocol does not include all tests included in the stability studies conducted to justify the shelf-life. The omission of these tests should be justified.

Adventitious agents

No raw materials of animal or human origin are used during the production of ZIOXTENZO drug product and therefore it is considered that any risk of contamination with viral adventitious agents

introduced by the raw materials or excipients can be excluded. The drug substance is manufactured in *E. coli*, which does not support the growth of viruses.

Comparability exercise for Finished Medicinal Drug Product

Comparability data to support changes made during DS product development and used in trials has been discussed (see DS section). In general the Company has followed the recommendations made in the ICH Q5E guidance; the comparability studies performed to link materials from different stages of the development program to the final manufacturing process are sound and comprehensive.

However, according to EMA guideline EMA/CHMP/BWP/247713/2012 any comparability exercise(s) for process changes introduced during development should be clearly identified in the dossier and addressed separately from the comparability exercise performed to demonstrate biosimilarity versus the reference medicinal product. Questions are raised in the DP process development section.

Biosimilarity exercise

The Company has described methodology and provided a summary of the criticality assessment performed for ZIOXTENZO quality attributes. The approach for criticality assessment and classification of ZIOXTENZO specific quality attributes is scientifically sound. The criticality score is the basis for classification of quality attributes into different tiers for which different statistical approaches are applied in the course of the biosimilarity assessment.

Comparability evaluation 1: As first step a risk based tiered approach analytical data from large scale ZIOXTENZO drug product batches and Neulasta EU and Neulasta US batches analysed over a period of several years were subjected to a statistical evaluation of comparability.

Quality attributes were categorised into different tiers as indicated above. Depending on the tier, different statistical tools were used to evaluate comparability between ZIOXTENZO and both Neulasta EU and Neulasta US.

The basis for the classification of a quality attribute is the respective criticality score as determined in the critical quality attribute assessment.

Statistical Methods

Different statistical approaches were applied for quality attributes assigned to different tiers. Descriptive statistical analyses were performed for all quality attributes, and the raw data were provided.

In a first step, a risk-based tiered approach analytical data from large scale ZIOXTENZO drug product batches and Neulasta EU and Neulasta US batches analysed over a period of several years were subjected to a statistical evaluation of comparability. Comparability between several batches of ZIOXTENZO drug product with batches of Neulasta EU and Neulasta US has been evaluated.

An array of, state-of-the-art methods for physicochemical characterization were used. In general, comparability was demonstrated between the different ZIOXTENZO drug product presentations, as well as to the reference product Neulasta EU and to Neulasta US although points for clarification have been raised as described below. Stability profiles have also been compared.

Raw data have been provided.

In principle, the application of methods with different stringency depending on the relevance of the quality attribute (as defined by the tiers) is supported, however, it is not clear in how far the rigor of similarity decision criteria differs between methods used for different tiers, in particular with regard to the probability of falsely claiming biosimilarity.

As regards equivalence testing, the determination of the equivalence acceptance criterion (EAC) is not fully understood; questions are raised. As regards power, it is not clear which true underlying mean difference between originator and biosimilar candidate is assumed.

Comparability evaluation 2: In a second step confirmatory head-to-head comparability study batches of ZIOXTENZO DP were compared with batches of the reference product, Neulasta EU, and of the comparator product, Neulasta US. The relevant physicochemical and biological quality attributes of the pegfilgrastim have been characterised by a panel of highly sophisticated and state-of-the art methods.

Despite their apparent comparability in terms of quality attributes, a surprising difference in PK was observed in the clinical studies between ZIOXTENZO and Neulasta. The applicant has thus analysed the potential impact of even slight differences in quality attributes in terms of their potential to affect PK.

In summary, the applicant has provided an in-depth characterisation of the physiochemical and characteristics and biological activity of ZIOXTENZO batches throughout development as well as of Neulasta EU and Neulasta US. A comprehensive head-to-head comparability exercise was also performed. The biosimilarity exercise was conducted in accordance with the relevant guidelines. The difference in PK results for ZIOXTENZO and Neulasta cannot be explained by differences in quality attributes. From a quality perspective, ZIOXTENZO can be considered comparable to Neulasta subject to the resolution of the raised points for clarification.

3.1.4. Discussion on chemical, biological and pharmaceutical aspects

A very extensive Module 3 including a considerable amount of information has been provided: although a relative high number of deficiencies and ambiguities have been identified due to the poor presentation of some sections. In principle, the drug substance and drug product manufacturing process including the relevant process controls have been adequately described and validated. The submitted data indicate that the manufacturing process is reliable and capable of delivering product of consistent quality. In general, an appropriate quality control system is in place which ensures that process intermediates, drug substance and drug product material of sufficient high quality will be released.

Regarding the biosimilarity, 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. The data derived from these studies demonstrate that for most of the quality attributes similarity to the reference medicinal product has been shown. Nevertheless, some deficiencies regarding methodologies used for the statistical comparability evaluation add some uncertainties in the final conclusion on biosimilarity at quality level.

Of note, some data indicate a slightly lower impurity profile of the biosimilar candidate. To determine whether these slight differences at quality level could account for the observed PK differences in the clinical part, a re-assessment and critical discussion of these structural differences has been performed by the Company. From the perspective of the quality assessor the conclusion of the Company that none of the slight differences in the quality are large enough to account for the apparent difference in

the PK data can be followed. From the current view there seems to be no root cause related to the quality which could sufficiently explain the differences in the PK.

One major objection (absence of GMP certificate for a manufacturing site) related to the quality part of the dossier is raised and a number of other concerns and questions have been identified which must be appropriately addressed by the Company.

3.1.5. Conclusions on the chemical, pharmaceutical and biological aspects

From the quality perspective ZIOXTENZO is not approvable at this stage due to the major objection raised related to the absence of a GMP certificate for a manufacturing site and the list of other concerns, as listed in the CHMP list of questions.

3.2. Non clinical aspects

3.2.1. Pharmacology

ZIOXTENZO is a pegfilgrastim consisting of recombinant methionyl human granulocyte colony-stimulating factor (r-met-HuG-CSF, filgrastim) covalently linked to a 20 kDa polyethylene glycol (PEG). It was developed as biosimilar to the EU-authorized reference product Neulasta. Both, ZIOXTENZO and Neulasta are produced in an E. coli expression system. The mechanism of action of G-CSF related to the indication of neutropenia and mobilization of neutrophilic granulocytes from the bone marrow requires binding of G-CSF to the G-CSF receptor. The results of the in vitro binding assay (SPR) show that binding of ZIOXTENZO and Neulasta to the G-CSF receptor is highly similar. The results of the cell based bioassay, which also requires binding of the products to the G-CSF receptor on NFS-60 cells to initiate signalling pathways that lead to their proliferation, are considered highly similar. Based on these results it can be concluded that ZIOXTENZO and Neulasta generate comparable effects. This is further supported by a demonstration that both products have the same physicochemical properties.

Early phase formulation and dose range finding studies in dogs and rabbits revealed similar PD effects for various ZIOXTENZO formulations, and Neulasta.

Following guideline EMEA/CHMP/BMWP/31329/2005 the pharmacodynamic effects of ZIOXTENZO and Neulasta were compared in neutropenic and non-neutropenic in vivo rodent models, i.e. rats. Naïve (non-neutropenic) rats were administered with single doses from 50 to 500 µg/kg b.w. ZIOXTENZO or Neulasta.

Neutropenic animals induced either by 5-FU or CPA were treated with single doses from 4 to 1000 µg/kg b.w. of either product.

The duration and extent of ANC increase were similar between ZIOXTENZO and Neulasta for all dose levels tested in naïve rats. The first peak levels were about 2 days after administration, followed by a decrease and second peak levels 6-9 days after administration in both, naïve and neutropenic rats. In neutropenic animals neutropenia was markedly reduced in all ZIOXTENZO- and Neulasta- treated groups as compared to placebo controls. These results indicate that both, in a naïve setting as well as in the case of pronounced chemotherapeutic bone marrow suppression, the PD effects of ZIOXTENZO and Neulasta were similar. But no pronounced dose-response effect on ANC was seen in neutropenic rats in study LA-EP06-004, using 5-FU in Wistar rats, mainly due to higher PK values for ZIOXTENZO in the 100 µg/kg dose group also showing a much higher degree of variability. Differences in the physiologic dose-related response to neutropenia induced by different agents (5-FU, 125 mg/kg b.w.

intraperitoneal vs. CPA, 50 mg/kg b.w. intraperitoneal) may give a possible explanation for this unexpected finding.

It is not clear whether the model of 5-FU induced neutropenia is adequate as the applicant argues in one study that in case of 5-FU treatment neutropenia was not fully established in the tested animals (study LA-EP06-004) and in another study the use of 5-FU is justified by the applicant on the basis of its capacity to induce a severe model of neutropenia (study LA-EP06-012). The degree of suppressing the production of non-lymphatic white blood cells may have been more pronounced by the one drug than by the other. The switch from 5-FU to CPA is argued by its use in the nonclinical studies for the reference product Neulasta. In addition, Wistar rats may be more sensitive to treatment with pegfilgrastim than Sprague-Dawley rats which have been used in the pivotal studies of Amgen with Neulasta.

Additionally, to cover the intended clinical regimen, male and female Sprague-Dawley rats were administered s.c. with repeated doses of ZIOXTENZO and EU-authorized Neulasta of 50, 100, 200 or 1000 µg/kg at different dosing regimens (every other day for 2 or 4 weeks, once weekly for 5 weeks). Across all dosing schedules and strengths as well as both genders repeated administration resulted in AUEC ratios ranging from 0.909 to 1.061. A comparative assessment of male and female rats could not be performed due to the lack of a considerable amount of data for male rats.

The inability of demonstrating PK bioequivalence between EU-authorized Neulasta and ZIOXTENZO as well as between EU-authorized and US-licensed Neulasta in clinical studies triggered a re-assessment of critical quality attributes potentially contributing to this observation. Specifically, an enhancing effect of dipegylated ZIOXTENZO on the ANC levels could be demonstrated. However, an even lower level of dipegylated variants is present in ZIOXTENZO than in Neulasta.

No secondary or dedicated safety pharmacology studies were performed, which is generally in accordance with the respective guidelines.

3.2.2. Pharmacokinetics

Exposure to ZIOXTENZO as compared to the reference product Neulasta was assessed after single and multiple dosing in naïve and neutropenic rats in various experimental settings.

The Bioanalytical assay applied for PK detection in serum measures not only pegfilgrastim, but is – due to the application of anti-GCSF antibodies in the PK assay – also able to detect partly metabolised, cleaved forms of pegfilgrastim and is expected to be especially sensitive for measuring released/cleaved G-CSF entities. In contrast, the assay may be less sensitive for detecting pegfilgrastim as PEG may to a certain degree mask the binding site. This aspect may hamper the sensitivity and variability of the nonclinical PK assessment.

Study LA-EP06-004

The rate and extent of absorption were comparable between the products at all dose levels for both naïve and neutropenic (5-FU induced) Wistar rats, with the exception of the 100 µg/kg dose in neutropenic rats (AUC ratio 1.73), where higher PK values were observed following the application of ZIOXTENZO than after Neulasta. Generally, the PK levels were higher for the neutropenic rats than for the naïve rats due to the receptor mediated clearance of G-CSF. There was a clear dose-concentration relationship for PK parameters with a similar profile for both treatments.

Study LA-EP06-008

As no pronounced PD effect was seen in 5-FU induced neutropenic Wistar rats (study LA-EP06-004) a different approach in CPA induced Sprague Dawley (SD) rats was used to assess also PK parameters. The rate and extent of absorption were comparable between the products at all dose levels for neutropenic rats with a tendency to slightly higher AUC and Cmax levels for ZIOXTENZO at the lower dose levels (4 µg: AUC ratio 1.2, Cmax ratio: 1.27; 14 µg: AUC ratio 1.13, Cmax ratio: 1.04) compared to Neulasta.

Study LA-EP06-012

A single subcutaneous treatment of 5-FU induced neutropenic Wistar rats with the test item ZIOXTENZO or the reference item Neulasta led to a clear dose-related G-CSF exposure. The rate and extent of absorption were comparable between the products at all dose levels in 5-FU-pretreated rats with a tendency to slightly higher AUC and Cmax-levels for ZIOXTENZO with one exception at a dose level of 200 µg/kg b.w. where the Cmax-level for ZIOXTENZO was slightly lower than the Cmax level of Neulasta.

Study LA-EP06-013

Repeated s.c. administration of ZIOXTENZO and Neulasta at 200 µg/kg for 2 weeks and at 100 and 200 µg/kg for 4 weeks (24 animals per dose group!) showed no difference in the PD effect.

Study LA-EP06-010

This study in rabbits examined not only PK/PD of dipegylated product variants, but also pegfilgrastim manufactured on the basis of Amgen´s filgrastim product Neupogen, using Sandoz' process and raw materials. While dipegylated variants showed a significant difference, all other pegfilgrastims showed no difference in the pharmacodynamic effect (compared against Neulasta and ZIOXTENZO (Phase I and III Lot)).

The PK of repeated dose studies was assessed within the scope of the toxicity studies. In study LA-EP06-003 male and female Wistar rats were treated with doses ranging from 100-1000 µg/kg b.w. of ZIOXTENZO or Neulasta for a planned period of four weeks. Due to severe adverse effects observed in male rats the animals had to be killed and a sufficient amount of data could not be analysed. The results in female rats showed a similar exposure at all dose levels. Gender related differences cannot be deduced from this study due to the mentioned shortcomings. It was hypothesized that the clinical signs (abnormal gait, reddening, swelling/thickening and dysfunction of the hind legs and/or ankle joint) observed in ZIOXTENZO- or Neulasta-treated Wistar rats are caused by strain-dependent reactions to pegfilgrastim interacting with the immune system and further studies examined SD rats, which were also used in the pivotal nonclinical studies performed by the originator Amgen. Nevertheless, no data were submitted supporting the assumption that immune-mediated effects were responsible.

It is noteworthy that whereas the clinical posology is to dose once per schedule of chemotherapy, the repeat dose studies in animal used frequent dosing, once every other day over 1 month. In terms of clearance, it is known that the neutrophilic response results in great expansion of G-CSF receptor in blood and this binds the pegylated (and no-pegylated) G-CSF. Thus, its clearance is accelerated consequent upon the primary pharmacodynamic effect becoming evident. This type of pattern was seen with each of Neulasta and Zioxtenzo in the Applicant's studies.

The challenges in studying nonclinical PK by serum analysis may also be influenced by the fact that the lymphatic system becomes an increasingly important mechanism for macromolecules, which is even more the case for pegylated modalities being subcutaneously administered. Thus the feasibility-driven limitation to assaying PK by analysing serum samples does not provide direct data from the lymphatic

system, which is – if compared to nonpegylated, small(er) molecules – of particular importance to accurately picture pharmacokinetics (e.g. Offman et al. 2016). This may partly explain the variability seen in nonclinical PK studies. Facing the need of showing bioequivalence in the clinics for purposes of biosimilar development, limitations for pegylated large proteins on measuring PK in blood but not in lymphatic organs needs to be considered. In summary, this remains to be one of the topics that need to be challenged on the clinical level.

3.2.3. Toxicology

Two comparative and one, non-comparative toxicity studies have been conducted to support the nonclinical development of ZIOXTENZO. One dose range finding study was conducted in a non-comparative way as well.

All repeated-dose toxicity studies included a toxicokinetic (TK) evaluation, while an immunogenicity assessment was only performed in studies ZIOXTENZO-003 and LA-EP06-006.

All toxicity studies were conducted in compliance with Good Laboratory Practice (GLP).

ZIOXTENZO-003:

In this comparative repeated dose study, toxicity of ZIOXTENZO was paralleled to EU-authorized Neulasta following s.c. administration every other day (q2d) at the dose levels of 0 (formulation buffer), 100, 500 (ZIOXTENZO only) and 1000 µg/kg to groups of 10 male and 10 female Wistar rats for 4 weeks.

After a total of five treatments in the RDTS performed with Wistar rats, several clinical signs were noted in males at all dose levels, including swelling of hindlegs and/or ankle joints that resulted in moderate to marked difficulties to move. Similar effects were also noted in the females after 7 - 8 treatments with ZIOXTENZO or Neulasta®. Although the treatment of the animals was stopped after five (males) and eight (females) administrations, the clinical signs persisted or even worsened.

Neither a no-observed-effect-level (NOEL) nor a no observed- adverse-effect-level (NOAEL) were established in this repeated dose study after a total of 5 (males) or 8 (females) administrations of ZIOXTENZO by subcutaneous injection to Wistar rats of both sexes.

As these findings appeared to be present in both administration groups (ZIOXTENZO and Neulasta®), this is more likely a consequence of different susceptibilities in the selected Wistar rat strain if compared to the originally used Sprague Dawley rat strain (used for the RDTS of Neulasta®) caused the deviating findings (Kühn et al., 1983; Paré WP, 1989). As a consequence, a dose range finding study was conducted in Sprague-Dawley rats (Study LA-EP06-005) followed by the pivotal repeat dose toxicity study in the same strain (Study LA-EP06-006).

LA-EP06-005:

This non-comparative dose-range finding toxicity study was initiated to define the no-observed adverse-effect level (NOAEL) through a treatment period of 4 weeks using an every other day (q2d) subcutaneous (s.c.) administration scheme. Furthermore, this study comprised two additional doses to allow a better comparability with historical data from toxicology studies with Neulasta®. 100 µg/kg q2d was administered for a period of 2 weeks which allowed a direct comparison with the reported NOAEL for Neulasta® based on a 2-week treatment period and, another subcutaneous dose of 1000 µg/kg q1w, reflecting the NOAEL for Neulasta® in toxicity studies with a duration of more than 2 weeks.

The Sprague-Dawley rat was chosen, as this strain was also used in the rodent toxicity studies performed with Neulasta® (EPAR), and no such unexpected results were reported in Sprague-Dawley rats, as discovered in the first repeat dose toxicity study (LA-EP06-003) with the Wistar rat strain.

Male rats were treated subcutaneously with ZIOXTENZO every other day at doses of 100 µg/kg for 13 days (7 applications), 12.5, 25, 50, 75 or 100 µg/kg for 29 days (15 applications) or with 1000 µg ZIOXTENZO/kg every week for 29 days (5 applications). Subcutaneous treatment with 75 µg ZIOXTENZO/kg every other day for 29 days (15 applications) or with 100 µg ZIOXTENZO/kg for 29 days (15 applications) caused systemic changes in form of swollen ankle joints of the hind legs resulting in limited mobility.

In succession, appropriate doses from this dose finding study were transferred to the pivotal comparative toxicity study of ZIOXTENZO vs. Neulasta® (LA-EP06-006).

The enclosed toxicokinetic data of this study were not assessed in comparison to the reference product Neulasta®. In absence of direct head to head comparison the decisive value on biosimilar assessment is lacking, but confirms the expected, originator-labelled effects for pegfilgrastim with the selected SD strain on a general level.

LA-EP06-006:

In this comparative repeated dose toxicity study, the toxicity profile of the biosimilar ZIOXTENZO and the EU-authorized originator Neulasta® was compared following s.c. administration to Sprague-Dawley rats (24 animals/sex/group) at dose levels of 100°µg/kg (q2d) for 2 weeks, 50, 100 or 200°µg/kg (q2d) for 4 weeks, or 1000°µg/kg once weekly for 5 weeks. A control group (10 animals/sex) received the vehicle (formulation buffer) q2d for 4 weeks. Reversibility of any effect was assessed after an 8-week treatment-free period.

For both, ZIOXTENZO and EU-authorized Neulasta®, no unscheduled mortality occurred during the study.

There were no treatment-related effects on body weight, food consumption, ophthalmoscopy or auditory examination. The hematological assessment showed the expected PD effect, which was overall similar for ZIOXTENZO and EU-authorized Neulasta®. Clinical signs and macroscopic findings of swollen ankle joints and/or feet were noted. An unexpected exitus was reported, but absence of an explanation for the animals´death is noted.

Clinical chemistry showed an increase in serum alkaline phosphatase (ALP) for both products. The microscopic examination showed arthritis, edema, inflammation of soft tissue, joint and bone destruction and fibro-osseous proliferation in the ankle joints and feet, as well as bone remodelling of the femur and tibia; these bone changes were similar for ZIOXTENZO and EU-authorized Neulasta®. Although these findings of joint swelling and microscopic osteopathy had not been described during the nonclinical development of Neulasta® (EPAR), peer-reviewed publications describe bone resorption detected in rat hind limbs accompanied by joint synovitis following treatment with rhG-CSF (Keller et al 1993; Suzuki et al 1997).

Immunogenicity of ZIOXTENZO upon repeated dosing was either the same or apparently lower as compared to EU-authorized Neulasta®. Considering the low sample size and limited predictivity of animal immunogenicity data for humans, this was considered as not clinically relevant. Whatsoever, the limited predictivity of comparative preclinical *in vivo* studies regarding clinical immunogenicity substantiate the assumption, that non-clinical *in vivo* studies might not add any substantial value to the biosimilar development program.

LA-EP06-011:

ZIOXTENZO was administered s.c. q2d to pregnant female Himalayan rabbits (26 animals/group) in an embryo-fetal developmental study (LA-EP06-011) during the critical phase of organogenesis from gestation Day 6 to 18, at dose levels of 0 (formulation buffer), 2, 5, 50 or 100 µg/kg.

ZIOXTENZO caused maternal toxicity at a dose of 100 µg ZIOXTENZO/kg bw, embryotoxicity at a dose of 50 and 100 µg ZIOXTENZO/kg bw. The increased incidences of developmental abnormalities observed starting at a dose level of 50 µg ZIOXTENZO/kg bw/day are considered to be test item-related.

Reported results are generally in line with data reported for the reference medicinal product, but no further conclusions can be drawn in absence of direct head to head comparison with Neulasta®.

No genotoxicity, carcinogenicity, reproductive and developmental toxicity, or dedicated local tolerance studies were performed, which is in line with relevant guidance on non-clinical development of biosimilars.

Local tolerability assessment was performed in the dose range finding study LA-EP06-005 in rats, as well as in the comparative repeated dose toxicity study LA-EP06-006. Both studies confirmed that ZIOXTENZO or Neulasta® have a comparable local tolerability.

The SmPC for Zioxtenzo reflects that of Neulasta. There is no indication of any need for different information to be provided in principle.

3.2.4. Ecotoxicity / environmental risk assessment

The Applicant submitted the Marketing Authorization Application for ZIOXTENZO following Article 10(4) of Directive 2001/83/EC as a similar biological medicinal product. All data generated for the reference product Neulasta® are applicable to ZIOXTENZO in the same way.

It is expected that ZIOXTENZO will substitute parts of the prescriptions for the reference product; however, no changes in the environmental risks that are not already identified are to be anticipated.

ZIOXTENZO, being developed as a biosimilar to Neulasta® and having pegfilgrastim as the active substance, is unlikely to be of environmental concern given the low projected supply, and a predicted rapid degradation in the environment because of the proteinaceous nature of Filgrastim, and as the PEG component is expected to be excreted in bile and urine and then subject to aerobic microbial degradation. The Applicant provided an appropriate justification with Module 1.6.1, in line with the CHMP guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 Corr 2).

3.2.5. Discussion on non-clinical aspects

Biosimilarity regarding receptor binding (SPR based approach) and in vitro functionality (NFS-60 cell-based proliferation assay) was shown. Additionally, in vivo studies in beagles, rabbits and rats were performed indicating no differences. Pivotal studies in naïve and neutropenic rats conducted in a comparative manner were not able to show differences, but suffered from high inter-individual variability, an overly susceptible rat strain (Wistar) and potential differences in myelosuppressive effects induced by 5-FU or CPA.

Overall, regarding primary pharmacology a considerable number of studies were performed, their added value on clarifying PK/PD issues identified at the clinical level seem limited, although sufficiently powered regarding the number of included animals. Unfortunately also the way of data presentation varies between studies (absolute ANC, neutrophil ratio LA-2006 vs. Neulasta, neutrophil ratio product vs. control), which complicates or completely eludes the chance to draw conclusions across different nonclinical studies.

Regarding nonclinical PK several studies indicated no differences between both compounds, but suffered as well from inter-individual variability. Above that, due to potential masking effects of pegylation, sensitivity of the PK assay may be hampered. Thus, the applicant is requested to provide data clarifying to which degree the loss of sensitivity is given, if pegfilgrastim – instead of unpegylated filgrastim is assayed. Especially as a PK assay system has been applied which was, initially generated for the detection of G-CSF. This should help to get an idea of the overall performance of nonclinically generated PK data.

For the studies with Wistar rats no data were presented confirming that increased susceptibility is indeed an immune-mediated effect. This would be of interest, especially as anti-PEG IgMs are frequently speculated of causing accelerated plasma clearance after s.c. administration (e.g. Chan et al. 2015). The toxicological assessment has been conducted and indicated no differences between ZIOXTENZO and Neulasta®. A similar safety profile was established for both, the biosimilar and the originator product with all treatment-related changes are caused by the exaggerated pharmacological effects of pegfilgrastim. No new unexpected toxicities were identified for ZIOXTENZO. Minor differences were observed what could be attributed to inter-individual variability.

The toxicity results can be seen as supporting data to the biosimilar developmental approach and are considered acceptable. However, the absence of an explanation for the death of an animal is recognised.

3.2.6. Conclusion on non-clinical aspects

In summary, the kinetic studies indicated differences such that there was generally higher exposure to Zioxtenzo than to Neulasta at the same dose. No objection to this is raised in respect of a claim of biosimilarity.

The submitted nonclinical data support the human use of ZIOXTENZO. Prior to a successful MA the “other concerns” should be addressed.

3.3. Clinical aspects

ZIOXTENZO was developed as a similar biological medicinal product to the European Union (EU) authorised reference product Neulasta in accordance with EMA guidelines and CHMP scientific advice.

The Applicant intends to claim the same therapeutic indication for ZIOXTENZO as granted for Neulasta in the European Union. The recommended dose is 6 mg, administered subcutaneously (SC). The proposed pharmaceutical formulation is 6 mg/0.6 ml solution for injection.

The clinical programme included a PK/PD study conducted in healthy volunteers using a three-arm parallel design to demonstrate PK bioequivalence and PD comparability based on the PD marker ANC between ZIOXTENZO and the chosen EU and US reference products; and two separate double-blind, parallel group comparative efficacy and safety studies in patients with breast cancer treated with myelosuppressive chemotherapy to demonstrate similar efficacy over six cycles of chemotherapy, and to assess safety and immunogenicity of ZIOXTENZO.

The Applicant's development programme to demonstrate pharmacokinetic and pharmacodynamic similarity between ZIOXTENZO and the reference product can in general be considered adequate; it is in line with the guidance on biosimilars and the recommendations given in the Scientific Advice provided by CHMP.

- ***Tabular overview of clinical studies***

The clinical programme supporting this MAA is summarised in the below table (Table 1-1).

Table 1-1 Summary of the clinical development program of LA-EP2006

Study No.	Study objective	Study population	Treatment duration	Dosage [batch number]
LA-EP06-101	Similarity of LA-EP2006 and Neulasta (EU and US) in terms of PK, PD, safety, immunogenicity, local tolerance, and possible influences on ECG parameters	Healthy volunteers Total: N=279 (156m/123f) LA-EP2006: N=93 (51m, 42f) Neulasta EU: N=93 (53m, 40f) Neulasta US: N=93 (52m, 41f)	Up to 49 days (including screening, dosing, PK, PD and safety assessments and follow-up)	LA-EP2006: 6 mg (10 mg/1 mL, glass vial), single s.c. injection [30114715] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection [1016759] Neulasta US: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection [1012807, 1017404]
LA-EP06-301	Efficacy and safety of LA-EP2006 compared to Neulasta EU with respect to the mean DSN, defined as the number of consecutive days with grade 4 neutropenia (ANC < 0.5 × 10^9/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen in patients with breast cancer	Patients with breast cancer Total: N=316f LA-EP2006: N=159f Neulasta EU: N=157f	44 weeks (18 weeks plus a 6-month SFU)	LA-EP2006: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [30324244, 30324245] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [10271811A, 1028053D, 1033639A]
LA-EP06-302	Efficacy and safety of LA-EP2006 compared to Neulasta EU with respect to the mean DSN, defined as the number of consecutive days with grade 4 neutropenia (ANC < 0.5 × 10^9/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen in patients with breast cancer	Patients with breast cancer Total: N=308f LA-EP2006: N=155f Neulasta EU: N=153f PK sub-study: N=58f ECG sub-study: N=54f	22 weeks (18 weeks plus a 4-week follow-up) including a PK/ECG sub-study	LA-EP2006: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [30324244, 30324245] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [1022627/1022626, 1028053D, 1033639A]

ANC=absolute neutrophil count; ECG=electrocardiogram; f=female; m=male; Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta; PD=pharmacodynamics; PFS=pre-filled syringe; PK=pharmacokinetics; s.c.=subcutaneous; SFU=safety follow-up; TAC=Taxotere® (docetaxel 75 mg/m^2) in combination with Adriamycin® (doxorubicin 50 mg/m^2) and Cytoxan® (cyclophosphamide 500 mg/m^2)

3.3.1. Pharmacokinetics

3.3.1.1. Bioanalytical methods

Analysis of pharmacokinetics

Study LA-EP02-101:

The analysis of the pharmacokinetic data is based on the per-protocol population.

Plasma concentrations were summarized by treatment and nominal time-point. Descriptive statistics were undertaken for plasma concentrations obtained at each nominal time-point. Pharmacokinetic parameters were also listed by subject and summarized by treatment. The PK parameters AUC0→last, AUC0→∞, Cmax, tmax, kel and t½ were summarized descriptively. Descriptive statistics (except tmax) included: the number of subjects (n), arithmetic mean, the SD, the coefficient of variation given as percentage (CV%) and the geometric CV%, median, geometric mean, minimum and maximum values. For tmax, only median, minimum and maximum values were presented, using actual sampling times.

Study LA-EP02-302:

Evaluation of concentration versus time data (summary tables for raw plasma concentrations):

- For all pre-dose samples at day 2, the sampling time will be set to 0;
- For post-dose samples, the planned sampling time will be used for figures of individual concentration-time curves, and the actual sampling times will be used for calculation of the PK parameters;
- All pre-dose concentration values < LLOQ (lower limit of quantification) will be set to zero;
- Post-dose concentration values < LLOQ will be set to ½ LLOQ if there are no further concentrations > LLOQ at later time points;
- Post-dose concentration values < LLOQ will be discussed in the BDRM if there are further concentrations > LLOQ at later time points;
- Missing post-dose values will not be replaced.

Determination of pharmacokinetic metrics (if different from above):

- For post-dose samples, the actual sampling time will be used;
- Post-dose concentration values < LLOQ in the absorption phase will be set to ½ LLOQ;
- Post-dose concentration values < LLOQ after tmax will be ignored if there are no further concentrations > LLOQ at later time points;
- Post-dose concentration values < LLOQ after tmax will be discussed in the BDRM if there are further concentrations > LLOQ at later time points;

Exclusion from PK analyses:

Patients can be excluded from the PK analyses for the following reasons:

- Major Protocol Deviation
- Potential bias because of presence of (neutralizing) antibodies
- administered dose unequal planned dose
- invalid PK profile caused by missing values

All PK results and patient profiles will be visually inspected during the BDRM.

3.3.1.2. Statistical methods

Sample size

Sample size calculations were based on both PK and PD biosimilarity testing and for both reference products (EU and US), and are summarized in the following table:

Table 2 Two-group t-tests (TOST) for ratio of means (using log scale) (equal n's)

	C_{max}	AUC	E_{max}	AUEC
Test significance levels, α (one-sided)	0.025	0.025	0.013	0.013
Lower bioequivalence limit for μ_T / μ_S, Δ_L	0.800	0.800	0.870	0.870
Upper bioequivalence limit for μ_T / μ_S, Δ_U	1.250	1.250	1.150	1.150
Expected ratio, μ_T / μ_S	1.050	1.050	1.010	1.010
Coefficient of variation, σ_S / μ_S	0.350	0.350	0.230	0.230
Power (%)	90	90	90	90
n per group	82	82	84	84

A stricter nominal alpha level was applied for PK testing (2.5% one-sided) and PD testing (1.25% one-sided) in order to warrant overall type I error control across regions (the study would have been considered successful if the comparison of ZIOXTENZO to at least one of the two reference products shows an equivalent PK and PD profile). The variability estimates, inter-subject coefficients of variation of 0.350 for AUC and 0.230 for AUEC, were based on another pegfilgrastim study (no further details were provided), and the final sample size of n=93 per arm are based on an expected exclusion rate from the per-protocol population of 10%.

279 healthy subjects were finally randomized in this study, 93 per treatment arm.

The procedure for type I error control is acknowledged, although the less stringent alpha-level of 5% and 2.5% (one-sided) for PK and PD evaluations respectively would be acceptable as well. In fact, the company additionally reports 90% CI for PK evaluations. The sample size calculations appear to be technically correct, and the planned sample size is large enough to achieve reasonable conjunctive power to simultaneously demonstrate PD and PK equivalence. The anticipated inter-subject variability is difficult to comprehend in view of published Neulasta studies (e.g. Yang et al 2008, Yang and Kido 2011) and with regard to the estimates observed in study 101. Overall, the sample size considerations are acceptable.

Populations for analyses

The primary PK and PD analyses were based on the Per-Protocol Analysis Set. It included all subjects who received IMP, provided evaluable PK and PD (ANC) profiles, and completed the study without a major protocol violation. Major protocol violations were agreed on in a blinded data review meeting before unblinding. The determination of protocol violations focused on protocol violations or intercurrent illnesses that may invalidate the study by interfering with the study objectives, subject who erroneously entered the study in violation of the protocol, administration of a treatment dose amount outside the accepted deviation range and/or misrandomised subjects, missing or invalid PK or PD (ANC) profiles (subjects with missing concentration data will be included in the PK analysis provided that, in the opinion of the PK analyst, a complete set of PK parameters can be calculated), missing or invalid/incomplete ECG triplicate measurements, or other significant protocol violation during the study.

In the analysis of the PK subgroup in study LA-EP06-302, the existence of antibodies was taken into account when assessing evaluability.

The definition of the PK analysis set is acceptable.

In study LA-EP06-101, two randomized subjects were excluded (as decided during the blinded data review meeting), both received Neulasta US product.

In the PK substudy of LA-EP06-302, two subjects were excluded from the statistical analysis (one Subject was excluded due to a major protocol deviation in cycle 1, dosing was delayed, another Subject was excluded, because all concentrations were below LLOQ).

Statistical Analysis of PK Data

The PK parameters of pegfilgrastim following single s.c. doses of 6 mg ZIOXTENZO and Neulasta (EU- and US-registered) were estimated by non-compartmental analysis. The calculation of the AUC was performed using the linear trapezoidal (linear/log interpolation) method assuming the theoretical dose (6 mg) administered. Actual sampling times relative to dosing rather than nominal times were used in the calculation of all derived PK parameters. Any subjects with missing concentration data were included in the PK analysis set provided that, in the opinion of the PK analyst, a complete set of PK parameters can be calculated. A minimum of three concentration-time points above the lower limit of quantification (LLOQ) were used in the estimation of K_{el} for determination of the elimination half-life. Terminal values below the limit of quantification were treated as missing for pharmacokinetic evaluation. The PD parameters were derived in a similar way.

Let μ_T and μ_R denote the population means for test (ZIOXTENZO) and reference (Neulasta®, EU- or US-registered), the null and alternative hypotheses were defined:

Pharmacokinetic: H_0: $\mu_T / \mu_R \leq 0.8$ or $\mu_T / \mu_R \geq 1.25$ versus H1: $0.8 < \mu_T / \mu_R < 1.25$.

Pharmacodynamic: H_0: $\mu_T / \mu_R \leq 0.87$ or $\mu_T / \mu_R \geq 1.15$ versus H1: $0.87 < \mu_T / \mu_R < 1.15$.

As the comparison between ZIOXTENZO and both Neulasta® products were performed independently, an alpha-adjustment was applied to both the PK and the PD equivalence assessments. Hence, biosimilarity between ZIOXTENZO and Neulasta® (EU- or US-registered) was defined to be demonstrated if the 95% CI for the ratio of ZIOXTENZO:Neulasta® is completely contained within the range 0.8 to 1.25 for AUC0→last of pegfilgrastim. Similarly, pharmacodynamic comparability between ZIOXTENZO and Neulasta® (EU- or US registered) was defined to be demonstrated if the 97.5% CI for the ratio of ZIOXTENZO:Neulasta® is completely contained within the range 0.87 to 1.15 for AUEC0→last of ANC.

Missing PK/PD values between two measurable values were linear interpolated for the calculations. Missing pre-dose PD values were replaced by the mean of all available pre-dose values of this parameter of the whole study population. No imputation schemes for other missing values were applied.

For the comparison of the primary pharmacokinetic parameter AUC0→last after logarithmic transformation an analysis of variance (ANOVA) with factor treatment using a linear model was performed. A point estimate and the corresponding 95% CI (pharmacokinetic endpoint) for the difference between ZIOXTENZO and 1) Neulasta® (EU registered) and 2) Neulasta® (US-registered) was calculated and anti-logged to obtain the point estimate and the 95% CI (pharmacokinetic endpoint) for the ratio of the geometric means on the untransformed scale. The same analysis was performed for the secondary parameters AUC0→∞ and Cmax. In addition a sensitivity analysis (for AUC0→last, AUC0→∞ and Cmax) was performed adjusting for weight class and gender. tmax was analyzed using Hodges-Lehman CIs (large sample size approximation) for estimation of the treatment group differences.

Analysis of the PD parameter AUEC0→last was performed in a similar way.

Analysis of PK parameters in the PK subgroup of study LA-EP06-302 was mainly based on descriptive statistics, and the ratio of the geometric means and 90% confidence interval of the ratio of geometric means of AUC0-last and Cmax was also calculated.

The multiplicity adjustment due to comparison to both the Neulasta US and EU product is acknowledged. However, the less stringent one-sided alpha-levels of 5% and 2.5% for PK and PD evaluations respectively is considered acceptable for the application of a marketing authorization in the EU. The additionally reported 90% CI for PK evaluations are acceptable and will be subject to the assessment. Statistical analysis methods based on log-transformed variables to estimate confidence intervals of the ratio of geometric means in an AN(C)OVA model are following the standard requirements outline in the bioequivalence guideline and are considered appropriate. The proposed sensitivity analysis is considered equally important as it takes the stratification factors into account.

As regards the PK substudy of LA-EP06-302, methods for statistical analysis of PK parameters are considered appropriate as well.

3.3.2. Clinical

Two studies investigated the pharmacokinetics of ZIOXTENZO. One study was a randomized, three-arm, parallel group PK/PD (study LA-EP06-101) in 279 healthy volunteers, using Neulasta EU and Neulasta US as active comparators. The other study was an exploratory PK sub-study of the confirmatory efficacy and safety study LA-EP06-302, where 60 patients with breast cancer were included.

3.3.2.1. Study LA-EP06-101:

The design, methods and description of conduct of the study are presented in this pharmacokinetics section. Endpoints and results regarding pharmacodynamics are presented in the pharmacodynamics section 3.4.2.

Design:

This was a randomized, double-blind, three-arm, parallel-group study to determine the pharmacokinetics, pharmacodynamics and safety of ZIOXTENZO and Neulasta (EU- and US registered) in healthy subjects
A parallel design was chosen by the Applicant due to the long half-life of the product, anticipated period effects and the complexity to attribute immunogenicity results to specific products.

Study period: 24-Jun-2010 - 28-Dec-2010

Population:

The study was powered to demonstrate PK equivalence of ZIOXTENZO and both Neulasta products, assuming an inter-subject variability of 35%. Allowing for a drop-out rate of 10% to achieve 84 completers per treatment arm, a total of 279 Caucasian subjects, 156 (55.9%) male and 123 (44.1%) female aged 18 to 55 years were randomized at a single centre in Germany with 93 subjects per treatment group (1:1:1).

A population of healthy subjects is considered appropriate to sensitively detect potential differences between the two treatments.

In- and exclusion criteria were considered appropriate.

Treatment:

Each enrolled subject received a single dose of pegfilgrastim 6 mg ZIOXTENZO (= Zioxtenzo) or EU sourced Neulasta or US sourced Neulasta, which is the only dose approved and currently used for the comparator product.Randomization was stratified by body weight (weight bands of 10 kg were applied, i.e. 50.0-59.9 kg, 60.0-69.9 kg, 70.0-79.9 kg, 80.0-89.9 kg, and 90.0-99.9 kg) and gender. The randomisation plan is considered adequate.

Each drug was administered as a 6 mg SC injection into the abdominal area.

The test product used was from a pre-commercial production process. A comparable quality profile of the ZIOXTENZO batch used in this PK/PD study with the intended commercial material has been provided, which is considered sufficient.

The study was performed in a -blinded manner. The measures taken to organise and keep the blinding are considered adequate.

Selection of dose:

The applicant provided a justification for the selection of the 6 mg dose: The dose-response relationship of pegfilgrastim and its effect on the ANC, the PD marker of drug's efficacy, has been well characterized in two clinical studies (Molineux et al 1999, Johnston et al 2000). Both studies were based on a single dose, dose-escalation design (30 µg/kg, 60 µg/kg, 100 µg/kg and 300 µg/kg), either in 32 healthy volunteers or in a healthy-volunteer like setting, using 13 pre-chemotherapy cancer patients. Both studies produced consistent results in terms of PK and PD responses. In healthy volunteers, a population which represents a highly sensitive setting with respect to the ANC response, the ANC profiles demonstrated a clear dose response, both in the magnitude and duration of the effect (Molineux et al 1999). Values of the median maximum ANC (ANCmax) and the median area over the baseline effect curve increased with increasing dose of pegfilgrastim.

Figure 1-1 **ANC response after single injection of pegfilgrastim at ascending doses in healthy volunteers (Molineux et al 1999)**

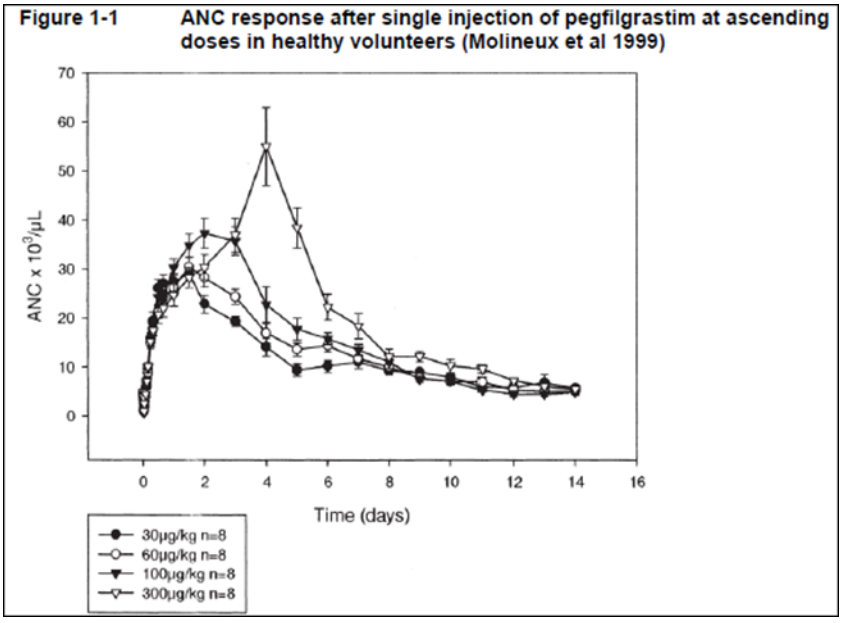
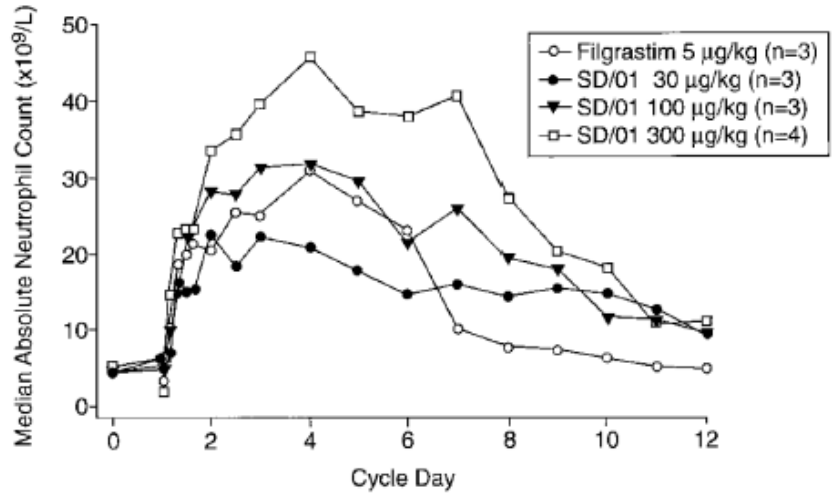


Figure 1-2 Median ANCs in cycle 0 (pre-chemotherapy) in cancer patients (Johnston et al 2000)



Source: Figure 2 of Johnston et al (2000)

The presented dose-response data in the pre-chemotherapy setting (Johnston et al. 2000, see figure 2 above) indicate that the 6 mg dose of pegfilgrastim (corresponding to a dose of approximately 100 µg/kg) provides an approximately 30-40% higher ANC response relative to the lowest dose studied (i.e. 30 µg/kg). At the highest studied dose of 300 µg/kg pegfilgrastim, the ANC response is approximately double relative to the 6 mg dose.

The conclusion of the applicant was that the 6 mg dose is positioned at the non-saturating portion of the dose-response curve and provides optimal sensitivity with respect to the ANC response within the range of doses evaluated during the clinical development of pegfilgrastim by the originator.

Sampling time points:

- PK: 24 blood samples for measurement of serum pegfilgrastim will be taken pre-dose and 0.5, 4, 8, 12, 16, 20, 24, 28, 32, 36, 48 , 60, 72, 84, 96, 108, 120, 144, 168, 192, 216, 264 and 336 h (d 14) post dose.
- Immunogenicity: Blood samples will be collected at 15 minutes pre-dose on Day 1, and on Days 15 and 28 for detection of antibody formation against pegfilgrastim.
- Laboratory and urinalysis: Blood and urine samples will be taken for laboratory safety tests at screening, and in the morning on Days –1, 3, 7 (±1 day) and 15 (follow-up visit).

The sampling time points are adequate to reflect the characteristics of pegfilgrastim and gain respective data for a comparative evaluation of the critical PK parameters.

Primary PK endpoint

$AUC_{0\text{-last}}$ (area under the concentration-time curve from dosing to the last measurable concentration) as co-primary endpoint with PD endpoint ANC $AUEC_{0\text{-last}}$

Secondary PK Endpoints:

Cmax	Maximum serum concentration
Tmax	Time to reach Cmax
AUC0–inf	Area under the concentration-time curve from dosing to infinity

λz Terminal rate constant

t1/2 Apparent terminal half-life

A comparability acceptance margin of 80% to 125% was selected for the PK analysis.

PK parameters were presented using a 95% CI to account for multiplicity adjustment, as well as a 90% CI. The assessment of the PK results is based the on the analysis using the 90% CI without multiplicity adjustment, as usually the 90% CI is accepted for PK comparison.

The selection of AUC0-tlast as primary PK endpoint is in line with the Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor (EMEA/CHMP/BMWP/31329/2005) and was supported by the SAWP Scientific advice (EMEA/H/SA/1419/1/1/2009/III). The last plasma sampling will be taken at d14, which is considered to also sufficiently represent the late elimination period (and was confirmed by the individual concentration-time curves, as in most cases pegfilgrastim concentration at the last sampling time point was below LLOQ).

Study conduct:

Demographic baseline characteristics:

The mean age of the population was 37 years and male and female were similarly distributed across treatment arms with slightly more male subjects enrolled (56% vs 44%). Also the other demographic characteristics (i.e. age, height, body mass index) were similarly distributed across all treatment groups of the safety population considering the overall mean values. But when looking at table 11-2, where the subjects are stratified to 5 weight classes, more subjects are assigned to the highest two weight classes of female subjects randomized to LA-EP 2006 (8 LA-EP 2006 vs. 2 Neulasta EU vs. 4 Neulasta US). This imbalance could possibly lead to lower mean pegfilgrastim concentrations in female LA-EP 2006 treated subjects.

Table 11-2 Stratification for randomization (Safety Population)

	LA-EP2006 (n=93) n (%)	Neulasta® EU (n=93) n (%)	Neulasta® US (n=93) n (%)
Male			
50.0–59.9 kg	0 (0.0)	0 (0.0)	1 (1.1)
60.0–69.9 kg	8 (8.6)	7 (7.5)	9 (9.7)
70.0–79.9 kg	20 (21.5)	22 (23.7)	17 (18.3)
80.0–89.9 kg	14 (15.1)	13 (14)	15 (16.1)
90.0–99.9 kg	9 (9.7)	11 (11.8)	10 (10.8)
Female			
50.0–59.9 kg	9 (9.7)	9 (9.7)	10 (10.8)
60.0–69.9 kg	13 (14)	15 (16.1)	16 (17.2)
70.0–79.9 kg	12 (12.9)	14 (15.1)	11 (11.8)
80.0–89.9 kg	6 (6.5)	2 (2.2)	3 (3.2)
90.0–99.9 kg	2 (2.2)	0 (0.0)	1 (1.1)

n: Number of subjects; (%): Percentage per treatment group

Source: Section 14, Table 14.1.2, Appendix 16.2, Listing 16.2.3.1

Patient flow:

515 subjects were screened, of which 236 subjects failed screening. The most frequent reasons why subjects failed the eligibility criteria were any previous exposure to G-CSF products (N=202) or

infections within 2 weeks before study entry (N=34). A total of 279 subjects were enrolled and randomized (1:1:1) to the three treatment arms:

ZIOXTENZO (N=93), Neulasta EU (N=93) or Neulasta US (N=93).

All enrolled patients completed the study, with the exception of one subject (subject 118) in the Neulasta US group who withdrew from the study due to personal reasons on the day he received study medication. Major protocol deviations were noted for two subjects in the Neulasta-US arm, who were hence excluded from the per-protocol population.

Table 10-1 Subject disposition and analysis populations

	LA-EP2006 n (%)	Neulasta® EU n (%)	Neulasta® US* n (%)
Subjects Randomized	93	93	93
Subjects Completed	93 (100)	93 (100)	92 (98.9)
Subjects Withdrawn	0 (0.0)	0 (0.0)	1 (1.1)
Analysis Populations			
Safety Population	93 (100)	93 (100)	93 (100)
Per-protocol Population	93 (100)	93 (100)	91 (97.8)
ECG Population	59 (63.4)	63 (67.7)	62 (66.7)

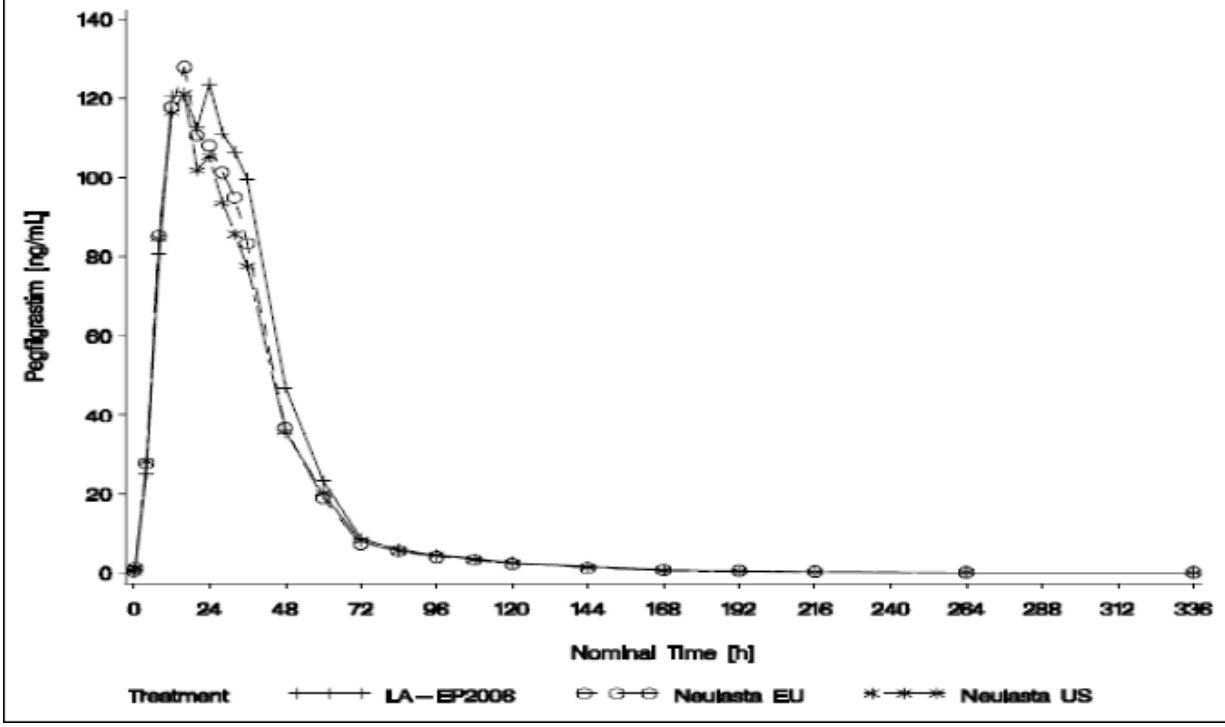
n: Number of subjects; (%): Percentage per treatment group

Source: Section 14, Table 14.1.1, Appendix 16.2, Listings 16.2.1.2 and 16.2.2.1

* Two batches; batch 1012807: N=52; batch 1017404: N=41 (see Table 9-1); values manually calculated.

Pharmacokinetic results:

Figure 11-6 Mean plasma concentration plot of pegfilgrastim: linear scale (Per-protocol Population)



The peak of the mean plasma concentrations of all three pegfilgrastim products were approximately 120 to 130 ng/mL and occured at about 24 hours post-dose. The curves showed a biphasic decline with a rapid phase until ~72 to 96 hours post-dose and a slow phase thereafter with concentrations below 20 ng/mL until the last sampling point.

In the first part of the elimination phase, approximately between the time of peak concentration and 72 hours post-dose, the mean pegfilgrastim concentration of ZIOXTENZO appeared to be mostly higher than the reference products (best seen in the linear representation, Figure 11-6), which would probably lead to a larger AUC of ZIOXTENZO if compared to both Neulasta products.

The initially applied 95% CI showed that all PK parameters had CIs outside the upper limit of 125%.

Table 11-5	Summary of derived pharmacokinetic parameters (Per-protocol Population)				
		n	Mean (SD, CV%)	Median (Range)	GeoMean
LA–EP2006	C_{max} [ng/mL]	93	228.9 (186.4, 81.5)	169.0 (12.0–717.0)	157.6
	t_{max} [h]	93	NA	24.02 (8.06–48.03)	NA
	$AUC_{0\rightarrow last}$ [h ng/mL]	93	8215.7 (7277.0, 88.6)	5810.1 (849.2–32740.9)	5766.7
	k_{el} [1/h]	92	0.018 (0.006, 32.9)	0.018 (0.006–0.037)	0.017
	$t_{1/2}$ [h]	92	42.9 (16.1, 37.6)	39.43 (18.65–109.36)	40.45
	$AUC_{0\rightarrow\infty}$ [h ng/mL]	92	8288.7 (7294.0, 88.0)	5835.5 (862.0–32754.0)	5833.7
Neulasta® EU	C_{max} [ng/mL]	93	214.9 (157.7, 73.4)	158.0 (10.6–713.0)	155.0
	t_{max} [h]	93	NA	20.00 (8.00–47.93)	NA
	$AUC_{0\rightarrow last}$ [h ng/mL]	93	7276.6 (5623.2, 77.3)	5394.1 (569.7–24002.1)	5244.9
	k_{el} [1/h]	93	0.018 (0.006, 35.6)	0.018 (0.006–0.040)	0.017
	$t_{1/2}$ [h]	93	44.6 (19.7, 44.3)	38.13 (17.42–112.13)	41.15
	$AUC_{0\rightarrow\infty}$ [h ng/mL]	93	7294.8 (5621.2, 77.1)	5408.6 (592.5–24011.4)	5277.4
Neulasta® US	C_{max} [ng/mL]	91	207.2 (170.6, 82.3)	161.0 (6.4–717.0)	145.1
	t_{max} [h]	91	NA	16.01 (8.00–59.48)	NA
	$AUC_{0\rightarrow last}$ [h ng/mL]	91	7213.4 (6600.0, 91.5)	5259.6 (370.2–33693.2)	4997.6
	k_{el} [1/h]	90	0.019 (0.008, 39.1)	0.017 (0.007–0.050)	0.018
	$t_{1/2}$ [h]	90	41.3 (16.9, 40.9)	40.00 (13.84–106.29)	38.41
	$AUC_{0\rightarrow\infty}$ [h ng/mL]	90	7251.4 (6633.1, 91.5)	5283.0 (386.9–33706.0)	5019.9

n: Number of subjects analyzed; SD: Standard deviation; CV%: Coefficient of variation as percentage; GeoMean: Geometric Mean
NA: Not applicable
Source: Section 14, Table 14.7.2.1, Appendix 16.2, Listing 16.2.9.2

The *post hoc* analyses, for the PK parameters using a 90% CI are tabulated below.

Table 2-5 Bioequivalence analysis for PK parameters – comparison of treatments – study LA-EP06-101 (PP population)			
Comparison	**Parameter**	**Ratio**	**[90% CI]**
LA–EP2006 vs. Neulasta EU			
	$AUC_{0\text{-last}}$ [a]	109.95	[88.90; 135.98]
	C_{max}	101.71	[81.24; 127.32]
	$AUC_{0\text{-}\infty}$	110.54	n.d.
	t_{max} [b]	0.136	n.d.
adjusted for weight class and gender	$AUC_{0\text{-last}}$	112.07[c]	[92.39; 135.93]
	C_{max}	103.57	[84.08; 127.58]
	$AUC_{0\text{-}\infty}$	112.10	n.d.
LA–EP2006 vs. Neulasta US			
	$AUC_{0\text{-last}}$ [a]	115.39	[93.19; 142.87]
	C_{max}	108.67	[86.70; 136.20]
	$AUC_{0\text{-}\infty}$	116.21	n.d.
	t_{max} [b]	3.998	n.d.
adjusted for weight class and gender	$AUC_{0\text{-last}}$	118.75	[97.79; 144.20]
	C_{max}	111.72	[90.59; 137.79]
	$AUC_{0\text{-}\infty}$	118.68	n.d.
Neulasta EU vs. Neulasta US			
	$AUC_{0\text{-last}}$	104.95	[84.76; 129.95]
	C_{max}	106.84	[85.24; 133.92]
adjusted for weight class and gender	$AUC_{0\text{-last}}$	105.96	[87.26; 128.68]
	C_{max}	107.87	[87.46; 133.03]

$AUC_{0\text{-}\infty}$=area under the curve measured from the time of dosing and extrapolated to infinity; $AUC_{0\text{-last}}$=area under curve measured from the time of dosing to the last measurable concentration; CI=confidence interval; C_{max}=measured maximum serum concentration after administration; n.d.=not determined; Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta; PK=pharmacokinetics; PP population=per protocol population; t_{max}=time point of C_{max}

For $AUC_{0\text{-last}}$ and C_{max}, the ratios and their margins are given as percentages. For t_{max}, the ratio and its bounds are given as absolute values.

[a] Primary PK endpoint

[b] Confidence intervals calculated using the Hodges-Lehman estimation (large sample size approximation)

[c] In the additional analyses, a ratio of 112.06 was obtained for this comparison.

Source: for ratios and 90% CI, additional analyses were performed

PK values were generally higher for ZIOXTENZO with a mean AUC0→last of 8215 h ng/mL (Neulasta EU: 7276.7 h ng/mL) and a mean Cmax of approximately 230 ng/mL (Neulasta EU: 214.9 ng/mL). In most subjects the pegfilgrastim concentration was below LLOQ at the last sampling time point at day 15, therefore mean AUC0→∞ values were only slightly different to the mean values of AUC0→last. The median tmax ranged between 16 and 24 hours among all three pegfilgrastim products with ZIOXTENZO showing the highest value. The elimination rate constant kel, and consequently t1/2, showed no remarkable differences with a mean value of about 0.018/h for kel and approximately 41 to 45 hours for t1/2.

Conclusion:

PK bioequivalence could not be demonstrated between ZIOXTENZO and the EU reference product Neulasta; the 90% CI for the primary PK parameter was not contained within the standard

bioequivalence margin of 80% - 125%. Drug exposure was observed to be higher following treatment with ZIOXTENZO, as the 90% CIs for the ratios of the geometric means for PK endpoints comparing ZIOXTENZO EU sourced Neulasta fell outside of the prespecified upper acceptance limit of 125%. This affected the primary endpoint AUC0-last, as well as the secondary endpoints AUC0-∞ and Cmax (10% difference according to exposure point estimate, see Table 2-5). This picture remained unchanged after the calculation was adjusted for weight class and gender (12% difference in exposure point estimate).

The inter-subject variability (CV%) was in the range of 77.3 - 91.5% for AUC0-last, and between approximately 73% and 82% for Cmax. For kel, and t1/2 the CV% was smaller with approximately 33% to 44%, and may support the observation, that the elimination phase was rather similar in ZIOXTENZO and Neulasta (EU and US registered). It has to be considered that clearance of pegfilgrastim via the neutrophile G-CSF receptor mediated pathway might be saturated at the used dose of 6 mg and therefore potential differences could be masked. The estimates of tmax following a Hodges-Lehman analysis between ZIOXTENZO and Neulasta EU or Neulasta US were 0.136 and 3.998, respectively, and showed lower 95%CI bounds of –0.024 and 0.034, while the upper 95%CI bounds were 4.017 and 7.986, respectively. Thus, the 95% CIs of the estimates of tmax did not support a statistically significant difference in both comparisons (the 90% boundaries were not presented by the applicant).

The observed variability of the PK parameters by far exceeds the anticipated variability for sample size planning (CV% was in the range of 77.3 - 91.5% for AUC0-last). This (and preference of a parallel-group design) is certainly an important factor why the upper bounds of the (90% and 95%) confidence intervals are clearly larger than 125%.

High inter-subject variability of 86.5% was also reported in a recent publication by the originator (Yang et al 2015) of a biosimilarity study in male and female subjects which also used a parallel-design.

A full PK report however could not be found and is requested to be submitted.

Only the comparison of the test and EU reference product is of interest but the differences with the US reference product are even more pronounced.

Furthermore it is noteworthy that the EU compared to the US reference products were not shown to be bioequivalent either, as the CI also exceeds the upper acceptance limit- most likely due to the high variability.

To further identify factors that explain the high variability of PK parameters, the applicant performed a Principal Component Analysis (PCA) of clinical PK/PD data.

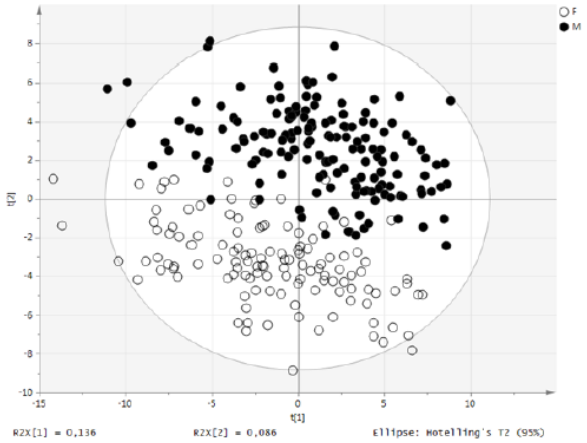
Review of potential root causes contributing to PK difference:

The applicant further investigated the underlying reasons for the observed apparent PK differences. The analyses focused on product quality attributes and subject specific factors that could have contributed to these observations, i.e. on (I) all critical quality attributes, (II) clinical baseline characteristics, (III) study conduct and (IV) immunogenicity results. Cumulatively, the applicant concluded that small differences that do exist between the products based on structural and functional characterization, cannot fully explain the observed PK difference of approximately 10-12% higher exposure with the test product in healthy volunteers. However, further data are requested on the effect of free filgrastim.

A comprehensive review of potential clinical parameters susceptible to have influenced PK parameters was undertaken with also no findings being able to fully explain the observed PK difference. However aspects of the analytical assays (unexpected matrix interferences detected during validation) have not been adequately addressed by the Applicant.

A retrospective Principal Component Analysis (PCA) was performed to explore the potential impact of subject specific covariates collected during the clinical study LA-EP06-101. The re-analysis of the data showed a gender effect and further revealed a shift between cohort 1 and 2 of EU and US-sourced Neulasta arms, which is not observed in the LA- EP 2006-arm.

Figure 2-5 **Gender as the key subject factor in healthy volunteers population in study LA-EP06-101 using PCA**



Closed circles=male subjects; open circles=female subjects; PCA=principal component analysis

Table 2-14 **Bioequivalence assessment of pegfilgrastim PK in male subjects of study LA-EP06-101 (PP population)**

	Test		Reference			
Parameter	**N**	**Adjusted GeoMean (CV%)[a]**	**N**	**Adjusted GeoMean (CV%)[a]**	**Ratio[b]**	**[90% CI]**
LA-EP2006 vs. Neulasta EU						
$AUC_{0\text{-last}}$ (ng×h/mL)	51	6267.45 (82.60)	53	6098.20 (71.00)	102.78	[78.71; 134.20]
C_{max} (ng/mL)	51	172.56 (75.06)	53	177.63 (68.03)	97.14	[72.97; 129.32]
LA-EP2006 vs. Neulasta US						
$AUC_{0\text{-last}}$ (ng×h/mL)	51	6267.45 (82.60)	50	6036.95 (90.05)	103.82	[79.20; 136.09]
C_{max} (ng/mL)	51	172.56 (75.06)	50	173.10 (79.48)	99.69	[74.57; 133.27]
Neulasta EU vs. Neulasta US						
$AUC_{0\text{-last}}$ (ng×h/mL)	53	6098.20 (71.00)	50	6036.95 (90.05)	101.01	[77.26; 132.08]
C_{max} (ng/mL)	53	177.63 (68.03)	50	173.10 (79.48)	102.62	[76.97; 136.82]

$AUC_{0\text{-last}}$=area under the drug-concentration curve measured from the time of dosing to the last measurable concentration; CI=confidence interval; ratios and their margins are given as percentages; CV%=coefficient of variation as percentage; C_{max}=measured maximum serum concentration after administration; GeoMean=geometric mean; N=number of evaluable subjects in a treatment group; Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta; PK=pharmacokinetics; PP population=per protocol population

[a] CV% of the arithmetic mean

[b] Ratios are based on the geometric means.

Table 2-15 Bioequivalence assessment of pegfilgrastim PK in female subjects of study LA-EP06-101 (PP population)

	Test		Reference			
Parameter	N	Adjusted GeoMean (CV%)[a]	N	Adjusted GeoMean (CV%)[a]	Ratio[b]	[90% CI]
LA-EP2006 vs. Neulasta EU						
$AUC_{0\text{-last}}$ (ng×h/mL)	42	5212.04 (97.40)	40	4295.34 (85.64)	121.34	[86.62; 169.99]
C_{max} (ng/mL)	42	141.23 (90.68)	40	129.37 (80.49)	109.17	[76.64; 155.53]
LA-EP2006 vs. Neulasta US						
$AUC_{0\text{-last}}$ (ng×h/mL)	42	5212.04 (97.40)	41	3969.18 (89.06)	131.31	[93.93; 183.57]
C_{max} (ng/mL)	42	141.23 (90.68)	41	116.94 (84.71)	120.78	[84.97; 171.68]
Neulasta EU vs. Neulasta US						
$AUC_{0\text{-last}}$ (ng×h/mL)	40	4295.34 (85.64)	41	3969.18 (89.06)	108.22	[77.09; 151.91]
C_{max} (ng/mL)	40	129.37 (80.49)	41	116.94 (84.71)	110.63	[77.50; 157.93]

$AUC_{0\text{-last}}$=area under the drug-concentration curve measured from the time of dosing to the last measurable concentration; CI=confidence interval; ratios and their margins are given as percentages; CV%=coefficient of variation as percentage; C_{max}=measured maximum serum concentration after administration; GeoMean=geometric mean; N=number of evaluable subjects in a treatment group; Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta; PK=pharmacokinetics; PP population=per protocol population

[a] CV% of the arithmetic mean

[b] Ratios are based on the geometric means.

Several observations were noticed when separate PK bioequivalence analyses were performed for male and female subpopulations:

In the male subgroup the mean ratios for AUC0-last were close to 100%, whereas the 90% CIs remained outside the 80-125% range, explained by the applicant mainly due to the high variability and the smaller number of subjects included in this subgroup analysis.

The ratio of geometric means of AUC0-last between ZIOXTENZO and Neulasta EU differs between men and women: it is 1.03 in male subjects (n=104) and 1.21 in female subjects (n=82).

Results of the female subgroup analysis suggest that the variability of PK response between ZIOXTENZO and Neulasta (EU and US) treatment groups is higher in female subjects as can be seen from the much wider CIs for the geometric mean ratios and the CV% for the arithmetic means (Table 2-15).

In addition, an overall lower systemic drug exposure was noted in female subjects compared to male subjects, although the ANC response was similar across all treatment and gender groups.

The applicant is asked to further elaborate on the gender effect; test the gender-by-group interaction, generate graphical displays of the gender-specific group differences (e.g. using boxplots / interaction plots of log-data) and discuss the plausibility of the observed differences.

The company further investigated the lower PK response in female patients and identified AUC differences between two recruitment cohorts defined by different clinical batch sources of Neulasta US: It became apparent that while the test product and the EU reference product were sourced from a single batch, Neulasta US was sourced from two clinical batches (patients receiving these two different batches are referred to as Cohort 1 and Cohort 2) with a time span of 3 month between release of the second batch during which the study was interrupted.

AUC0-last was compared between the two batches (cohort 1 and cohort 2). A shift was noted in AUC0-last between the two cohorts within the female population receiving Neulasta EU and Neulasta US. No

such difference was observed for the male population and for both genders receiving ZIOXTENZO. It was suggested that the shift towards lower AUC0-last values observed in Cohort 2 of the female subjects receiving both reference products accounted for the overall lower PK exposure in females compared to males, creating an apparent gender effect.

An additional bioequivalence analysis was performed without the female subjects in Cohort 2 (EU or US sourced Neulasta), showing geometric mean ratios of AUC of 98.5 for the test product vs the EU reference product (compared to 109.5 for the PP population), suggesting an impact of this female subgroup on the overall study population. The 90% CI still remained out of the 80%-125% [79.2; 122.5]

It is acknowledged that the applicant evaluates possible sources of PK variability; however, the explanation of failed PK similarity on the basis of differences due to study cohorts (defined by two recruitment periods in which different batches of Neulasta US product were administered) appears questionable. It is not clear whether this can be considered a chance finding due to the investigation of a large number of potentially influencing factors in this data-driven post hoc analysis. Also, it is not comprehensible why Neulasta EU samples have also been excluded and not clear whether cohorts 1 and 2 are comparable with regard to all parameters that could potentially have an impact on PK, or why a possible period or batch effect is only observed in females, but not in males.

3.3.2.2. Study LA-EP06-302

Design:

A randomized, double-blind, parallel-group, active-controlled, multi-center Phase III study in patients with histologically proven breast cancer having an indication for neo-adjuvant or adjuvant treatment with TAC (Taxotere [docetaxel] in combination with Adriamycin [doxorubicin] and Cytoxan [cyclophosphamide]) chemotherapy, eligible to receive six cycles of chemotherapy.

PK is to be evaluated in a PK/ECG subgroup.

Study population of PK-Subset:

The evaluation of PK and triplicate ECG assessment during Cycle 1 of chemotherapy were performed on a subset 58 study patients randomized 1:1 in Neulasta or ZIOXTENZO treatment arm, stratified by chemotherapy category (adjuvant or neo-adjuvant) and weight class (< 65kg; ⩾ 65kg to < 80kg; ⩾ 80kg).

A total of 60 patients were randomised to the PK subgroup.

The ECG/PK subgroup had specific additional cardiac exclusion criteria concerning significant cardiac disease, arrhythmias, QTCF >480 ms or concomitant use of medications known to have effect on any of the above ECG parameters and primary or secondary endpoints.

Treatments:

Patients received ZIOXTENZO or Neulasta EU at a dose of 6 mg/0.6 mL s.c. on day 2 following TAC (Taxotere [docetaxel 75 mg/m2] in combination with Adriamycin [doxorubicin 50 mg/m2] and Cytoxan [cyclophosphamide 500 mg/m2]) chemotherapy on day 1 for up to six cycles.

A full double-masking was technically not possible. The method to keep investigator and patient blinded is considered acceptable.

PD Endpoints (secondary objective within this study):

- PK profile consisting of a pre-dose measurement of PEG-filgrastim serum and daily measurements after the first administration of study drug in cycle 1 of chemotherapy.
- Cmax and AUC0-last of filgrastim concentrations within 24 hours after the first administration of study drug in cycle 1 of chemotherapy.
- Trough concentrations on Day 1 of Cycles 2 to 6.

Sampling time points:

- Cycle 1: PK profile pre-dose on Day 1, on Day 2 and on the following days until Day 15 prior to pegfilgrastim administration, and on Day 1 of the subsequent five cycles.
- Subsequent cycles: Trough concentrations on Day 1 of Cycles 2 to 6.

For Cmax and AUC0-last, the ratio between ZIOXTENZO and Neulasta and a 90% CI were to be calculated. Descriptive statistics were to be determined for Cmax and AUC0-last, the daily pegfilgrastim concentrations in Cycle 1, as well as Ctrough from Cycle 1 to Cycle 6.

Analysis data sets:

PK Analysis (PK) Set: All patients who participated in the PK sub-study with a valid (as defined during the blind data review meeting [BDRM]) PK profile.

Conduct of study:

Patient flow:

60 patients were included (29 ZIOXTENZO vs. 31 Neulasta) in the ECG/PK subset.

58 patients had valid PK profiles, 2 Subjects (both ZIOXTENZO) were excluded from the PK analysis due to major protocol deviations: Subject 916-06 had a delayed drug administration on Day 3, subject 921-04 showed concentrations below LLOQ at all time points.

After data base lock, further protocol deviations were identified, all of which were felt not to have led to exclusion of patients from the PP set.

Pharmacokinetic results:

Table 11-15 Summary of derived PK parameters (PK set)

	$AUC_{0\text{-last}}$ (ng×h/mL)			C_{max} (ng/mL)			t_{max} (h)		
	LA-EP	Neu	Total	LA-EP	Neu	Total	LA-EP	Neu	Total
	N=27	N=31	N=58	N=27	N=31	N=58	N=27	N=31	N=58
Geo. mean	9612.46	7929.51	8672.77	143.58	116.53	128.42	31.91	35.61	33.84
CV%	112.66	110.39	110.96	92.74	113.15	103.48	41.07	52.69	47.45

$AUC_{0\text{-last}}$ = area under the concentration-time curve from zero up to the last concentration ≥ lower limit of quantification; C_{max} = measured maximum serum concentration after administration; CV% = percentage of coefficient of variance; Geo. mean = geometric mean; LA-EP = LA-EP2006; Neu = Neulasta; PK set = pharmacokinetics analysis set; t_{max} = sampling time of C_{max}

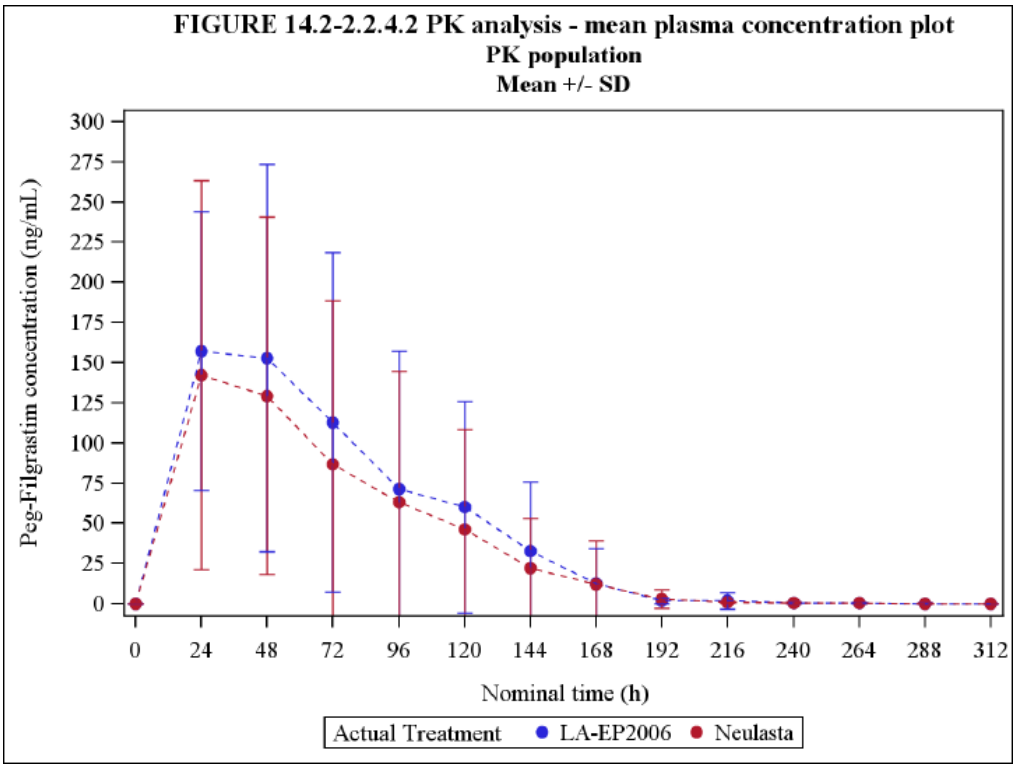
Source: Table 14.2-4.2

Table 11-16 Comparison of PK parameters of LA-EP2006 and Neulasta (PK set)			
Parameter	**Point estimate[a] [%][a]**	**90% CI [%]**	**CV (%)**
$AUC_{0\text{-last}}$ (ng×h/mL)	121.22	[81.62; 180.05]	89.85
C_{max} (ng/mL)	123.22	[84.59; 179.47]	85.42

$AUC_{0\text{-last}}$ = area under the concentration-time curve from zero up to the last concentration ≥ lower limit of quantification; CI = confidence interval; C_{max} = measured maximum serum concentration after administration; CV (%) = coefficient of variance in percent; PK set = pharmacokinetics analysis set

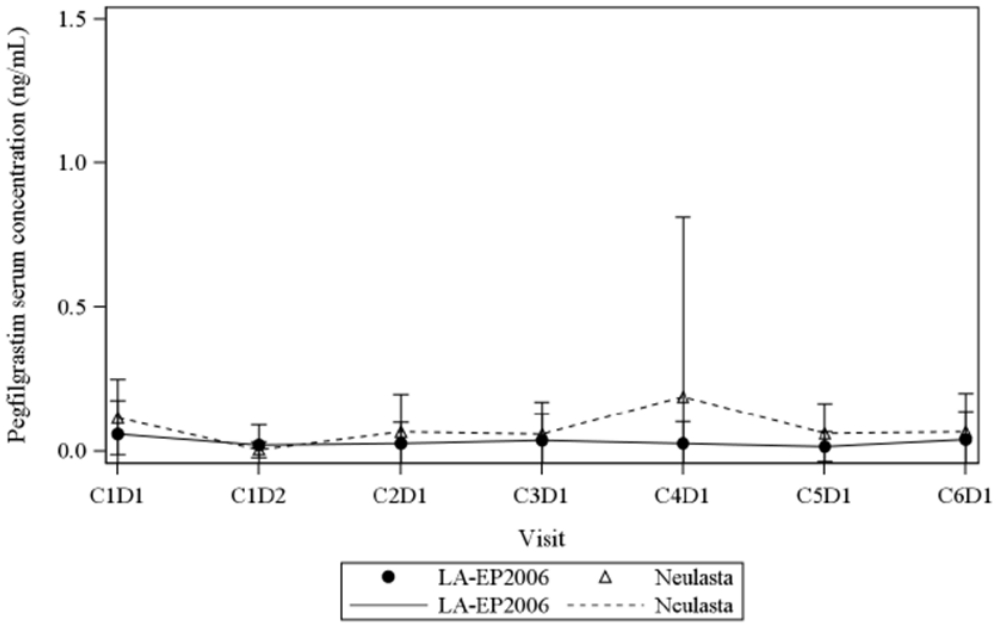
[a] The comparison is based on the geometric means of $AUC_{0\text{-last}}$ and C_{max} and reflects the ratio of LA-EP2006 to Neulasta multiplied by 100.

Source: Table 14.2-4.3



Pegfilgrastim concentrations in Cycle 1 showed similar time-courses, but were numerically higher in patients allocated to ZIOXTENZO than in patients allocated to Neulasta. Tmax were similar for ZIOXTENZO and Neulasta (31.91h and 35.61h respectively). Afterwards, pegfilgrastim concentration slowly declined to approach pre-dose values. The ratio between ZIOXTENZO and Neulasta for AUC0-last was 121.22% with a 90% CI of [81.62%; 180.05%] and the ratio for Cmax was 123.22% with a 90% CI of [84.59%; 179.47%]. 90% CIs were wide due to a small sample size and the high variability in the PK.

Figure 11-4 Mean (+/- SD) pre-dose pegfilgrastim serum concentrations (Cycle 1, Days 1 to 2, to Cycle 6, Day 1) (PK set)



C1D1, C1D2,..., C6D1 = Cycle, 1 Day 1, Cycle 1, Day 2,..., Cycle 6, Day 1; PK set = pharmacokinetics analysis set; SD = standard deviation

Source: Table 14.2-4.1

Pegfilgrastim serum concentrations at the beginning of each chemotherapy cycle were low and comparable between the ZIOXTENZO and Neulasta treatment groups indicating that there was no accumulation of pegfilgrastim.

Conclusion:

The observed variability of ZIOXTENZO and Neulasta EU was very high in the PK subset. Nevertheless, a higher mean exposure of ZIOXTENZO as compared to Neulasta was observed, with the point estimate (121.2%) being consistent with the results seen in (women of) study ZIOXTENZO-101. Furthermore the Ctrough results were influenced by the delivery problems during this study: 10 patients received commercial Pegfilgrastim after cycle 1 in single cycles. 6 patients randomized to LA-EP-2006 were treated with Pegfilgrastim in cycle 2, 1 patient in cycle 3, 1 in cycle 4 and 1 patient in cycle 5.

3.3.3. Pharmacodynamics

Study LA-EP06-101:

This was a randomized, double-blind, three-arm, parallel-group study to determine the pharmacokinetics, pharmacodynamics and safety of ZIOXTENZO and Neulasta (EU- and US registered) following a single s.c. injection in healthy subjects, stratified by body weight and gender, using reference treatment Neulasta EU-registered 6 mg given as s.c. injection, Neulasta US registered 6 mg given as s.c. injection and test treatment ZIOXTENZO as single s.c. injection.

The general study design and methods are presented in section 2.1.3 and not repeated here. Issues especially related to the pharmacodynamics evaluation are discussed below.

Pharmacodynamic endpoints:

- Primary endpoint (co-primary to PK endpoint):
  - AUEC0→last: The area under the effect curve measured from the time of dosing to the last measurable concentration [h • 103/µl]
- Secondary endpoints:
  - ANC Emax: The maximum effect attributable to the IMP [103/µl]
  - ANC tmax,E: The time to the maximum effect attributable to the IMP [h]
  - CD34+ cell response

Pharmacodynamic comparability between ZIOXTENZO and Neulasta will be demonstrated if the 97.5% CIs for the respective ratios of AUEC0→last for ANC are completely contained within the predefined boundary of 87-115%, as an alpha-adjustment was applied to account for the multiplicity of comparing two formulations to the reference product. Furthermore an analysis with a 95% CI was repeated by the applicant, as recommended by the SAWP (EMA/CHMP/BMWP/572828/2011). The acceptance interval of 0.87 to 1.15 was based on the treatment difference between filgrastim and placebo for AUEC (ANC), as described by Borleffs and colleagues (1998) with an observed minimal clinical important difference in ANC response of 15% of the effect observed with filgrastim compared to placebo.

The calculation of the pharmacodynamic parameters was performed based on baseline (pre-dose, Day 1) corrected values of ANC using the actual sampling time. The PD parameters were estimated by non-compartmental analysis. AUEC0→last of the ANC was calculated using the linear trapezoidal method. E_{max} and $t_{max,E}$ of the ANC were directly read from the data. No formal comparison of the CD34+ cell count was performed due to high inter-individual variability in a parallel-group study design.

The selection of the Endpoints is acceptable and according to the Guidance on G-CSF.

Selection of dose:

The applicant argued that the 6 mg dose is positioned at the non-saturating portion of the dose-response curve and provides optimal sensitivity with respect to the ANC response within the range of doses evaluated during the clinical development of pegfilgrastim by the originator (Moulineux et al., 1999; Johnston et al., 2000), see also section 3.4.1.2.1 of this Overview.

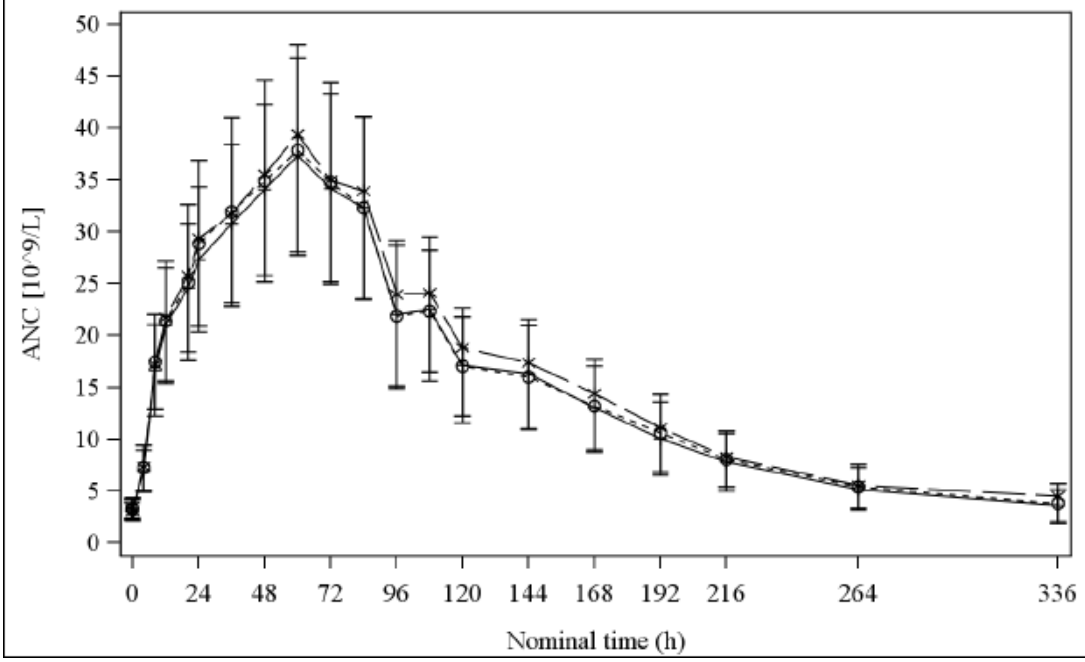
Nevertheless current literature is inconclusive concerning the question whether the used standard dose (6mg) is most sensitive (i.e. in the steep part of the dose response curve) to detect differences in PD. The guidance on similar medicinal products containing rG-CSF states that studies at more than one dose level may be useful" (EMEA/CHMP/BMWP/31329/2005).

The PD results have to be assessed bearing in mind that, although ANC being a widely accepted PD surrogate efficacy endpoint, the sensitivity to show potential differences between test and reference product at the investigated dose might be reduced compared to PK.

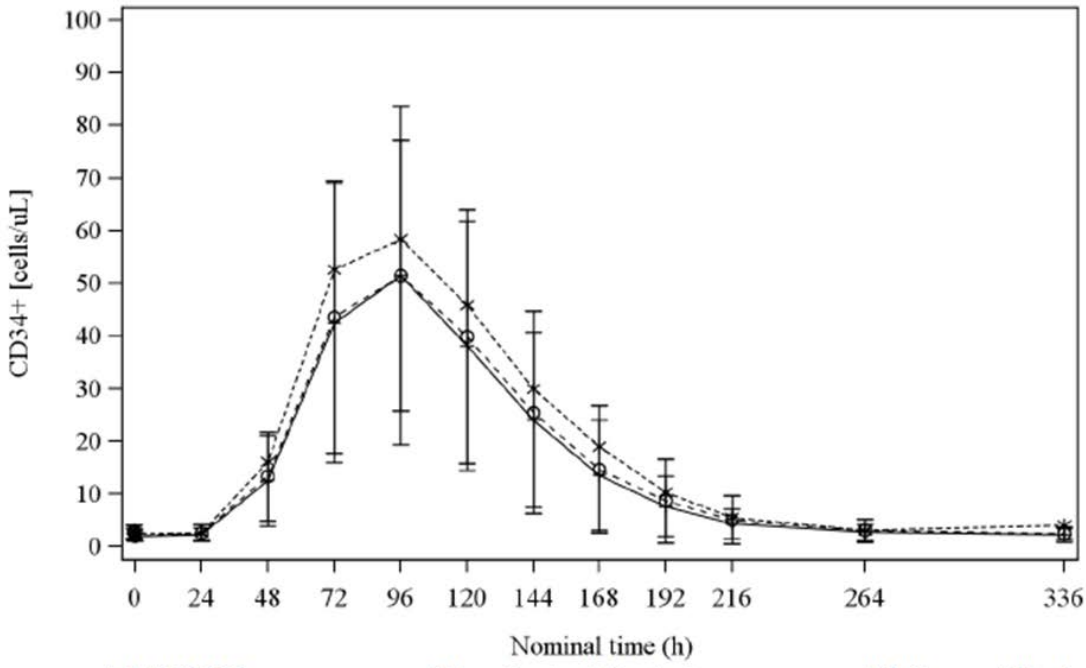
Pharmacodynamic results:

Figure 2-4 Mean (+/- SD) response curves of ANC (a) and CD34+ (b) – study LA-EP06-101) (PP population)

a)



b)



+–+–+ =LA-EP2006; o – – o – – o =EU-authorized Neulasta; * - - * - - * =US-licensed Neulasta; ANC=absolute neutrophil count; CD34+=cluster of differentiation 34 positive; PP=per-protocol; SD=standard deviation

Source: [Module 5.3.4.1 LA-EP06-101-Figure 11-4], [Module 5.3.4.1 LA-EP06-101-Figure 11-5], modified by adding SD

Table 2-12	Summary of ANC derived PD parameters – study LA-EP06-101 (PP population)		
	LA–EP2006 (n=93)	**Neulasta EU (n=93)**	**Neulasta US (n=91)**
$AUEC_{0\text{-last}}$ ($h \times 10^9/L$)			
Mean (SD, CV%)	5124.37 (1015.70, 19.8)	5220.76 (1212.75, 23.2)	5458.28 (1446.71, 26.5)
Median (range)	5077.91 (3282.36–8900.78)	5038.04 (3041.53–8863.98)	5400.82 (2558.56–8595.78)
GeoMean	5028.25	5090.53	5265.27
E_{max} ($10^9/L$)			
Mean (SD, CV%)	39.01 (9.05, 23.2)	38.89 (10.02, 25.8)	40.06 (10.30, 25.7)
Median (range)	38.90 (20.90–66.60)	37.50 (22.90–73.00)	39.20 (18.10–72.40)
GeoMean	37.93	37.71	38.78
$t_{max,E}$ (h)			
Mean (SD, CV%)	n.a.	n.a.	n.a.
Median (range)	59.81 (35.99–107.33)	59.78 (35.97–108.16)	59.82 (23.94–95.80)
GeoMean	n.a.	n.a.	n.a.

CV%=coefficient of variation as percentage; $AUEC_{0\text{-last}}$=area under the effect curve measured from the time of dosing to the last measurable concentration; E_{max}=maximum effect attributable to the study drug; n=number of subjects; n.a.=not applicable; Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta; PD=pharmacodynamics; PP population=per protocol population; SD=standard deviation; $t_{max,E}$=time point of E_{max}

Source: [Module 5.3.4.1 LA-EP06-101-Table 11-3]

Comparability of ZIOXTENZO with the reference product Neulasta was demonstrated for the primary PD endpoint ANC AUEC0-last and as well as for the secondary endpoint ANC Emax and ANC tmax,E.

The comparison of the geometric means of AUEC0→last between ZIOXTENZO and Neulasta EU resulted in a ratio of 100.75%, whereas the ratio of this parameter was 98.59% between ZIOXTENZO and Neulasta US.

The ratio of the geometric means of Emax was 102.31 if ZIOXTENZO was compared Neulasta EU. The ratios of AUEC0→last and Emax adjusted for weight class and gender did not show remarkable deviations in relation to the unadjusted values.

For both comparisons and both parameters the lower and upper bounds of the 95% CIs ranged approximately between 94% and 110% and were contained within the proposed ranges of acceptance (i.e. 87–115%) for PD biosimilarity.

PD results in relation to observed gender difference:

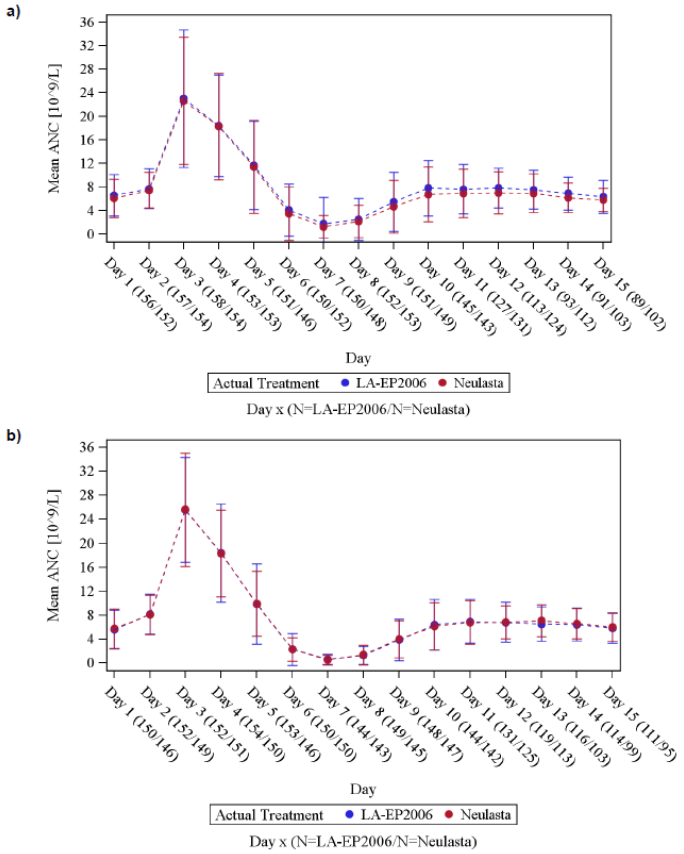
Following the potential gender difference postulated to account for the PK differences, PD comparability analyses were performed separate for the male and female PP population. All ANC PD parameters were within the proposed range of acceptance (87% - 115%) of the 97.5% CIs.

Clinical efficacy trials LA-EP06-301 & 302

ANC time course

The entire time course of the mean ANC during Cycle 1 indicates almost superimposable ANC profiles for ZIOXTENZO and EU Neulasta with early ANC increase at Day 3 to > 20 × 10^9/L, followed by a subsequent decrease with nadir at Day 7 and subsequent recovery and stable values from Day 10 on (see Figure 2-3 below).

Figure 2-3 **Time course of arithmetic mean (+/- SD) of ANC count – studies LA-EP06-301 and LA-EP06-302 (FAS set)**



a) Study LA-EP06-301; b) Study LA-EP06-302
FAS set=full analysis set; SD=standard deviation
Source: [Module 5.3.5.1 LA-EP06-301-Figure 11-2], [Module 5.3.5.1 LA-EP06-302-Figure 11-2], modified

As biosimilarity of ZIOXTENZO to the reference product Neulasta could be concluded for pharmacodynamics, the observed higher PK levels (AUC and Cmax) of ZIOXTENZO are not reflected in the PD results. In this respect is has to be considered that pharmacodynamic endpoints might be less sensitive to detect differences between the study drugs compared to pharmacokinetic endpoints in the case of pegfilgrastim.

Exploratory analysis of ANC derived PD endpoint

PD equivalence could be shown for both studies, using the proposed margin for the pivotal PK/PD study (87% - 115%) for the area under the ANC effect curve ($AUEC_{0\text{-last}}$) during Cycle 1.

Table 2-13 **$AUEC_{0\text{-last}}$: comparison of treatment arms – studies LA-EP06-301 and LA-EP06-302 (FAS set)**

	Adjusted GeoMean (ng×h/mL)		Point estimate [a] [%]	95% CI [LL, UL]	CV%
	LA-EP2006	Neulasta EU			
LA-EP06-301	94.43	91.45	103.26	[92.93, 114.72]	47.11
LA-EP06-302	92.78	90.45	102.58	[94.89, 110.89]	34.50

ANC=absolute neutrophil count; $AUEC_{0\text{-last}}$= area under the ANC effect curve, calculated from time of investigational medicinal product administration to last scheduled blood sample during Cycle 1; CI=confidence interval; CV%=coefficient of variation as percentage; GeoMean=geometric mean; LL, UL=lower limit, upper limit; Neulasta EU=EU-authorized Neulasta
[a] The comparison is based on the geometric means of $AUEC_{0\text{-last}}$ and reflects the ratio of LA-EP2006 to Neulasta multiplied by 100.
Source: [Module 5.3.5.3 Statistical Overview-Tables 6.2.2.1

3.3.4. Discussion on clinical pharmacology

Two studies investigated the clinical pharmacology of ZIOXTENZO. One study was a randomized, three-arm parallel group PK/PD (study LA-EP06-101) in 279 healthy volunteers, using Neulasta EU and Neulasta US as active comparators. The other study was an exploratory PK sub-study of the confirmatory efficacy and safety study LA-EP06-302, where 58 evaluable patients with breast cancer were included.

Pharmacokinetics:

The pharmacokinetics of pegylated filgrastim is complex and differs between healthy subjects and the target population of oncology patients with chemotherapy induced neutropenia.

Pegfilgrastim shows a non-linear, higher than dose proportional pharmacokinetics (Neulasta EPAR; Roskos et al, 2006; Yang and Kido, 2011) and receptor-mediated, feedback regulated clearance by neutrophils (pharmacodynamics-mediated drug disposition). Roskos (J Clin Pharmacol, 2006) investigated the PK of 30-300 µg/kg pegfilgrastim in healthy volunteers. A 10-fold increase in the dose resulted in an approximately 25-fold increase in Cmax and approximately 75-fold increase in AUC when regarding the full range of 30-300 µg/kg and the differences in AUC and Cmax between the 60 µg/kg and 100 µg/kg are reported to be approximately 4- and 3-fold, respectively. This non-linearity makes pharmacokinetic parameters very sensitive to small differences.

The rate of serum clearance of pegfilgrastim decreased with increasing dose, which is attributed to saturation of the neutrophil-mediated clearance pathway. Terminal t1/2 was independent of dose, suggesting that the concentration at the terminal phase fell below levels saturating G-CSF receptors. Beside the receptor-mediated clearance, renal clearance is nearly negligible in healthy volunteers with normal neutrophil counts. Based on the above data, the 6 mg dose lies in the steep part of the dose-concentration curve and is therefore appropriate.

The choice of a flat dose instead of a dose adjusted to bodyweight is counterintuitive when trying to reduce inter-subject variability and could have been discussed. However, with weight-based doses, average pegfilgrastim concentrations were found to be higher in subjects with higher bodyweights than in those with lower bodyweights. A possible explanation for this observation was that patients with higher bodyweight had a lower volume of distribution relative to their bodyweight. Proteins of the size of pegfilgrastim distribute to the extracellular water space, and the extracellular water volume does not increase proportionally with bodyweight, especially in overweight or obese subjects. Therefore, it was hypothesized that a fixed dose, in addition to simplifying treatment, would also provide consistent exposure to pegfilgrastim. If this is the case, the choice of a flat dose would be justified.

Nevertheless, the Applicant has not described the impact of bodyweight on the PK profile of a flat dose of pegfilgrastim. A representation of Cmax and AUC by bodyweight tertile and treatment should be provided and commented.

The choice of population and design for the pivotal PK trial LA-EP06-101 is endorsed. The reasoning for the chosen parallel group design for the pivotal PK trial is acknowledged: long half-life, period effects and complexity to attribute potential immunogenicity results to a specific compound. On one hand, given the known high inter-individual variability observed for pegfilgrastim, a cross-over design would have been preferable. On the other hand, a three-arm cross-over trial, although feasible, would have been more difficult to conduct; a high number of non-completers would have been likely given the required trial duration, the action of pegfilgrastim being much longer than its presence in blood. Since a period effect is known (return to baseline of the bone marrow does not occur during repeated administrations), there would be a risk of unbalanced randomisation sequences in the completers.

However, in a parallel design, the most homogeneous population would have been preferred to decrease known inter-subject PK variability of pegfilgrastim, even in the absence of evidence of a gender effect. Nevertheless it should be noted that for another pegfilgrastim, bioavailability was shown to be higher in male than in female subjects (EPAR lipegfilgrastim).

The study was adequately powered to demonstrate PK equivalence of ZIOXTENZO and both Neulasta compounds, based on an inter-subject variability assumption of 35% CV. However, this assumption proved to be incorrect as the actual CVs observed in the trial ranged between 73-89% for the test and EU reference product, and therefore, the study was underpowered.

The primary PK parameter was AUC0-last (drug concentration measured from the time of dosing to the last measurable concentration) with a co-primary PD endpoint of AUEC0-last (effect curve measured from the time of dosing to the last measurable concentration). Secondary PK endpoints included Cmax and AUC to infinite; secondary PD endpoints included Emax as well as development of CD34+ cell count following IMP administration.

A more stringent confidence interval of 95% was predefined for the PK comparison with the similarity margin being the standard bioequivalence margin of 80-125%, because ZIOXTENZO was compared to both the EU and US reference products independently. From the perspective of the EU assessment it is sufficient to use 90% CIs, respectively 95% CIs for PD equivalence, as there is no multiplicity caused by the presence of the US reference product in the trial; there is only one chance for the study to be positive from an EU perspective as a positive result against the US reference could not rescue negative results against the EU reference.

Comparability between ZIOXTENZO and Neulasta could neither be concluded for the primary PK endpoint AUC0-tlast nor for the secondary endpoints AUC0-inf and Cmax in study LA-EP06-101 as the 90% CIs for key PK parameters AUC0-last and Cmax were not contained within the standard equivalence margin of 80% - 125%.

Regarding the primary endpoint AUC0-t, the point estimate of the geometric mean ratio (ZIOXTENZO vs. Neulasta) was 109.95% with a 90% confidence interval of 88.90 – 135.98%.

Also Cmax and AUC0-∞ exceeded the pre-determined acceptance limits: 101.71% (81.24 – 127.32%) and 110.54% (90% CI: not presented, the 95% CI was reported with 85.81-142.41) respectively. In most subjects the pegfilgrastim concentration was below LLOQ at the last sampling time point at day 15, therefore mean AUC0→∞ values were only slightly different to the mean values of AUC0→last.

The median tmax ranged between 16 and 24 hours among all three pegfilgrastim products with ZIOXTENZO showing the highest value. The elimination rate constant (kel) and the apparent terminal half-life of elimination phase (t1/2) showed no substantial differences between treatments.

It should be noted that formally PK similarity could not be concluded for EU Neulasta compared to US Neulasta, as the upper limit of the 90% CI exceeded the upper acceptance limit of 125% for AUC. But despite the high variability, the mean AUC difference between US-licensed Neulasta and EU-approved Neulasta was only 5% (compared to 10% between ZIOXTENZO and EU-Neulasta); for Cmax, the difference was larger (7%) between US- and EU-Neulasta than between ZIOXTENZO and EU-Neulasta (2%).

To determine whether there is a molecular difference between the study drugs or difference regarding clinical parameters that could account for the observed differences in PK, the applicant performed a thorough root cause analysis of critical quality attributes, clinical baseline characteristics, study conduct and immunogenicity results: Only non-PEGylated filgrastim showed discernible characteristics or differences that may be attributed to the observations seen in the nonclinical and clinical settings.

Therefore the PK/PD physiological model of pegfilgrastim (Roskos et al, 2006) was re-produced by the applicant. The simulations revealed that the induction of small (3%) differences in systemic exposures (Cmax, AUC) is possible, when accounting for contribution of free filgrastim (0.1 to 0.3%) seen in marketed EU-authorized and US-licensed Neulasta drug products, but not in ZIOXTENZO. However, the applicant concluded that the magnitude of this particular effect was considered to be too small to account alone for the differences observed in clinical studies and some animal studies. Furthermore, no clinical factors (e.g. immunogenicity) were identified to explain the differences.

Cumulatively, the evaluation revealed that small differences that do exist between the products based on structural and functional characterization, cannot account for the observed differences in PK.

A post-hoc multivariate analysis (PCA) was performed to retrospectively characterize the PK data of study LA-EP06-101 in more detail.

This analysis seemed to indicate that gender translates into an additional subject specific covariate influencing PK variability and exposure for all treatment arms. Differences were hypothesised to be driven by the high variability of response in the female population, in particular a shift towards a lower AUC, even for the EU reference product, in a subgroup of female subjects after release of a second batch for the US reference product (Cohort 2). In this multivariate analysis (PCA) 200 subject-specific covariates were considered. The applicant is asked to further analyse, which of these variables cause the gender-specific difference. The dependency of the PK parameters from these factors should be described and explained. It should be further assessed whether the distribution of these factors differs between treatment arms (however, substantial differences cannot be expected due to randomisation).

Existing evidence indicates that mainly weight and ANC count appear to be covariates influencing the PKs of pegfilgrastim although a similar gender effect (higher exposure in males than females) has previously been described with another pegylated filgrastim (lipegfilgrastim, EMA EPAR). It is not clear, if one of the covariates, possibly leading to the observed PK difference included the baseline absolute neutrophil count. It should be discussed by the applicant, if relevant differences in baseline absolute neutrophil count between the treatment arms exist, and to which extend baseline ANC and bodyweight could influence the exposure, since the elimination of pegfilgrastim is predominantly neutrophil-mediated.

Up to date no gender-related differences were observed in the pharmacokinetics of Neulasta (Product monograph Neulasta, 2015). The applicant is asked to further elaborate on the gender effect; test the gender-by-treatment group interaction, generate graphical displays of the gender-specific group differences (e.g. using boxplots / interaction plots of log-data) and discuss the plausibility of the observed differences.

The explanation of failed PK similarity on the basis of systematic differences due to study cohorts appears questionable. It is not clear whether this can be considered a chance finding due to the investigation of a large number of potentially influencing factors in this data-driven post hoc analysis.

It is noteworthy that high inter-subject variability was observed for all three products with CV% ranging between 77.1% and 91.5%, which was much higher than assumed for the calculation of trial samples (CV 35%). Underestimation of the variability of the estimate and preference of a parallel-group design might be one reason that comparability of exposure (as measured by AUC0-last) could not be demonstrated. Importantly, the EU and US Neulasta products were not shown to be bioequivalent either.

However, there is some evidence that the exposure is consistently larger in ZIOXTENZO than in Neulasta treated subjects:

In study LA-EP06-101 the point estimate of geometric mean ratio of AUC was 1.10.

Also in the PK subset of study ZIOXTENZO-302, exposure to pegfilgrastim in terms of AUC0-last and Cmax was higher for ZIOXTENZO as compared to Neulasta: The point estimate was 121.22 (90% CI: 81.62-180.05) and 123.22 (90%CI: 84.59-179.47) for AUC0-tlast and Cmax respectively. Again, the pegfilgrastim serum concentrations showed high variability of CV between 89.5% and 116.5%.

The outcome for exposure in non-clinical studies was variable and less uniform, with values for area under the serum concentration curve (AUC) and maximum serum concentration (Cmax) for ZIOXTENZO being higher compared to Neulasta EU in some animal studies while demonstrating similar or lower exposure in other animal studies.

Regarding physiochemical characterisation, ZIOXTENZO showed slight differences compared to Neulasta in several quality attributes with a potential impact on PK.

Although no single causative factor could be detected in the physiochemical analysis, minor product differences may cumulatively translate into distinct PK differences, taken into account the higher than dose proportional pharmacokinetics of pegfilgrastim.

Pharmacodynamics:

Comparability of ZIOXTENZO with the reference product Neulasta was demonstrated for the primary PD endpoint ANC AUEC0-last and as well as for the secondary endpoint ANC Emax and ANC tmax,E. The point estimates as well as the 95% CI were well within the predefined acceptance range of 87-115%. The medians for tmax,E were approximately 60 hours for all three pegfilgrastim products.

The PD response of pegfilgrastim seems to be lower than dose proportional. A study of Neulasta in healthy volunteers showed that a ten-fold increase in the dose (from 30 µg/kg to 300 µg/kg) results approximately in a doubling of ANC (Cmax: from 30 to 51 $x10^6$/mL and AUC: from 101 to 223 $x10^6$ day/mL) with intermediate results at 100 µg/kg: 37 and 141, respectively. The applicant argued that the 6 mg dose is positioned at the non-saturating portion of the dose-response curve and provides optimal sensitivity with respect to the ANC response within the range of doses evaluated during the clinical development of pegfilgrastim by the originator (Moulineux et al., 1999; Johnston et al., 2000).

According to the guidance on similar medicinal products containing rG-CSF *"The selected dose should be in the linear ascending part of the dose-response curve. Studies at more than one dose level may be useful"* (EMEA/CHMP/BMWP/31329/2005).

The PD results are assessed bearing in mind that, although ANC being a widely accepted PD surrogate efficacy endpoint, the sensitivity to show potential differences between test and reference product might be lower compared to pharmacokinetics.

3.3.5. Conclusions on clinical pharmacology

From a PK perspective, two issues have been identified.

• Zioxtenzo failed to demonstrate biosimilarity in respect to pharmacokinetic results. Comparability between ZIOXTENZO and Neulasta could neither be concluded for the primary endpoint AUC0-tlast, nor for the secondary endpoints AUC0-inf and Cmax in the PK/PD study LA-EP06-101. The 90%CIs of the AUCs and Cmax ratios were not contained within the standard equivalence interval but the study was clearly underpowered given that the assumed inter-subject variability used to calculate the sample size (CV 35%) was much lower than the observed variability (CV 73-89% for the test and EU reference

product). Moreover, the study also failed to demonstrate bioequivalence of the EU and US reference products whereas these are highly similar at the quality level as would be expected.

• The exposure to pegfilgrastim was found to be higher with ZIOXTENZO than EU Neulasta in healthy subjects (up to 36%). This picture remained unchanged after the calculation was adjusted for weight class and gender.

• The observation of a larger exposure observed in the healthy volunteers study was replicated in a very limited subgroup of female breast cancer patients (up to 80% higher AUC) on the basis of the upper limit of the respective 90% confidence intervals (point estimate of the geometric mean ratio in $AUC_{0\text{-last}}$ was around 1.2).

The applicant performed a thorough root cause analysis and concluded that small observed differences in structural and functional characterization cannot account for the observed differences in PK. Although the variability turned out to be substantially larger than in the stage of study planning, the exposure seems to be larger in ZIOXTENZO treated subjects. This might also be enhanced by the supra-proportional PK of pegfilgrastim.

The difference between exposure to ZIOXTENZO as compared to Neulasta EU appears to depend on gender. However, this heterogeneity has to be further discussed by the applicant (OC). Also, since a large number of potential PK influencing factors were investigated in a post-hoc manner, the credibility of the presented subgroup findings (with regard to gender and production batch cohort) is questionable.

However, despite PK data suggesting differences in exposure, comparable PD response was demonstrated in healthy volunteers as well as in neutropenic patients. In view of available PK/PD modelling from the literature (Yang, 2011), this finding could be expected given that, at the level of pegfilgrastim concentrations achieved with a 6 mg dose, a very large difference in drug concentration would be needed to translate into a noticeable difference in ANC response. The 6 mg dose produces concentrations around EC90, and at this level, the PD response increases only slightly over a large range of pegfilgrastim concentrations. An increase in dose from 60 to 100 µg/kg (roughly equivalent to a flat 6 mg dose), which would result in a 3-fold increase in the median pegfilgrastim average concentration in patients with breast cancer, would only lead to a 12% increase (from 82% to 94%) in the response. Of note, this finding would support the relevance of a PK/PD trial at a lower dose (as recommended in the CHMP Scientific Advice), in the steep part of the concentration-response curve, where a small difference in concentration would be expected to translate into a notable difference in the ANC response.

PK results did not translate into PD response of study LA-EP06-101, as biosimilarity could be demonstrated for ANC, supported by the results of the two efficacy studies and comparative safety and immunogenicity.

The Applicant claims biosimilarity based on the totality of data, in particular as the PK differences did not translate into PD differences.

The absolute neutrophil count (ANC) is a relevant pharmacodynamic marker for the activity of r-GCSF (GL). ANC-based PD parameters are seen as acceptable primary endpoints to confirm clinical biosimilarity, but also a similar PK profile, including 90 % CI within pre-specified acceptance range, is considered essential to demonstrate and claim biosimilarity.

3.3.6. Clinical efficacy

Dose-response studies and main clinical studies

Summary of main efficacy results

Clinical efficacy and safety of ZIOXTENZO were compared with that of Neulasta EU in two independent double-blind, randomized, parallel-group, multi-center studies of nearly identical design. Studies [LA-EP06-301] and [LA-EP06-302] were both designed to assess equivalence and non-inferiority of ZIOXTENZO to Neulasta EU in 302 female patients with breast cancer treated with myelosuppressive chemotherapy (TAC).

The major features of these studies are summarized in Table 1-1.

- Studies LA-EP06-301 and LA-EP06-302 were designed to be adequate and well-controlled:
- the study objectives and method of analysis were stated in the protocol and study report
- the study design permitted valid comparison with a control situation
- the method of patient selection adequately assured they had the condition being studied
- treatment assignment minimized bias to assure comparability of treatment groups
- adequate measures were taken to minimize bias by subjects, observers and data analysts
- the measures used to assess the subject's response were well-defined and reliable
- the analysis of study results was adequate to assess the effects of the drug

Those two trials represent sufficiently large entities to compare the efficacy of ZIOXTENZO and Neulasta EU in a meaningful way. It could be discussed whether a second, replicate design trial really adds valuable information to the comparability exercise since the EMA regulatory framework for biosimilars does not request this.

Table 1-1 Summary of controlled confirmatory efficacy and safety studies

Study No.	Study objective	Study population	Treatment duration	Dosage [batch number]	Efficacy endpoints
LA-EP06-301	Efficacy and safety of LA-EP2006 compared to Neulasta EU with respect to the mean DSN, defined as the number of consecutive days with grade 4 neutropenia (ANC < 0.5 × 10^9/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen in patients with breast cancer	Patients with breast cancer Total: N=316f LA-EP2006: n=159f Neulasta EU: n=157f	44 weeks (18 weeks plus a 6-month safety follow-up)	LA-EP2006: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [30324244, 30324245] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [10271811A, 1028053D, 1033639A]	Primary endpoint: • (1) Equivalence and (2) non-inferiority of LA-EP2006 and Neulasta in terms of mean DSN in Cycle 1 Secondary efficacy endpoints: • Incidence of FN • Number of patients with at least one fever episode • Depth of ANC nadir in Cycle 1 • Time to ANC recovery in Cycle 1 • Frequency of infection • Mortality due to infection
LA-EP06-302	Efficacy and safety of LA-EP2006 compared to Neulasta EU with respect to the mean DSN, defined as the number of consecutive days with grade 4 neutropenia (ANC < 0.5 × 10^9/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen in patients with breast cancer	Patients with breast cancer Total: N=308f LA-EP2006: n=155f Neulasta EU: n=153f	22 weeks (18 weeks plus a 4-week follow-up)	LA-EP2006: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [30324244, 30324245] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [1022627/ 1022626, 1028053D, 1033639A]	Primary endpoint: • (1) Equivalence and (2) non-inferiority of LA-EP2006 and Neulasta in terms of mean DSN in Cycle 1 Secondary efficacy endpoints: • Incidence of FN • Number of patients with at least one fever episode • Depth of ANC nadir in Cycle 1 • Time to ANC recovery in Cycle 1 • Frequency of infection • Mortality due to infection

ANC=absolute neutrophil count; DSN=duration of severe neutropenia; f=female; FN=febrile neutropenia; n=number of patients in a treatment group; N=number of randomized patients; Neulasta EU=EU-authorized Neulasta; PFS=pre-filled syringe; s.c.=subcutaneous; TAC=Taxotere® (docetaxel 75 mg/m^2) in combination with Adriamycin® (doxorubicin 50 mg/m^2) and Cytoxan® (cyclophosphamide 500 mg/m^2)

Source: [Module 5.3.5.1 LA-EP06-301], [Module 5.3.5.1 LA-EP06-302]

The Applicant states that due to transient shortages of IMP in the two studies, 70 patients in study LA-EP06-301 (ZIOXTENZO: 28 patients; Neulasta EU: 42 patients) and 73 patients in study LA-EP06-302 (ZIOXTENZO: 32 patients; Neulasta EU: 41 patients) received one or more administrations of commercial G-CSF containing product instead of IMP. In case this happened in treatment cycle 1 (before the primary endpoint) this was considered a major protocol deviations and patients were excluded from the PP set.

Table 2-2 Analysis patient sets – studies LA-EP06-301 and LA-EP06-302 (all randomized patients)

	LA-EP06-301			LA-EP06-302		
	LA-EP2006	Neulasta EU	Total	LA-EP2006	Neulasta EU	Total
	N=159	N=157	N=316	N=155	N=153	N=308
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
FAS set	159 (100.0)	157 (100.0)	316 (100.0)	155 (100.0)	153 (100.0)	308 (100.0)
PP set	146 (91.8)	149 (94.9)	295 (93.4)	148 (95.5)	144 94.1)	292 (94.8)
FAS-C set	130 (81.8)	116 (73.9)	246 (77.8)	123 (79.4)	112 (73.2)	235 (76.3)

FAS set=full analysis set; FAS-C set=a subset of patients of the FAS set who received only assigned investigational medicinal product throughout the study; n=number of patients in an analysis set; N=number of randomized patients; Neulasta EU=EU-authorized Neulasta; PP set=per protocol set

3.3.7. Recruitment

In both studies, the study population consisted of women of 18 years or older with histologically proven breast cancer who were eligible for six cycles of neo-adjuvant or adjuvant treatment with TAC chemotherapy. Each study aimed to randomize a total of 302 patients (to be treated with either ZIOXTENZO or Neulasta in a 1:1 ratio) in approximately 65 sites worldwide. This goal was slightly superseded in both trials (316 and 308, respectively).

Inclusion criteria:

The patients had to fulfil all of the following inclusion criteria to be eligible for admission to the study:

1. Written informed consent before any assessment was performed
2. Patients with histologically proven breast cancer, eligible for neo-adjuvant or adjuvant TAC chemotherapy
3. Women ⩾ 18 years of age
4. Estimated life expectancy of more than six months
5. Eastern cooperative oncology group (ECOG) performance status ⩽ 2
6. Adequate bone marrow function on Cycle 1 Day 1 prior to chemotherapy administration:
   - ANC ⩾ 1.5 × 109/L
   - Platelet count ⩾ 100 × 109/L
   - Hemoglobin ⩾ 10 g/dL
7. Total bilirubin not higher than the upper limit of normal (ULN), unless the patient had Gilbert's syndrome
8. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level ⩽ 2 × ULN
9. Liver-derived alkaline phosphatase level ⩽ 3 × ULN
10. Creatinine ⩽ 1.5 × ULN
11. For all women of childbearing potential: negative serum pregnancy test within seven days prior to randomization, and using a highly effective method of birth control.

During the screening period of the study, the eligibility of the patients to participate in the study was assessed based on safety evaluations

Exclusion criteria

General exclusion criteria

Patients meeting any of the following exclusion criteria were not to be enrolled in the study:

1. History of chronic myeloid leukemia or myelodysplastic syndrome
2. History or presence of sickle cell disease
3. Previous or concurrent malignancy except non-invasive non-melanomatous skin cancer, in situ carcinoma of the cervix, or other solid tumor treated curatively, and without evidence of recurrence for at least ten years prior to study entry
4. Any serious illness or medical condition that may have interfered with safety, compliance, response to the products under investigation or chemotherapy and their evaluation

The inclusion and exclusion criteria were considered acceptable.

Objectives

Primary objective(s)

The primary objective of both studies was to assess the efficacy of ZIOXTENZO compared to Neulasta (EU-authorized) with respect to the mean duration of severe neutropenia (DSN), defined as the number of consecutive days with Grade 4 neutropenia (absolute neutrophil count [ANC] less than 0.5 × 109/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen (Taxotere® [docetaxel 75 mg/m2] in combination with Adriamycin® [doxorubicin 50 mg/m2] and Cytoxan® [cyclophosphamide 500 mg/m2]) in breast cancer patients.

Secondary objectives:

The secondary objectives were to further compare ZIOXTENZO and Neulasta with respect to the efficacy, safety, and immunogenicity of both products. The following assessments were performed:

Efficacy assessments

• Incidence of febrile neutropenia (FN), defined as oral temperature ⩾ 38.3C while having an ANC < 0.5 × 10^9/L (both measured on the same day) by cycle and across all cycles

• Number of days of fever, defined as oral temperature ⩾ 38.3 [illegible] (The analysis of this efficacy assessment was modified.)

• Depth of ANC nadir, defined as the patient's lowest ANC in Cycle 1

• Time to ANC recovery, defined as the time in days from the chemotherapy administration until the patient`s ANC increased to ⩾ 2 × 10^9/L after the nadir, in Cycle 1

• Frequency of infection by cycle and across all cycles

• Mortality due to infection

The choice of primary and secondary efficacy measures can be accepted and seem to cover relevant outcomes to characterize biosimilarity in terms of clinical efficacy. Also the guidance outlined in EMEA/CHMP/BMWP/31329/2005 (biosimilar r-gcsf guideline) has been followed in the definition of the primary endpoint (mean duration of severe neutropenia (DSN)), defined as the number of consecutive

days with Grade 4 neutropenia (ANC below 0.5 x 109/l)). The comparison was to be established in a hierarchical way: ZIOXTENZO is equivalent (margin: ±1 day) to Neulasta with respect to DSN in Cycle 1 and, if this was successfully established, that ZIOXTENZO is non-inferior (margin: -0.6 days) to Neulasta with respect to DSN in Cycle 1. One secondary endpoint "days of fever" was retrospectively adapted since the assessment of a fever episode contained a self-reported component, probably resulting in underreporting. Hence, it was decided to modify the endpoint "number of days of fever" to "number of patients with at-least one fever episode", which is in general acceptable, however information might have been lost (see list of OC)

Sample size

Sample size considerations were identical for study LA-EP06-301 and LA-EP06-302. The following assumptions were made for the sample size determination based on available literature [Holmes et al 2002; Green et al 2003]:

- Equivalence/Non-inferiority limit: ±1 day /-0.6 days
- Expected difference in the means: 0 days
- Common standard deviation: 1.6 day
- one-sided significance level: 2.5%
- Randomization ratio: 1:1 (ZIOXTENZO:Neulasta®)

Then, 302 evaluable subjects were deemed sufficient to achieve at least 90% power for each set of hypothesis tests, i.e. for testing equivalence with respect to a margin of ± 1 day using a two-group t-test (TOST) procedure for equivalence in means where each test is made at the 2.5% level as well as for the two-group 2.5% one-sided t-test to assess non-inferiority with respect to a margin of -0.6 days. Since the primary analysis was planned to be based on the ITT population (all randomized patients), no additional patients were planned to be recruited.

Rationale and calculations of the sample size are comprehensible. As regards equivalence testing alone (which is considered sufficient for EU registration), the resulting power values are above 99%. Furthermore, the observed standard deviation (SD) in the studies (SD was around 0.9 and 1.1 days in study 301 and 302 respectively) was clearly smaller than the assumed SD of 1.6 days. Therefore, the confidence interval of the group difference is expected to be narrow and to fall clearly in the ±1 day interval (in case the true difference between groups is close to 0). On the other hand, analysis based on both the FAS and PPS are considered equally important in equivalence trials. The equivalence margin has been accepted in the scientific advice letter from 2009. Overall, sample size considerations are acceptable.

Randomisation

Patients who had given written informed consent, who were eligible for study entry, and who received full doses of chemotherapy regimen on Day 1 were randomly allocated to the treatment arms on Day 2 using an interactive voice response system/interactive web-based randomization system. Randomization was stratified by chemotherapy category (adjuvant or neoadjuvant) and region (study 301: Europe/Asia/America, study 302: US/Asia/rest of world).

The randomization procedure is appropriate. In study 301 the stratification factor "region" has been changed during trial conduct (Amendment 1 dated 03SEP2012) from Europe/rest of world to Europe/Asia/America. Ultimately, the trial was conducted in 42 sites of 6 countries: Brazil, India, Mexico, Romania, Russia, and Ukraine. In study 302, region was changed from US/non-US to

US/Asia/rest-of-world (Amendment 1 dated 10MAY2012) and the trial was ultimately conducted in Argentina, Chile, India, Malaysia, Puerto Rico, Russia, Spain, USA. Overall, the stratified randomization procedure can be acceptable.

Blinding (masking)

Due to a different appearance of the primary packaging of the used IMP, pre-filled syringes of ZIOXTENZO and of Neulasta®, a full double-masking is technically not possible. An unblinded drug dispenser was established who is not involved in any study assessments and documents drug administration such that the blinded investigator has no access to this documentation. Investigator and patient were kept blinded.

The plan to keep investigators and subjects blinded is supported, whereas a complete masking may not be possible for the latter. The planned procedure appears to be acceptable to reduce biased results towards equivalence.

Statistical methods

Statistical methods were laid down in Statistical Analysis Plans (SAPs) based on the corresponding study protocols; SAP of study 301 dated 18-DEC-2013, SAP of study 302 dated 18-Mar-2014. The analysis plans are virtually the same. No interim analyses were performed.

The statistical analysis plans of both studies were completed before the blinded data review meeting took place and before un-blinding. The main characteristics of the statistical analysis (analysis data sets, hypotheses and significance level, primary efficacy analysis model and missing data handling) were already laid down in the original protocol versions. Changes in the planned analyses were not related to the primary efficacy analysis, apart from the consideration of the FAS defined as all randomized subjects who received at least one dose of the study drug for the main analysis (and not the ITT set, which was defined as all patients randomized). In both the 301 and the 302 study, ITT and FAS differed by 2 patients who had been randomized and immediately withdrawn on Cycle 1, Day 2, without administration of IMP. Statistical analysis based on the FAS is acceptable.

Analysis Data Sets

The following analysis sets were defined (the table is taken from the SAP of study 302, where the in addition a PK substudy was conducted):

Analysis Set	Definition
Safety analysis (SAF) Set:	The safety (SAF) population will consist of all patients who received at least one dose of study drug (LA-EP2006 or Neulasta®) and had at least one post-baseline safety assessment.

Full Analysis (FAS) SET:	All randomized patients who received at least one dose of study medication, i.e. of either LA-EP2006 or Neulasta®. Following the intent-to-treat principle, patients will be analyzed according to the treatment they were assigned to at randomization.
Per Protocol (PP) Set:	The per-protocol (PP) population is a subset of the FAS who completed the first chemotherapy cycle without major protocol deviations. To assess the validity of the patients for the PP population the data will be checked for the following potential protocol deviations: Deviation from entry criteria Errors in treatment assignment Use of commercial (peg)filgrastim Use of excluded/forbidden/un-allowed medication Poor compliance Loss to follow-up Missing ANC data
Valid Case (VC) Set:	The valid case (VC) population is a subset of the FAS who received only assigned IMP throughout the study.
PK Analysis (PK) Set:	All patients participating in the PK substudy with a valid PK profile.
ECG Analysis (ECG) Set	All patients with at least one available baseline triplicate measurement and at least one on-treatment ECG triplicate measurement (after IMP administration). Patients without violation of the cardiac exclusion criteria.

The decision whether a patient is belonging to each of the above mentioned populations was made during a blind data review meeting (BDRM). Safety was planned to be analyzed based on the SAF set with sensitivity analyses carried based on the VC set. The primary and secondary efficacy parameters (regarding cycle 1) were planned to be analyzed based on the full analysis set (FAS) and the per protocol (PP) set. Further efficacy endpoints regarding cycles 2-6 were planned to be analyzed based on the FAS with sensitivity analyses carried out for the VC set. Immunogenicity analyses were planned to be performed based on the SAF and the VC populations. PK and ECG analyses were based on the PK and ECG set, respectively.

The definitions of the analysis sets are generally acceptable. The per protocol set excludes subjects on the basis of eligibility deviations, compliance criteria, use of commercialized (peg)filgrastims and loss to follow-up / missing data. The applicant defines the FAS as the basis for the primary efficacy analysis, and considers the PPS for one of the sensitivity analysis. It is noted that the analysis based on both the FAS and the PPS are considered equally important and should lead to similar conclusions in a trial to demonstrate equivalence. In this context, the analysis based on the valid case set (which is also refered to as the FAS-C set) should also be taken into consideration.

Statistical Analysis of Efficacy Endpoints

The primary efficacy endpoint is the duration of severe neutropenia (DSN) in cycle 1. The testing procedure was set up in a hierarchical structure, where first equivalence between ZIOXTENZO and Neulasta® is assessed (margin ±1 day) and only if this is successfully established, non-inferiority between the two products will be tested using a tighter margin of -0.6 days.

Step 1, Equivalence assessment: The following set of hypotheses was tested using the two one-sided test procedure at a significance level of 2.5%:

H_{10}: | $\mu_{Neulasta} - \mu_{ZIOXTENZO}$ | ≥ 1 day
H_{11}: | $\mu_{Neulasta} - \mu_{ZIOXTENZO}$ | < 1 day,

where μ is the mean DSN under Neulasta® and ZIOXTENZO, respectively. The main efficacy parameter were analyzed by means of an ANCOVA with factors treatment group, region, chemotherapy, and co-variate "baseline ANC count", with the corresponding 95% confidence intervals being based on the residual standard error and adjusted least-square means of the ANCOVA. Equivalence was concluded, if the confidence interval lies entirely within the equivalence margins of ± 1 day.

Step 2, Non-inferiority assessment: The following set of hypotheses was tested at a one-sided significance level of 2.5%:

H_{20}: $\mu_{Neulasta} - \mu_{ZIOXTENZO} \leq$ -0.6 days
H21: $\mu_{Neulasta} - \mu_{ZIOXTENZO} >$ -0.6 days,

where μ is the mean DSN under Neulasta® and ZIOXTENZO, respectively. The non-inferiority analyses were conducted by means of the same ANCOVA as described for the equivalence assessment (Step 1), but comparing the lower bound of the 95% confidence interval with the non-inferiority margin of 0.6 days. Non-inferiority of ZIOXTENZO was concluded if the lower limit of the two-sided 95% confidence interval of the treatment difference did not exceed the -0.6 day margin (means the lower bound lies entirely above the non-inferiority margin of -0.6 day).

The primary analysis of the main efficacy endpoint was based on the FAS population. The following sensitivity analysis and further analyses of the main efficacy parameter were performed:

- An additional analysis using ANCOVA as mentioned before based on the PP, will serve as a sensitivity analysis to ensure the robustness of the results.
- ANOVAs for the primary efficacy endpoint including factor treatment group only will be analyzed (FAS and PP).
- Frequency of severe neutropenia will be presented with counts/ percentages for each day and overall in cycle 1 (FAS)

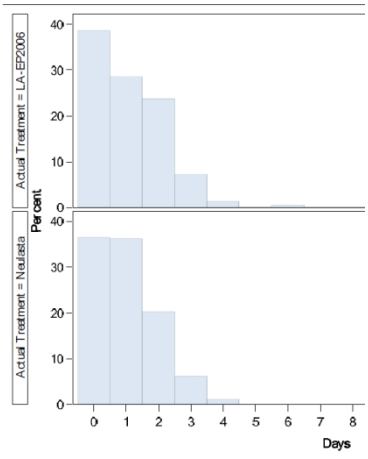
No testing for the assumption of normality will be done. The robustness of the AN(C)OVA is assumed based on simulation results for 3 to 5 point ordinal outcomes scale [Sullivan LM, D'Agostino RB, 20035]. The DSN is expected to have a similar ordinal outcome scale.

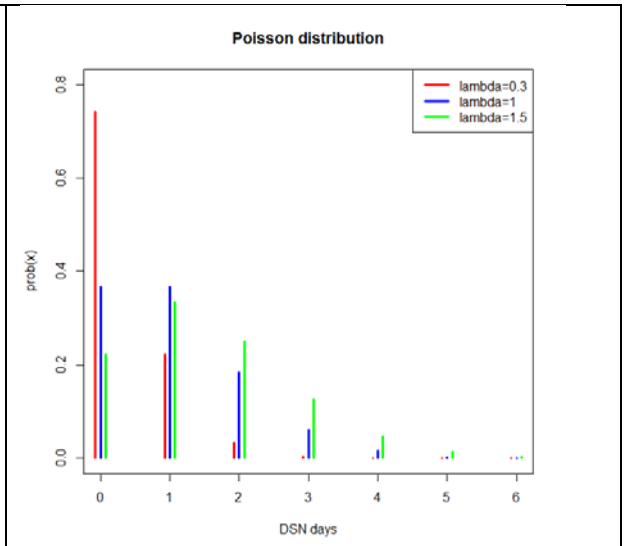
Secondary endpoints were analysed by means of descriptive statistics.

The primary endpoint DSN in days in cycle 1 has only 4 to 5 levels (0 to 3/4 days), whereas in study 301 at least 75% of the subjects in each treatment arm have a DSN of 0 or 1 days, and around 95% of the subjects 0-2 days. In study 302, the mean DSN of patients from both groups is larger (1.28 as compared to 0.79 in study 301), the 3rd quartile is 2, and the maximum is 6. The DSN has a limited number of levels, the distribution is skewed and does clearly not follow a normal distribution. The applicant argues that the linear model is robust for ordinal outcomes with a small number of levels. This is acknowledged, however, a sensitivity analysis that addresses the obvious departure from the normality assumption and that takes the limited number of outcome levels into account is considered useful. In fact, the applicant conducted a Poisson regression as an additional sensitivity analysis in the context of a pooled analysis (please refer to the respective comments provided in the section on pooled analysis). These additional analyses also contain the frequency distribution of days of SN, which was not (or only partly) presented in the reports of the individual studies.

The observed DSN distribution also indicates that DSN may not be a sensitive parameter to detect differences with regard to clinical efficacy and casts some doubt on the choice of the equivalence margin of 1 day. The mean (and the median) days of severe neutropenia in the Neulasta arm is close to 1 (in the pooled analysis of both studies) and the DSN of more than 90% of the subjects is 0, 1 or 2 days (see Figure 1.3.2.2 from the pooled analysis, shown below). Therefore, a mean difference of 1 day appears large. Assuming that the DSN follows a Poisson distribution, differences of 0.5 to 0.7 already show large differences (see right figure below, where the distribution is shown in red for mean DSN of 0.3 and shown in green for a mean DSN of 1.5), and such differences could not be detected in the proposed statistical analysis, i.e. the 95% confidence interval of the difference between means would fall entirely within [-1;1] with high probability.

FIGURE 1.3.2.2 Histogram of Duration of Severe Neutropenia in Cycle 1 - Pool 1
Overall
PP population





It is however noted that the observed confidence interval is very narrow, the sample size is reasonably large and the point estimate is close to 0. Hence, the results of the primary variable, has to be read in conjunction with those of secondary measures.

Adjustment of the statistical model for stratification factors and baseline ANC value is endorsed.

Examination of the consistency of findings in relevant subgroups analyses, were not planned. The choice of the stratification factors indicates that region and chemotherapy (adjuvant/neoadjuvant) may have an impact on the efficacy outcome. Therefore, the applicant is asked to conduct subgroup analyses for the stratification factors (and other factors that may be considered prognostic or predictive). Efficacy variables should be analysed by subgroup with respect to the LA-EP06 vs Neulasta difference, but also with respect to the absolute levels of the efficacy outcomes (e.g. mean days of severe neutropenia per treatment arm, OC).

Missing Data Handling

No missing values were imputed. For the determination of DSN, the following rules applied:

The missing value imputation refers only to the determination of severe neutropenia and not to the replacement of the ANC value itself. In case an ANC value is missing the following rules may be used:

- The ANC before and after the missing day is ≥ 0.5 x 109/L: the day can likely be ignored as a potential day of severe neutropenia. However, there were exceptions to this rule, in case the potential of the missing ANC to fulfil the severe neutropenia definition was high, e.g. if the missing

ANC value could have been the nadir. Such cases were reviewed, decided upon and documented at the BDRM in a completely blinded way.

- If at both neighbouring days the ANCs are < 0.5 x 109/L, then set the missing day to severe neutropenia.
- If the day before is < 0.5 x 109/L and the day after ≥ 0.5 x 109/L, then the missing day is set to severe neutropenia.
- If the day before is ≥ 0.5 x 109/L and the day after < 0.5 x 109/L, then the missing day is set to severe neutropenia.
- If any of the neighbouring days (i.e. 2 or more missing values in a row) is also missing, severe neutropenia cannot be determined automatically. These cases were discussed in the BDRM.

The handling of missing DSN values is based on the underlying ANC profile, which is supported. The proposed method appears to be acceptable. Bias towards equivalence could in principle be introduced (during BDRM) by similar determination of severe neutropenia of all patients. However, the number of cases which may be ambiguously and requiring a decision of the BDRM are limited (according to the listings on protocol deviations as defined in the blind data review meeting and the individual ANC time courses). Overall, handling of missing values is acceptable.

Results

Most frequent reasons for the exclusion of patients from the PP set were use of commercial (peg)filgrastim, IMP-related reasons, and missing ANC data.

Table 10-4 Patients with major protocol deviations leading to exclusion from the PP set (all randomized patients)

Treatment Patient number	Protocol deviation	
	Category	Details
LA-EP2006		
102-05	ANC not evaluable	
404-07	ANC not evaluable	
502-02	Prohibited medication	Administration of filgrastim during study
507-01	Randomization	Randomization error
507-02	IMP-related	Under quarantine
507-04	ANC not evaluable	
508-02	IMP-related	Under quarantine
508-08	IMP-related	Under quarantine
508-09	IMP-related	Under quarantine
508-11	IMP-related	Under quarantine
511-13	ANC not evaluable	
518-02	ANC not evaluable	
718-01	Commercial (peg)filgrastim	Neulastim®, Roche
Neulasta		
407-09	ANC not evaluable	
507-03	ANC not evaluable	
508-01	IMP-related	Under quarantine
508-10	ANC not evaluable	
508-10	IMP-related	Under quarantine
718-02	Commercial (peg)filgrastim	Neulastim®, Roche
718-03	Commercial (peg)filgrastim	Neulastim®, Roche
718-04	Commercial (peg)filgrastim	Neulastim®, Roche
901-01	ANC not evaluable	

ANC = absolute neutrophil count, IMP = investigational medicinal product; PP set = per protocol set
Source: [Appendix 16.2-2.2], [Appendix 16.1.9-BDRM Minutes]

Across both trials, the company did not provide data of 14 patients which were in the FAS but not included in the tables of the primary efficacy measure due to "Missing due to blind data review meeting decision (absolute neutrophil count not available)" The applicant should justify, why dose ANC profiles were not available and included in the analysis.

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

Title: 2 Pivotal studies in breast cancer patients investigating efficacy and safety of ZIOXTENZO and Neulasta® (identical design)

Study identifier	Protocol no. LA-EP06-301, EudraCT no. 2011-004532-58 Protocol no. LA-EP06-302, EudraCT no. 2012-002039-28		
Design	Two randomized, double-blind, parallel-group, active-controlled, multi-center Phase 3 study in patients with histologically proven breast cancer having an indication for neo-adjuvant or adjuvant treatment with TAC (Taxotere® [docetaxel] in combination with Adriamycin® [doxorubicin] and Cytoxan® [cyclophosphamide]) chemotherapy, eligible to receive six cycles of chemotherapy. The investigational medicinal product (IMP) was to be injected subcutaneously with a dose of 6 mg pegfilgrastim in 0.6 mL		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	18 weeks not applicable 22 weeks (LA-EP06-301) 4 weeks (LA-EP06-302)	
Hypothesis	Equivalence,Non-inferiority		
Treatments groups	Group 1: ZIOXTENZO (investigational drug treatment) (	A dose of 6 mg of ZIOXTENZO was administered once per chemotherapy cycle, which is the recommended dose of pegfilgrastim for reduction in the duration of neutropenia and the incidence of FN in patients treated with cytotoxic chemotherapy for malignancy according to the Neulasta SmPC and for decreasing the incidence of infection, as manifested by FN, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of neutropenia according to the Neulasta USPI.	
	Group 2: Neulasta (control drug treatment 153 patients)	A dose of 6 mg of EU-Neulasta was administered once per chemotherapy cycle, which is the recommended dose of pegfilgrastim for reduction in the duration of neutropenia and the incidence of FN in patients treated with cytotoxic chemotherapy for malignancy according to the Neulasta SmPC and for decreasing the incidence of infection, as manifested by FN, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of neutropenia according to the Neulasta USPI.	
Endpoints and definitions	Primary endpoint	mean duration of severe neutropenia (DSN) during Cycle 1	ZIOXTENZO compared to Neulasta (EU-authorized) defined as the number of consecutive days with Grade 4 neutropenia (absolute neutrophil count [ANC] less than 0.5 × 109/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen (Taxotere® [docetaxel 75 mg/m2] in combination with Adriamycin® [doxorubicin 50 mg/m2] and Cytoxan® [cyclophosphamide 500 mg/m2]) in breast cancer patients.

	Secondary endpoint	Incidence of febrile neutropenia (FN),	defined as oral temperature ⩾ 38.3 while having an ANC < 0.5 × 109/L (both measured on the same day) by cycle and across all cycles
	Secondary endpoint	Number of days of fever	defined as oral temperature ⩾ 38.3C°, for each cycle
	Secondary endpoint	Depth of ANC nadir	defined as the patient’ s lowest ANC in Cycle 1
	Secondary endpoint	Time to ANC recovery	defined as the time in days from the chemotherapy administration until the patient`s ANC increased to ⩾ 2 × 109/L after the nadir, in Cycle 1
	Secondary endpoint	Frequency of infection by cycle and across all cycles	See safety
	Secondary endpoint	Mortality due to infection	See safety
	Secondary endpoint	Incidence, occurrence, and severity of adverse events (AEs)	See safety
	Secondary endpoint	Assessment of local tolerability at the injection site	See safety
	Secondary endpoint	Systemic tolerance (physical examination and safety laboratory assessments)	See safety
	Secondary endpoint	Safety follow-up including immunogenicity assessment four weeks after the last administration of the investigational medicinal product (IMP)	See safety
Results and Analysis			
Analysis description			

Analysis population and time point description	FAS		
Descriptive statistics and estimate variability		**ZIOXTENZO**	**Neulasta**
Descriptive statistics and estimate variability Effect estimate per comparison	Number of subject	314	310
	Primary endpoint Mean (SD)	1.05 (1.055)	1.01 (0.958)
	Median (Range)	1.00 (0.0-6.0)	1.00 (0.0-4.0)
	Mean for difference Zioxtenzo – Neulasta (95% CI)	**-0.04** **[-0.19, 0.11] [Equ.Margin: +/-1day, NI margin -0,6d]**	

	Observed incidence of febrile neutropenia in C1; n (%)	18 (5,7)	26 (8,4)
	Overall incidence of febrile neutropenia across all cycles; n (%)	25 (8.0)	32 (10.3)
	Time to ANC recovery in C1 (days); Mean (std)	2,00 (0,0-4,0)	2,00 (0,0-6,0)
	Depth of ANC nadir in C1; Mean (std)	0.800 (± 1.2436) × 109/L	0.687 (± 0.9586) × 109/L
	Number of patients with at least one day of fever	26 (16,4%)	26 (16,6%)

The Applicant demonstrated similarity in terms of efficacy between the biosimilar candidate and Neulasta-EU in terms of efficacy across primary and secondary endpoints. The primary objective, duration of severe neutropenia, has been analysed and compared in both LA-EP06-301 and LA-EP06-302. In general the mean duration of SN was lower in study LA-EP06-301, however both studies (and all treatment arms) presented comparable median values (1,00d). The 95% CI of the difference in DSN was easily preserved within the predefined equivalence margins of +/- 1d and consecutively within the more narrow NI margin (-0,6d) for both studies in the FAS as well as in the PP. Similarity was for the primary measure is also shown in the pooled analysis across the two trials(-0.04 [-0.19, 0.11]) .

Table 2-7 Primary efficacy variable: Difference in the duration of severe neutropenia (DSN) in days in Cycle 1 – Poisson regression (95% CI) – studies LA-EP06-301 and LA-EP06-302 (FAS and PP sets)

Difference in DSN Neulasta EU minus LA-EP2006	LA-EP06-301		LA-EP06-302	
	FAS	PP	FAS	PP
n	310	295	300	292
Difference	0.08	0.05	-0.12	-0.11
95% CI [LL, UL]	[-0.17, 0.33]	[-0.22, 0.31]	[-0.32, 0.08]	[-0.32, 0.09]

CI=confidence interval; DSN=duration of severe neutropenia; FAS=full analysis set; LL, UL=lower limit, upper limit; n=number of evaluable patients; Neulasta EU=EU-authorized Neulasta; PP=per protocol set

The Poisson regression model assessing the treatment point estimate and corresponding CIs was adjusted for chemotherapy, region, study, and baseline ANC count. Baseline ANC is defined as the ANC value at Day 1 of Cycle 1

Table 2-8 Primary efficacy variable: Duration of severe neutropenia (DSN) in days in Cycle 1 – studies LA-EP06-301 and LA-EP06-302 (FAS set)

	LA-EP06-301			LA-EP06-302		
	LA-EP2006	Neulasta EU	Total	LA-EP2006	Neulasta EU	Total
DSN	N=159	N=157	N=316	N=155	N=153	N=308
n[a]	155	155	310	151	149	300
Mean (SD)	0.75 (0.878)	0.83 (0.898)	0.79 (0.887)	1.36 (1.133)	1.19 (0.984)	1.28 (1.063)
Median (range)	1.00 (0.0-3.0)	1.00 (0.0-4.0)	1.00 (0.0-4.0)	1.00 (0.0-6.0)	1.00 (0.0-4.0)	1.00 (0.0-6.0)

DSN=duration of severe neutropenia; FAS set=full analysis set; n=number of evaluable patients; N=number of patients in a treatment group; Neulasta EU=EU-authorized Neulasta; SD=standard deviation

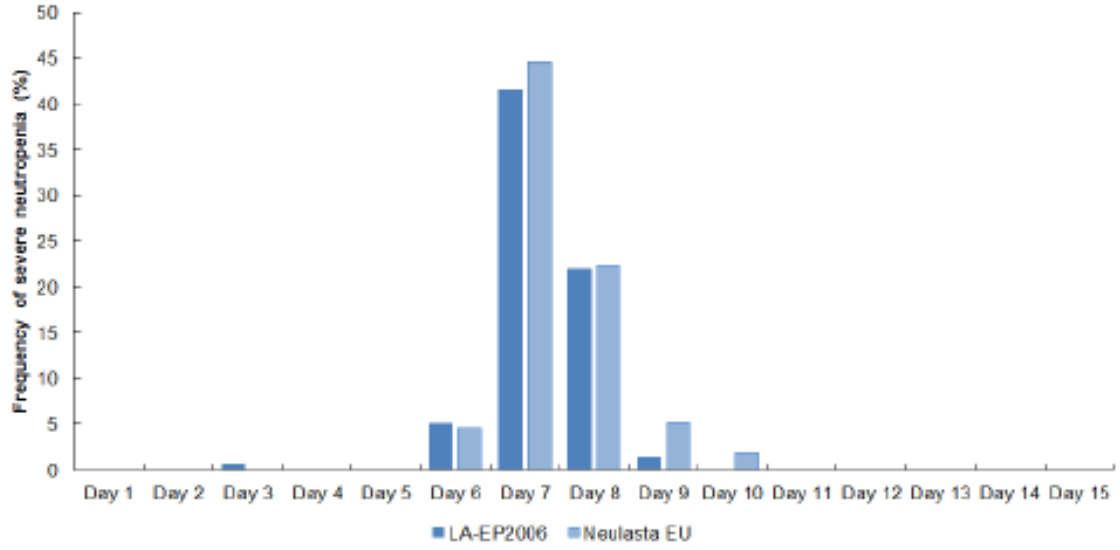
[a] Overall, 14 patients had missing ANC profiles and were excluded from the analysis.

Source: [Module 5.3.5.1 LA-EP06-301-Table 11-7], [Module 5.3.5.1 LA-EP06-302-Table 11-7]

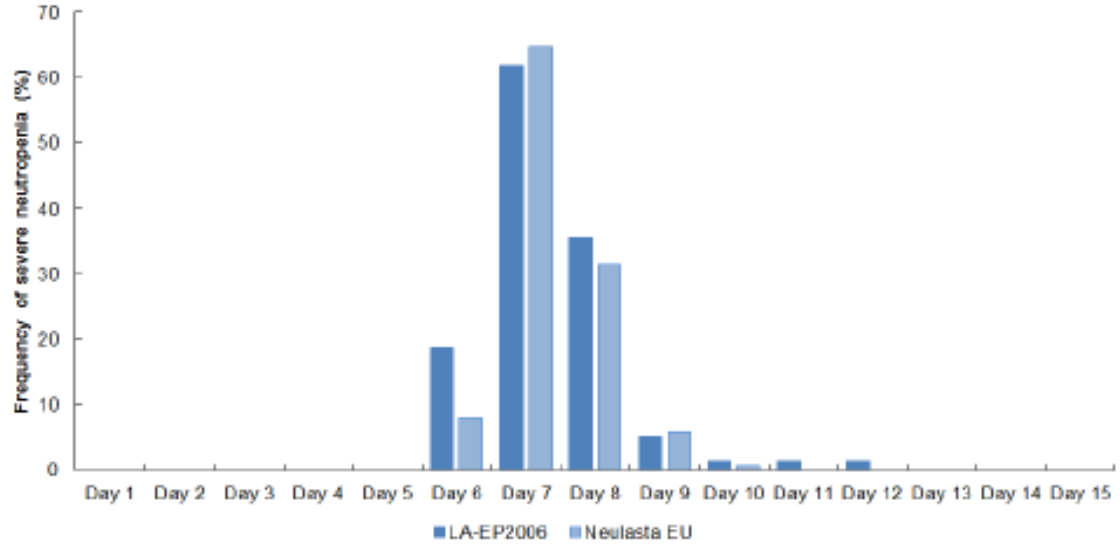
Not only the duration of SN was comparable but also its respective incidence and timing.

Figure 2-2 Incidence (%) of severe neutropenia at each day in Cycle 1 – studies LA-EP06-301 and LA-EP06-302 (FAS set)

Study LA-EP06-301



Study LA-EP06-302



FAS set=full analysis set; Neulasta EU=EU-authorized Neulasta

Source: [Module 5.3.5.1 LA-EP06-301-Table 14.2-2.1.4.1], [Module 5.3.5.1 LA-EP06-302-Table 14.2-2.1.4.1]

Results in the PP set were similar to that of the FAS set [Module 5.3.5.1 LA-EP06-301-Table 14.2-2.1.4.2], [Module 5.3.5.1 LA-EP06-302-Table 14.2-2.1.4.2].

Secondary Endpoints:

The Applicant also presented comparative data of predefined secondary measures for both efficacy trials. The presented data was mainly derived from the FAS set, however, especially for measures, that look beyond cycle one, such as frequency of infections, mortality due to infections, and incidence of fever as well as febrile neutropenia, the FAS set alone is not considered the relevant analysis set. It

includes patients, who were treated with commercial GCSF due to shortages of IMP. Looking at the whole study duration, the FAS-C set is considered most sensitive, since it excludes all patients treated with commercial products. The company has additionally provided tables for the FASC set widely confirming the results seen in the FAS set.

Incidence of febrile neutropenia and incidence of fever can be considered similar between treatments and across trials.

Table 2-11 Number of patients with at least one episode of febrile neutropenia by cycle and across all cycles – studies LA-EP06-301 and LA-EP06-302 (FAS set)

	LA-EP06-301			LA-EP06-302		
	LA-EP2006 N=159 n (%)	Neulasta EU N=157 n (%)	Total N=316 n (%)	LA-EP2006 N=155 n (%)	Neulasta EU N=153 n (%)	Total N=308 n (%)
Cycle 1	6 (3.8)	11 (7.0)	17 (5.4)	12 (7.7)	15 (9.8)	27 (8.8)
Cycle 2	2 (1.3)	1 (0.6)	3 (1.0)	0	3 (2.0)	3 (1.0)
Cycle 3	2 (1.3)	1 (0.6)	3 (1.0)	3 (2.1)	1 (0.7)	4 (1.4)
Cycle 4	1 (0.7)	0	1 (0.3)	2 (1.4)	1 (0.7)	3 (1.1)
Cycle 5	2 (1.4)	0	2 (0.7)	0	1 (0.7)	1 (0.4)
Cycle 6	1 (0.7)	1 (0.7)	2 (0.7)	2 (1.5)	1 (0.7)	3 (1.1)
Overall (at least one incidence)[a]	9 (5.7)	12 (7.6)	21 (6.6)	16 (10.3)	20 (13.1)	36 (11.7)

FAS set=full analysis set; n=number of evaluable patients; N=number of patients in a treatment group; Neulasta EU=EU-authorized Neulasta

[a] Patients with more than 1 event during the study are counted only once

Clinical studies in special populations

N/A

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

Statistical Analysis of Primary Efficacy

For the analysis of the primary efficacy endpoint DSN in cycle 1 an ANCOVA model similar to the model specified for individual studies extended by a “study” effect was performed. The model included the following terms:

- Fixed effects: Treatment, Chemotherapy (neo-adjuvant or adjuvant), and Region
- Random effect: Study
- Baseline ANC as a covariate

Baseline ANC count was the ANC value at Day 1 of Cycle 1 and the categorization of Region is given in the following table:

Table 3-4 Categorization of countries into regions

Category for Region	Case Report Form (CRF) for Countries
America	Argentina, Brazil, Chile, Mexico, Puerto Rico, United States
Europe	Spain, Romania, Russian Federation, Ukraine
Asia	India, Malaysia

Equivalence was assessed with the two-sided 95% confidence interval (CI) around the treatment difference in mean DSN, outputted from the ANCOVA model. ZIOXTENZO was considered equivalent to Neulasta if the 95% CI was entirely within the equivalence margins of ±1 day.

Two additional sensitivity analyses were performed on the primary endpoint to assess the robustness to study discontinuations and the normality assumption of DSN:

- Study discontinuations: The robustness to study discontinuations of the primary analysis results was evaluated by a study level assessment by performing the protocol specified analysis after excluding patients who discontinued in Cycle 1 (PP completers set). The PP set (which included patients who discontinued in Cycle 1) results were compared to the PP completers set (which excluded patients who discontinued in Cycle 1 but had been included in the PP set). The protocol specified results were deemed robust to study discontinuations if the results were similar and were still contained within the equivalence margins of ±1 day. This analysis was performed by study only.
- Normality assumption: The protocol-specified analysis of DSN assumes DSN follows a normal distribution. As a graphical assessment of DSN suggested a non-normal distribution, a Poisson regression was performed as a sensitivity analysis for Pool 1 and by study.

Due to the identical design of both efficacy studies the applicant performed pooled analyses from both trials of primary and secondary endpoints. This is endorsed, and due to the similarity in design aspects considered meaningful.

In the pooled analysis the factor “study” (2 levels: 301 and 302) is considered a random factor in the primary efficacy analysis model. The two studies are very similar with respect to study conduct and population. Nevertheless, the distribution of the primary endpoint differed between studies; the mean (SD) days of severe neutropenia was 0.79 (0.89) in study 301 as compared to 1.28 (1.06) in study 302. This difference may be explained by the different geographical regions considered in the two studies, such that the (random or fixed) factor study only has a minor impact on the analysis. Differences between regions/studies with regard to the mean days of severe neutropenia as well as the originator-biosimilar difference should be investigated by subgroup analysis.

The poisson regression appears to be a reasonable sensitivity analysis as the distribution of the primary endpoint does not follow a normal distribution, and the Poisson distribution appears plausible (see above figure in comment on the primary endpoint analysis). The results are very similar to those obtained from the linear (mixed) model based on normal data. According to the results tables, GLIMMIX has been used and the model was adjusted for the same factors that have been used in the linear model. However, no further details were provided, e.g. which factors are considered fixed and random, the link function, the program code.

As regards poisson regression for sensitivity analysis, the company is asked to provide a detailed specification of the model and the corresponding program code.

3.3.8. Discussion on clinical efficacy

Design and conduct of clinical studies

The Applicant compared clinical efficacy and safety of ZIOXTENZO with that of Neulasta EU in two independent double-blind, randomized, parallel-group, multi-center studies of nearly identical design. Studies [LA-EP06-301] and [LA-EP06-302] were both designed to assess equivalence and non-inferiority of ZIOXTENZO to Neulasta EU in 302 female patients with breast cancer treated with myelosuppressive chemotherapy (TAC).Those two trials represent sufficiently large entities to compare the efficacy of ZIOXTENZO and Neulasta EU in a meaningful way. The Applicant states that due to transient shortages of IMP in the two studies, 70 patients in study LA-EP06-301 (ZIOXTENZO: 28 patients; Neulasta EU: 42 patients) and 73 patients in study LA-EP06-302 (ZIOXTENZO: 32 patients;

Neulasta EU: 41 patients) received one or more administrations of commercial G-CSF containing product instead of IMP. In case this happened in treatment cycle 1 (before the primary endpoint) this was considered a major protocol deviations and patients were excluded from the PP set. To characterise the impact of the use of commercial G-CSF the Applicant implemented a FAS-C, where all patients who received the commercial product at some point, were excluded, which raises the questions how shortages of IMP can happen in the first place and how far the exclusion of patient's data has an impact on the comparison, which is probably rather a safety than an efficacy issue, since most patients completed Cycle 1 without temporarily "switching" to commercial products. Furthermore results of the PP and FAS-C are (as far as presented) consistent with the FAS. It can be agreed that the exclusion of this low number of patients is not considered to compromise the validity of the primary endpoint. Considering also the more "minor" deviations in other treatment cycles the company has addressed the issue by providing results not only for the FAS and PP set, but also the FAS-C, in which all patients who received commercial GCSF at some point were excluded. The Applicant recruited women of 18 years or older with histologically proven breast cancer who were eligible for six cycles of neo-adjuvant or adjuvant treatment with TAC chemotherapy for both studies, which was deemed acceptable The distribution of major protocol deviations between the treatment groups of studies LA-EP06-301 and LA-EP06-302 does not suggest an impact on the analysis of efficacy. In both studies, 38 major protocol deviations were noted in 36 patients. The deviations occurred in 19 patients from the ZIOXTENZO treatment arms and in 17 of the Neulasta treatment arms. Most frequent reasons for the exclusion of patients from the PP set were use of commercial (peg)filgrastim, IMP-related reasons, and missing ANC data.

Across both trials, the company did not provide data of 14 patients which were in the FAS but not included in the tables of the primary efficacy measure due to "Missing due to blind data review meeting decision (absolute neutrophil count not available)" The applicant should justify, why those ANC profiles were not available and included in the analysis.

One secondary endpoint "days of fever" was retrospectively adapted since the assessment of a fever episode contained a self-reported component, probably resulting in underreporting. Hence, it was decided to modify the endpoint "number of days of fever" to "number of patients with at-least one fever episode", which is considered acceptable, although the categorization may lead to information loss and the modified variable may be less sensitive to detect differences. If available, the information on the initial endpoint should be provided. Additionally, more information is needed about the instructions given to the patient about self-medication with antipyretics, analgesics or anti-inflammatories.

Efficacy analyses were performed to demonstrate therapeutic equivalence for duration of severe neutropenia (DSN) during Cycle 1, with the 95% CIs for the differences in mean DSN within a margin of ± 1 day. Both the endpoint and equivalence margin are considered acceptable and in line with CHMP Scientific Advice. The primary efficacy endpoint is calculated based on ANC, which was determined in local laboratories. It remains unclear whether the participating labs used validated techniques (e.g., certification, participation in quality control scheme) and how it was ensured that the methods were standardised across the laboratories. The Applicant is asked to elaborate on this further. Results were adjusted for the stratification factors chemotherapy and region and the covariate baseline ANC. A sensitivity analysis using Poisson regression was performed as DSN was not normally distributed, which did not change the conclusions. All primary endpoint analyses were performed using the FAS and the PP population, as required for equivalence trials. Overall the statistical approach is considered acceptable. The studies were conducted outside the European Union, which is considered acceptable; no racial or ethnical differences are known to influence the dose-response relationship of pegfilgrastim. As the pre-filled syringes (including the colour of the products) were different, blinding of the trial was

performed through the injection of the study products by an unblinded administrator. More details are requested about the procedures in place to ensure effective blinding in practice, in particular in the out-patient setting.

Efficacy data and additional analyses

The Applicant demonstrated similarity between the biosimilar candidate and Neulasta-EU in terms of efficacy as measured by the duration of severe neutropenia.

The primary endpoint, duration of severe neutropenia, has been analysed and compared in both LA-EP06-301 and LA-EP06-302. In general the mean duration of SN was lower in study LA-EP06-301, however both studies (and all treatment arms) presented comparable median values (1,00d). The 95% CI of the difference in DSN was easily preserved within the predefined equivalence margins of +/- 1d and consecutively within the more narrow NI margin (-0,6d) for both studies in the FAS as well as in the PP. Similarity for the primary measure was also shown in the pooled analysis across the two trials(-0.04 [-0.19, 0.11]).

The choice and definition of the primary measure is derived from the EMA Guideline on Biosimilar GCSF and has also been accepted during EMA scientific advice. Equivalence margins have been discussed regarding their clinical relevance and possible preservations of clinical effect.

Not only the duration of SN was comparable but also its respective incidence and timing.

Secondary Endpoints:

The Applicant also presented comparative data of predefined secondary measures for both efficacy trials. The presented data was mainly derived from the FAS set, however, especially for measures, that look beyond cycle one, such as frequency of infections, mortality due to infections, and incidence of fever as well as febrile neutropenia, the FAS set alone is not considered the relevant analysis set. It includes patients, who were treated with commercial GCSF due to shortages of IMP. Looking at the whole study duration, the FAS-C set is considered most sensitive, since it excludes all patients with major protocol deviations or treated with commercial products. The company has additionally provided tables for the FAS-C set widely confirming the results seen in the FAS set.

Incidence of febrile neutropenia and incidence of fever can be considered similar between treatments and across trials.

The incidence of febrile neutropenia suggests a small trend towards a lower incidence for the biosimilar candidate, a trend, that in both trials originates from numerical differences observed in Cycle one, while after that, the trend vanishes or slightly reverses (without meaningful differences however). The depth of ANC nadir was comparable between treatments with rather high SD and was in most cases reached around day 7. Interestingly the mean for this measure for both treatments was about 2-3 fold lower in study LA-EP06-302. [(Study LA-EP06-301, mean ($\pm$ standard deviation [SD]) ANC nadir was 1.102 ($\pm$ 1.5398) $\times$ 10^9/L in patients treated with ZIOXTENZO and 0.921 ($\pm$ 1.1771) $\times$ 10^9/L in patients treated with Neulasta EU.) In study LA-EP06-302, ANC nadir was 0.490 ($\pm$ 0.7205) $\times$ 10^9/L in patients treated with ZIOXTENZO and 0.444 ($\pm$ 0.5684) $\times$ 10^9/L in patients treated with Neulasta EU)]. Time to ANC recovery was almost identical between treatments.

The Applicant has compared the FAS set with PP and FAS-C set without encountering relevant differences between analysis sets.

In both studies, the comparison of secondary endpoints showed a favourable trend for ZIOXTENZO compared to Neulasta-EU with higher mean ANC nadir (0.8 vs 0.7 x109/L, respectively), lower

incidence of febrile neutropenia (10% vs 8%, respectively) and infections (16% vs 18%, respectively). However, the Applicant failed to present the comparison of concomitant therapies (antipyretics and antibiotics), which could potentially confound these results.

Overall, efficacy outcomes were worse in study LA-EP06-302 compared to study LA-EP06-301. The Applicant should provide subgroup analyses for the main efficacy outcomes (incidence and duration of severe neutropenia, incidence of febrile neutropenia) by geographical region.

3.3.9. Conclusions on clinical efficacy

Despite some minor concerns (use of commercial G-CSF in some patients instead of study drug) the Applicant has shown similarity in terms of efficacy in two confirmatory trials of nearly identical design. Therapeutic equivalence has been robustly shown in two clinical studies conducted in the target population. The remaining uncertainties are not expected to influence this conclusion in a meaningful way, however it has to be stated that both studies where comparable efficacy was investigated are not adequate to demonstrate biosimilarity on their own due to their limited sensitivity (see Discussion on Benefit Risk)

3.3.10. Clinical safety

Introduction

The studies or sources of data which contributed to the assessment of safety comprised (Table 1-1):

- One PK/PD study in healthy volunteers comparing ZIOXTENZO with Neulasta EU and
- Neulasta US (LA-EP06-101)
- Two confirmatory efficacy and safety studies in patients with breast cancer comparing
- Study LA-EP06-101

Safety assessments consisted of monitoring and recording all adverse events, including serious adverse events, the monitoring of haematology, blood chemistry, and urine, and the regular monitoring of vital signs, and physical condition.

12-lead ECGs were performed at Screening, at pre-dose and 1 and 4 hours post dose on Day 1, and in the morning on Days 2, 3, 7 (±1 day) and 15 (Follow-up visit). For a part of the subjects additional ECG recordings were performed in triplicate.

A spleen ultrasound examination was performed at a separate screening visit for subjects who fulfilled all other inclusion criteria and none of the exclusion criteria.

Local tolerability at the injection site was evaluated by the subjects themselves using a VAS and by the Investigator using the ISR Score.

ZIOXTENZO with Neulasta EU (LA-EP06-301 and LA-EP06-302)

The requirements for the claim of safety are included in this Summary of Clinical Safety. In studies LA-EP06-301 and LA-EP06-302, the safety assessments were performed during treatment with ZIOXTENZO or Neulasta EU administered as prevention of febrile neutropenia in patients with breast cancer receiving myelosuppressive chemotherapy as neo-adjuvant or adjuvant therapy. Study LA-EP06-301 included a 6-month safety follow-up (SFU) as required by “Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor”

(EMEA/CHMP/BMWP/31329/2005) and CHMP/EMA Initial Scientific Advice (EMEA/H/SA/1419/1/2009/III, 19-Nov-2009).

The potential effects of ZIOXTENZO, Neulasta EU and Neulasta US on cardiac repolarization and morphology were evaluated in healthy volunteers in study LA-EP06-101 and that of ZIOXTENZO and Neulasta EU in a subset of patients in study LA-EP06-302. This electrocardiogram (ECG) evaluation was performed following a request by FDA. Study LA-EP06-101 assessed the safety of ZIOXTENZO in comparison with both Neulasta EU and Neulasta US in healthy volunteers at a dose of 6 mg after a single subcutaneous (s.c.) administration. Safety of ZIOXTENZO was monitored through adverse event (AE) reporting, clinical laboratory testing, vital signs, physical examinations, and ECG. Immunogenicity was assessed in all studies in terms of monitoring for anti-pegfilgrastim antibodies.

Study No.	Study objective	Study population	Treatment duration	Dosage [batch number]	Safety endpoints

LA-EP06-302	Efficacy and safety of LA-EP2006 compared to Neulasta (EU-authorized) with respect to the mean DSN, defined as the number of consecutive days with grade 4 neutropenia (ANC < 0.5 × 10^9/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen in patients with breast cancer	Patients with breast cancer
		Total: N=308f
		LA-EP2006: n=155f
		Neulasta EU: n=153f
			22 weeks (18 weeks plus a 4-week follow-up)	LA-EP2006: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [30324244, 30324245]
				Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [1022627/ 1022626, 1028053D, 1033639A]
					• AEs, including SAEs
					• Physical examination
					• Vital signs, height and weight
					• Laboratory evaluations of blood and urine samples (hematology, biochemistry, urinalysis)
					• ECG (12-lead)
					• Local tolerability
					• Anti-pegfilgrastim antibodies
					• ECG subset (triplicate measurements)
					  • QT (corrected and uncorrected)
					  • heart rate
					  • PR
					  • QRS
					  • ECG morphological patterns
					  • correlation between the QTcF change and pegfilgrastim serum concentration

AE=adverse event; ANC=absolute neutrophil count; DSN=duration of severe neutropenia; ECG=electrocardiogram; f=female; m=male; Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta; PD=pharmacodynamics; PFS=pre-filled syringe; PK=pharmacokinetics; QTcF=QT interval, Fridericia's correction; SAE=serious adverse event; s.c.=subcutaneous; SFU=safety follow-up; TAC=Taxotere® (docetaxel 75 mg/m^2) in combination with Adriamycin® (doxorubicin 50 mg/m^2) and Cytoxan® (cyclophosphamide 500 mg/m^2)

Source: [Module 5.3.4.1 LA-EP06-101], [Module 5.3.5.1 LA-EP06-301], [Module 5.3.5.1 LA-EP06-302]

Table 1-1 Studies which contributed to the assessment of safety

Study No.	Study objective	Study population	Treatment duration	Dosage [batch number]	Safety endpoints

LA-EP06-101	Evaluate the similarity of LA-EP2006 and Neulasta (US-licensed and EU-authorized) in terms of PK, PD, safety, immunogenicity, local tolerance, and possible influences on ECG parameters	Healthy volunteers
		Total: N=279 (156m/123f)
		LA-EP2006: 93 (51m, 42f)
		Neulasta EU: 93 (53m, 40f)
		Neulasta US: 93 (52m, 41f)
			Up to 49 days (including screening, single dosing, PK, PD and safety assess-ments and follow-up)	LA-EP2006: 6 mg (10 mg/1 mL, glass vial), single s.c. injection [30114715]
				Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection [1016759]
				Neulasta US: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection [1012807, 1017404]
					• AEs, including SAEs
					• Physical examination
					• Vital signs, height and weight
					• Laboratory evaluations of blood and urine samples (hematology, biochemistry, urinalysis)
					• ECG (12-lead)
					• Local tolerability
					• ECG subset (triplicate measurements)
					  • RR
					  • PR
					  • QRS
					  • QT (corrected and uncorrected)
					• Ultrasound examination of the spleen
					• Anti-pegfilgrastim antibodies

LA-EP06-301	Efficacy and safety of LA-EP2006 compared to Neulasta (EU-authorized) with respect to the mean DSN, defined as the number of consecutive days with grade 4 neutropenia (ANC < 0.5 × 10^9/L), during Cycle 1 of the neo-adjuvant or adjuvant TAC regimen in patients with breast cancer	Patients with breast cancer Total: N=316f LA-EP2006: n=159f Neulasta EU: n=157f	44 weeks (18 weeks plus a 6-month SFU)	LA-EP2006: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [30324244, 30324245] Neulasta EU: 6 mg (6 mg/0.6 mL, PFS), single s.c. injection 1 day after TAC chemotherapy for up to six cycles [10271811A, 1028053D, 1033639A]	• AEs, including SAEs • Physical examination • Vital signs, height and weight • Laboratory evaluations of blood and urine samples (hematology, biochemistry, urinalysis) • ECG (12-lead) • Local tolerability • Anti-pegfilgrastim antibodies

Summarizing, the Applicant has collected safety date from a PK/PD study in healthy volunteers and two confirmatory efficacy trials of nearly identical design in female breast cancer patients. Immunogenicity as regards anti-pegfilgrastim antibodies has been evaluated throughout all studies. Potential effects of ZIOXTENZO and Neulasta EU on cardiac repolarization and morphology have been evaluated in the PK/PD study in healthy volunteers and a subset of patients due to an FDA request. This data is only regarded as supportive information for this biosimilar exercise. The general magnitude and type of safety data gathered and presented is acceptable.

Patient exposure

Analysis Sets

Table 1-2 Analysis sets defined for safety analysis of studies LA-EP06-301 and LA-EP06-302 and for combined safety analysis (Pool 1)

Analysis Set	Definition
Full analysis (FAS) set[1]:	The FAS population consists of all randomized patients who received at least one dose of study medication, i.e. of either LA-EP2006 or Neulasta. Following the intent-to-treat principle, patients were analyzed according to the treatment they were assigned to at randomization.
Safety analysis (SAF) set:	The SAF population consists of all patients who received at least one dose of IMP (LA-EP2006 or Neulasta) and had at least one post-baseline safety assessment.
Safety analysis set minus C (SAF-C) set:	The SAF-C population is a subset of the SAF who received only assigned IMP throughout the study (i.e. excluding all patients who received commercial G-CSF products instead of IMP).

G-CSF= granulocyte colony-stimulating factor; IMP=investigational medicinal product

[1] This analysis set was used for efficacy analyses and population characteristics.

Source: [Module 5.3.5.3-Statistical Overview-Table 3-2]

Table 1-3 Analysis sets of studies LA-EP06-301 and LA-EP06-302 and the combined studies (Pool 1)

Study No.	Analysis set	LA-EP2006	Neulasta	Total
LA-EP06-301	FAS	159	157	316
	SAF	159	157	316
	SAF-C	130	116	246
LA-EP06-302	FAS	155	153	308
	SAF	155	153	308
	SAF-C	123	112	235
Pool 1	FAS	314	310	624
	SAF	314	310	624
	SAF-C	253	228	481

FAS=full analysis set; SAF=safety analysis set; SAF-C=a subset of patients of the SAF set who received only assigned investigational medicinal product throughout the study

Source: [Module 5.3.5.3-Statistical Overview-Table 3-1]

Due to shortages of IMP (see clinical efficacy, trial conduct) some patients in the two efficacy trials were treated with commercial GCSF products at some point. This is considered to compromise the data integrity of FAS and the SAF. Hence, the most sensitive analysis set to determine on comparable safety is the so called SAF-C, where all patients who received commercial GCSF at some point, were excluded. Exposure data (where exposure is defined as number of applications) are available for the following studies and subject populations:

- Healthy volunteers receiving 6 mg ZIOXTENZO s.c. (study LA-EP06-101; in this study, ZIOXTENZO was provided in glass vials containing 10 mg/1.0 mL ZIOXTENZO)
- Patients with breast cancer receiving 6 mg ZIOXTENZO s.c. (studies LA-EP06-301 and LA-EP06-302; in these studies, pre-filled syringes [PFS] containing 6 mg/0.6 mL ZIOXTENZO were used)

During the clinical development program, 0.25 years of exposure were obtained in 93 healthy volunteers in study LA-EP06-101, and 4.62 years of exposure in 314 patients with breast cancer in studies LA-EP06-301 and LA-EP06-302, based on the SAF population (see Table 1-4).

Table 1-4 LA-EP2006 exposure to healthy volunteers and patients

Study	Population	N	Days of exposure	Patient-years
LA-EP06-101	Healthy volunteers	93	93	0.25
LA-EP06-301 & LA-EP06-302	Patients	314	1687	4.62
All studies combined	Patients & healthy Volunteers	407	1780	4.87

Patient-years=days/365.25

Exposure data are based on the individual study reports

Exposure in healthy volunteers

In study LA-EP06-101 in healthy volunteers, 279 subjects were included, 93 of whom received ZIOXTENZO, 93 of whom received Neulasta EU and 93 of whom received Neulasta US. The planned dose of all three pegfilgrastim products was 6 mg, administered s.c. as a single injection. In the three treatment groups, actual mean (± standard deviation, SD) doses were 6.09 (± 0.17) mg for ZIOXTENZO, 6.00 (± 0.19) mg for Neulasta EU, and 6.08 (± 0.21) mg for Neulasta US.

Exposure in patients with breast cancer

Exposure to IMP

A summary of the overall number of patients exposed and the overall cumulative dose in patients with breast cancer is presented in Table 1-5 and Table 1-6. This dataset included 624 patients, 314 of whom received ZIOXTENZO and 310 of whom received Neulasta EU.

Each patient received single s.c. administrations of 6 mg ZIOXTENZO or Neulasta EU for up tosix cycles.

Table 1-5 Number of patients treated with a single dose of 6 mg LA-EP2006 or EU-authorized Neulasta – Pool 1 (SAF-C set)

	LA-EP2006 N=253 n (%)	Neulasta EU N=228 n (%)	Total N=481 n (%)
Cycle 1	253 (100.0)	228 (100.0)	481 (100.0)
Cycle 2	243 (96.0)	221 (96.9)	464 (96.5)
Cycle 3	237 (93.7)	221 (96.9)	458 (95.2)
Cycle 4	229 (90.5)	217 (95.2)	446 (92.7)
Cycle 5	223 (88.1)	213 (93.4)	436 (90.6)
Cycle 6	215 (85.0)	209 (91.7)	424 (88.1)

n=number of dosed patients; N=number of patients in a treatment group; Neulasta EU=EU-authorized Neulasta; SAF-C=a subset of patients of the safety analysis set (SAF) who received only assigned investigational medicinal product throughout the study

Source: [Module 5.3.5.3 Statistical Overview-Tables 4.1.1.2]

Table 1-6 Cumulative absolute dose administered (mg) over all cycles in patients – Pool 1 (SAF-C set)

	LA-EP2006 N=253	Neulasta EU N=228	Total N=481
Mean (SD)	33.2 (7.51)	34.4 (5.85)	33.8 (6.80)
Median (range)	36.00 (6.0-36.0)	36.00 (6.0-36.0)	36.00 (6.0-36.0)

N=number of patients in a treatment group; Neulasta EU=EU-authorized Neulasta; SAF-C= a subset of patients of the safety analysis set (SAF) who received only assigned investigational medicinal product throughout the study; SD=standard deviation

Source: [Module 5.3.5.3 Statistical Overview-Tables 4.2.1.2]

Exposure to chemotherapy

In studies LA-EP06-301 and LA-EP06-302 in patients with breast cancer, chemotherapy doses were calculated according to the baseline body surface area (BSA) for all cycles according to the Mosteller formula. If there was a 10% or greater change in body weight compared to baseline, the BSA was to be recalculated. During the first cycle and across all cycles, mean actual doses of chemotherapy were below planned doses. There were no relevant differences between the two treatment groups.

In Summary:

Exposure to Neulasta EU and the biosimilar candidate was similar in the Applicant's PK/PD trial in healthy volunteers. However, in breast cancer patients the SAF-C is stronger deminished (compared to the SAF) in Neulasta patients, than for ZIOXTENZO. For this analysis set cumulative mean dose is similar between treatments. Exposure to chemotherapy did not differ in a meaningful way. Overall patient exposure is considered similar between treatments.

Adverse events

AEs were coded according to the Medical Dictionary for Regulatory Activities (MedDRA), Version 12.1 (study LA-EP06-101), Version 16.0 (study LA-EP06-301), and Version 16.1 (study La-EP06-302).

If not indicated otherwise, the following sections refer to treatment-emergent AEs (TEAEs) in the three clinical studies.

Overall assessment of adverse events

After administration of ZIOXTENZO, Neulasta EU or Neulasta US, the incidence and nature of TEAEs were similar between the different treatment groups.

Study LA-EP06-101 in 279 healthy volunteers showed that a single s.c. dose of 6 mg ZIOXTENZO, Neulasta EU, or Neulasta US was well tolerated. The safety results were similar for all three pegfilgrastim products, with the highest proportion of events being reported in the system organ class (SOC) "musculoskeletal and connective tissue disorders" as could be expected based on the pharmacological action of a pegfilgrastim product.

In **studies LA-EP06-301 and LA-EP06-302** the overall frequency and nature of TEAEs were similar between ZIOXTENZO and Neulasta EU treatment groups in a total of 624 patients with breast cancer. Most TEAEs reported in both studies were typical chemotherapy-related events (e.g. alopecia, nausea, asthenia, and vomiting) while the IMP-related (as per investigator assessment) TEAEs were largely caused by pegfilgrastim's pharmacological effect, e.g. bone pain and myalgia.

In the clinical studies with ZIOXTENZO, the overall safety profile of ZIOXTENZO was similar to that of Neulasta EU and Neulasta US.

Seven patients receiving ZIOXTENZO and 4 patients receiving Neulasta EU died during studies LA-EP06-301 and LA-EP06-302. One of the 7 patients treated with ZIOXTENZO patients died during the 6-month SFU period of the LA-EP06-301 study. None of the TEAEs leading to death as outcome were suspected to be related to study drug.

Healthy volunteers

Table 2-1 Overview of all TEAEs in healthy subjects – study LA-EP06-101 (Safety population)

	LA-EP2006 (N=93)	Neulasta EU (N=93)	Neulasta US (N=93)	Overall (N=279)
Total number of subjects with:	**n (%)**	**n (%)**	**n (%)**	**n (%)**
TEAEs	82 (88.2)	84 (90.3)	81 (87.1)	247 (88.5)
TEAEs suspected to be due to study drug	77 (82.8)	82 (88.2)	81 (87.1)	240 (86.0)
Severe TEAEs	1 (1.1)	0 (0.0)	0 (0.0)	1 (0.4)
Serious TEAEs	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TEAEs leading to withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TEAEs leading to death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total number of:				
TEAEs	276	271	283	830
TEAEs suspected to be due to study drug	248	239	265	752
Severe TEAEs	1	0	0	1
Serious TEAEs	0	0	0	0
TEAEs leading to withdrawal	0	0	0	0
TEAEs leading to death	0	0	0	0

n (%)=number of subjects and percentage in relation to dose group; N=number of subjects in a treatment group; Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta; TEAE= treatment-emergent adverse event

Adverse events were generally equally distributed between treatments. 82,8% of patients in the ZIOXTENZO group experienced TEAES suspected to be due to study drug as compared to 88,2% in the Neulasta EU group. On the whole 248 TEAES related to study drug were observed under Zioxtenzo treatment as compared to Neulasta. One single serious TEAE occurred in the whole study, in the ZIOXTENZO treatment arm (headache). No deaths occurred. The overall incidences of TEAES can be considered comparable in the healthy volunteer population.

Breast Cancer Patients

Studies LA-EP06-301 and LA-EP06-302 had almost identical designs. A brief side-by-side presentation of the TEAEs in both studies is presented below.

Table 2-3 Overview of adverse events in patients with breast cancer – Pool 1 (SAF and SAF-C sets)

	SAF set			SAF-C set		
Number of patients with at least 1	LA-EP2006 (N=314) n (%)	Neulasta EU (N=310) n (%)	Total (N=624) n (%)	LA-EP2006 (N=253) n (%)	Neulasta EU (N=228) n (%)	Total (N=481) n (%)
TEAE	289 (92.0)	276 (89.0)	565 (90.5)	233 (92.1)	206 (90.4)	439 (91.3)
Study-drug related TEAE	71 (22.6)	66 (21.3)	137 (22.0)	53 (20.9)	45 (19.7)	98 (20.4)
Chemotherapy-related AE	283 (90.1)	269 (86.8)	552 (88.5)	228 (90.1)	200 (87.7)	428 (89.0)
Serious TEAE	45 (14.3)	53 (17.1)	98 (15.7)	37 (14.6)	39 (17.1)	76 (15.8)
Study drug-related serious TEAE	7 (2.2)	1 (0.3)	8 (1.3)	3 (1.2)	1 (0.4)	4 (0.8)
Chemotherapy-related serious AE	38 (12.1)	43 (13.9)	81 (13.0)	30 (11.9)	30 (13.2)	60 (12.5)
TEAE leading to study-drug discontinuation	6 (1.9)	7 (2.3)	13 (2.1)	6 (2.4)	7 (3.1)	13 (2.7)
Study-drug related TEAE leading to study drug discontinuation	1 (0.3)	0	1 (0.2)	1 (0.4)	0	1 (0.2)
TEAE leading to death as outcome	6 (1.9)[a]	4 (1.3)	10 (1.6)	6 (2.4)	4 (1.8)	10 (2.1)

n=number of patient with an event; N=number of patients in a treatment group; n.a.=not applicable; Neulasta EU=EU-authorized Neulasta; post-TEAE=post-treatment-emergent adverse event; SAF set=safety analysis set; SAF-C=a subset of patients of the SAF set who received only assigned investigational medicinal product throughout the study; TEAE=treatment-emergent adverse event

Patients could have events in more than one category.

[a] In study LA-EP06-301, 1 further patient in the LA-EP2006 treatment group died because of disease progression during the 6-month safety follow-up.

Incidence and severity were roughly comparable between Neulasta-EU and ZIOXTENZO. Slightly more TEAES (overall and study drug related) occurred in the ZIOXTENZO group in both individual studies and consequently also in the pooled data of both trials. The incidence of study drug related TEAES in the pooled data was 71(22,6%) in the ZIOXTENZO group versus 66(21,3%). The difference of 1,3% roughly stays the same when looking at the more sensitive SAF-C set (1,2%). This difference per se is not considered meaningful.

Table 2-6 TEAEs with a suspected causal relationship to IMP in healthy volunteers, by preferred term – study LA-EP06-101 (Safety population)

Preferred term[a]	LA-EP2006 (N=93) n (%)	Neulasta EU (N=93) n (%)	Neulasta US (N=93) n (%)
Back pain	58 (62.37)	65 (69.89)	65 (69.89)
Headache	48 (51.61)	52 (55.91)	42 (45.16)
Myalgia	23 (24.73)	26 (27.96)	22 (23.66)
Arthralgia	13 (13.98)	11 (11.83)	14 (15.05)
Chest pain	4 (4.30)	6 (6.45)	8 (8.60)
Nasopharyngitis	0 (0.00)	0 (0.00)	0 (0.00)
Neck pain	2 (2.15)	7 (7.53)	7 (7.53)
Abdominal pain	6 (6.45)	3 (3.23)	5 (5.38)
Oropharyngeal pain	0 (0.00)	0 (0.00)	0 (0.00)
Nausea	3 (3.23)	3 (3.23)	6 (6.45)
Dizziness	2 (2.15)	1 (1.08)	8 (8.60)
Diarrhea	2 (2.15)	0 (0.00)	5 (5.38)
Hyperhydrosis	3 (3.23)	2 (2.15)	3 (3.23)
Pain	4 (4.30)	1 (1.08)	3 (3.23)
Pain in extremity	2 (2.15)	1 (1.08)	5 (5.38)
Insomnia	1 (1.08)	5 (5.38)	1 (1.08)
Vomiting	3 (3.23)	1 (1.08)	1 (1.08)
Palpitations	1 (1.08)	1 (1.08)	3 (3.23)
Injection site erythema	1 (1.08)	0 (0.00)	2 (2.15)
Ear pain	0 (0.00)	0 (0.00)	2 (2.15)
Hot flush	2 (2.15)	0 (0.00)	2 (2.15)
Ocular hyperemia	1 (1.08)	2 (2.15)	0 (0.00)
Feeling hot	2 (2.15)	0 (0.00)	1 (1.08)
Malaise	2 (2.15)	0 (0.00)	1 (1.08)
Toothache	0 (0.00)	0 (0.00)	0 (0.00)
Musculoskeletal chest pain	2 (2.15)	0 (0.00)	0 (0.00)

IMP=investigational medicinal product; n (%)=number of subjects with percentage in relation to dose group (%); Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta; TEAE=treatment-emergent adverse event

Subjects could have events in more than one category.

[a] Only TEAEs with a frequency >2% in any treatment group are listed

Even though only a single dose trial, the sensitivity of the healthy volunteer trial in terms of safety (no chemotherapy involved) has to be acknowledged. The most frequently affected SOC (i.e. >10% of subjects in any treatment group) was musculoskeletal and connective tissue disorders (primarily back pain, myalgia and arthralgia), followed by nervous system disorders (primarily headache), general disorders and administration site conditions (primarily chest pain and pain), and gastrointestinal disorders (primarily nausea, diarrhea and vomiting). Nasopharyngitis was the only infection andinfestations event reported by more than 1 subject in any treatment group. When looking at TEAES in terms of SOC the incidence is in most of SOCs equal or smaller for the biosimilar candidate, with some exceptions: For general disorders and administration site conditions the incidence was 16 (17,20%) for ZIOXTENZO versus 10 (10,75%) for Neulasta EU. Although not of primary interest for a

European MA there was hardly any difference between US originator and biosimilar 17(18.28%). A similar picture is observed for the SOC "Gastrointestinal disorders" which are 3,22% more abundant for the biosimilar candidate compared to Neulasta EU, while again, the highest incidence is observed for Neulasta US. When looking at TAES organized after "preferred term" the difference in Gastrointestinal Disorders is most likely derived from a 3.22% higher incidence in vomiting and a 1.07% (1 patient) higher incidence in diarrhoea. According to the applicant most of the AEs in the SOC general disorders and administration site constitute of "chest pain" and "pain", and indeed, the incidence of unspecific pain is 3,22% higher for the biosimilar candidate, which at least partly explains the difference on SOC level.

The most frequently reported TEAE was back pain, followed by headache, myalgia, and arthralgia, all of which were reported with frequencies >10% across the treatment groups. All other TEAEs were reported with frequencies < 10% by subjects in a treatment group. The most frequently reported TEAEs (back pain, myalgia and arthralgia) are reflective of the pharmacological effects of pegfilgrastim. On the whole for most presumably study related TEAES in healthy volunteers the biosimilar candidate exhibits a similar to favourable pattern compared to Neulasta EU with the possible exception of gastrointestinal disorders (vomiting, diarrhea) and unspecific pain which were slightly more abundant in the ZIOXTENZO group.

Table 2-9 TEAEs with a suspected causal relationship to IMP in at least 1% of patients with breast cancer, by system organ class and preferred term – Pool 1 (SAF set)

System organ class Preferred term[a]	LA-EP2006 (N=314) n (%)	Neulasta EU (N=310) n (%)
Total number of patients with TEAEs with suspected relationship to study drug	**71 (22.6)**	**66 (21.3)**
Musculoskeletal and connective tissue disorders	32 (10.2)	30 (9.7)
Bone pain	14 (4.5)	19 (6.1)
Pain in extremity	9 (2.9)	5 (1.6)
Arthralgia	5 (1.6)	4 (1.3)
Myalgia	3 (1.0)	4 (1.3)
Back pain	4 (1.3)	2 (0.6)
General disorders and administration site conditions	24 (7.6)	23 (7.4)
Asthenia	10 (3.2)	12 (3.9)
Pain	5 (1.6)	4 (1.3)
Pyrexia	2 (0.6)	4 (1.3)
Blood and lymphatic system disorders	16 (5.1)	10 (3.2)
Leukocytosis	8 (2.5)	6 (1.9)
Neutrophilia	6 (1.9)	5 (1.6)
Neutropenia	4 (1.3)	2 (0.6)
Gastrointestinal disorders	11 (3.5)	12 (3.9)
Nausea	7 (2.2)	6 (1.9)
Diarrhea	2 (0.6)	6 (1.9)
Vomiting	2 (0.6)	3 (1.0)
Nervous system disorders	8 (2.5)	7 (2.3)
Headache	5 (1.6)	6 (1.9)

The comparison of specific AEs in breast cancer patients is considered less straightforward, considering the heavy side effects inherent to the concomitant chemotherapy. However al lot of data has been

generated in two confirmatory trials which, even if in terms of AEs it is hard to discriminate between study drug and chemotherapy related events, can provide deeper insight in the similarity of Zioxtenzo and Neulasta. Studies LA-EP06-301 and LA-EP06-302 independently showed comparable safety results: The overall incidences and pattern of TEAEs were widely similar in the ZIOXTENZO treatment groups compared with the Neulasta EU treatment groups in both studies. Expectably, TEAEs with the highest incidences were typical chemotherapy induced events (alopecia, nausea, asthenia, and vomiting). The most frequently affected SOC (i.e. >10% of patients in either treatment group) in Pool 1 was "gastrointestinal disorders" (primarily nausea, vomiting and diarrhea), followed by "general disorders and administration site conditions" (primarily asthenia, fatigue and pyrexia), "skin and subcutaneous tissue disorders" (primarily alopecia), "blood and lymphatic system disorders" (primarily neutropenia, leukopenia, anemia, and febrile neutropenia), "musculoskeletal and connective tissue disorders", "infections and infestations", "nervous system disorders", "metabolism and nutrition disorders", "respiratory, thoracic and mediastinal disorders", and "investigations". The remaining SOCs affected were reported by <10% of patients in either treatment group. Findings in the SAF-C set were similar to that of the SAF set.

When trying to discriminate IMP related AEs, the most common, presumably treatment related AEs are musculoskeletal and connective tissue disorders (10,2% for ZIOXTENZO vs 9,7% in Neulasta EU). No clear pattern of dissimilarity can be deduced from this SOC. Incidences are either similar, more abundant in the Neulasta group (e.g. bone pain 4,1% vs. 9,7%) or more abundant in the Zioxtenzo group (e.g. pain in extremity 2,9% vs. 1,6%).

Also other IMP related SOCs like GI disorders, General Disorders and Administration Site Conditions and Nervous system disorders, Investigations, Skin and sc. Disorders, as well as Respiratory, thoracic and mediastinal disorders display similar incidences.

Most "prominent" (>1%) differences in IMP related TEAEs occur in the SOC Blood and lymphatic tissue disorders (16/5,1% vs10/3,2%). There most notably, the incidence of the AE Neutropenia is about double the count for ZIOXTENZO (4/1,3% vs 2/0,6%) However, thorough evaluation of neutropenia has been closely linked to efficacy assessment (duration, incidence, febrile neutropenia, depth of nadir, etc.) and no relevant differences could be detected. Hence, the differences are not considered to be relevant.

Serious adverse events and deaths

Deaths

No death occurred in the Applicant's healthy volunteer study. In a setting of life threatening disease (breast cancer) and with concomitant treatment (TAC regimen) associated with many AEs it is hard to comparatively evaluate and attribute incidences of death between Neulasta and ZIOXTENZO. Analyses are additionally masked by occasional use of commercial products due to lack of IMP. In absolute numbers, there were 7 deaths in ZIOXTENZO treated patients and 4 deaths in Neulasta EU treated patients. One death case in the Zioxtenzo group had previously received commercial product. Most deaths were due to cardiovascular events or infections, and were likely related to chronic underlying diseases or chemotherapy. Two deaths that occurred in the patients receiving ZIOXTENZO were reported as cardiac arrest and cardio-respiratory arrest, but no details on patients' underlying diseases or conditions causing the death are available. By the investigators no death was judged as IMP related, however one patient died in the course of febrile neutropenia, which can, while probably caused by TAC regimen, at least be linked to the study drugs efficacy. However, neutropenia has been thoroughly characterized in efficacy assessment, where no difference between the two products was evident. It

can be concluded, that while there is a small difference in death cases in the two pivotal efficacy studies (7 vs. 4) none (or perhaps only one) of them can be attributed to be caused by study drug and are most likely associated to the severity of the disease and the concomitant chemotherapy.

Serious adverse events

No SEAES were observed in the Applicants healthy volunteer trial. However, in breast cancer patients, several SEAES occurred, where it is (as outlined previously) extremely difficult to attribute AES to either the severe disease, the TAC regimen or the study drug.

Overall, serious TEAEs were reported with slightly lower frequency in the ZIOXTENZO than in the Neulasta EU treatment group (45 [14.3%] vs. 53 [17.1%]). The most frequently affected SOCs were “blood and lymphatic system disorders” (primarily febrile neutropenia and neutropenia), “gastrointestinal disorders” (primarily abdominal pain, diarrhea and vomiting), “infections and infestations” (primarily neutropenic sepsis), and “general disorders and administration site conditions” (primarily pyrexia). Serious TEAEs that were recorded in $\geqslant$ 2% of patients in a treatment group were febrile neutropenia and neutropenia.

Serious TEAEs with a suspected causal relationship to study drug as per investigator assessment occurred with a low incidence in both treatment groups. Overall, febrile neutropenia was reported with similar frequencies in the two treatment groups (ZIOXTENZO: 8.0%; Neulasta EU: 10.0%;), however were considered more frequently to be related to IMP in the ZIOXTENZO treatment group as compared to the Neulasta EU treatment group (5 [1.6%] vs 0) (as per investigator assessment). All serious AEs were linked to neutropenia, and all were 0,3% to 1,6% more common in the Zioxtenzo group. It would be interesting to know, on what basis the same SAE was in some cases considered IMP related and in others not.

Table 2-12 Serious TEAEs in patients with breast cancer, by system organ class and preferred term – studies LA-EP06-301 and LA-EP06-302 (SAF set)

	LA-EP06-301		LA-EP06-302		Pool 1	
System organ class Preferred term[a]	LA-EP2006 N=159 n (%)	Neulasta EU N=157 n (%)	LA-EP2006 N=155 n (%)	Neulasta EU N=153 n (%)	LA-EP2006 (N=314) n (%)	Neulasta EU (N=310) n (%)
Total number of patients with serious TEAEs	**16 (10.1)**	**21 (13.4)**	**29 (18.7)**	**32 (20.9)**	**45 (14.3)**	**53 (17.1)**
Blood and lymphatic system disorders	10 (6.3)	17 (10.8)	19 (12.3)	23 (15.0)	29 (9.2)	40 (12.9)
Febrile neutropenia	9 (5.7)	12 (7.6)	16 (10.3)	19 (12.4)	25 (8.0)	31 (10.0)
Neutropenia	3 (1.9)	6 (3.8)	4 (2.6)	6 (3.9)	7 (2.2)	12 (3.9)
Anemia	1 (0.6)	2 (1.3)	1 (0.6)	0	2 (0.6)	2 (0.6)
Thrombocytopenia	0	1 (0.6)	1 (0.6)	1 (0.7)	1 (0.3)	2 (0.6)
Gastrointestinal disorders	2 (1.3)	1 (0.6)	6 (3.9)	10 (6.5)	8 (2.5)	11 (3.5)
Abdominal pain	0	0	3 (1.9)	5 (3.3)	3 (1.0)	5 (1.6)
Diarrhea	0	1 (0.6)	2 (1.3)	5 (3.3)	2 (0.6)	6 (1.9)
Vomiting	0	1 (0.6)	2 (1.3)	4 (2.6)	2 (0.6)	5 (1.6)
Infections and infestations	5 (3.1)	2 (1.3)	1 (0.6)	7 (4.6)	6 (1.9)	9 (2.9)
Gastroenteritis	1 (0.6)	0	0	2 (1.3)	1 (0.3)	2 (0.6)
Neutropenic sepsis	2 (1.3)	0	0	1 (0.7)	2 (0.6)	1 (0.3)
General disorders and administration site disorders	2 (1.3)	3 (1.9)	1 (0.6)	5 (3.3)	3 (1.0)	8 (2.6)
Pyrexia	1 (0.6)	1 (0.6)	1 (0.6)	2 (1.3)	2 (0.6)	3 (1.0)
Asthenia	1 (0.6)	0	0	3 (2.0)	1 (0.3)	3 (1.0)
Cardiac disorders	3 (1.9)	0	3 (1.9)	1 (0.7)	6 (1.9)	1 (0.3)
Cardio-respiratory arrest	2 (1.3)	0	1 (0.6)	0	3 (1.0)	0
Cardiac arrest	1 (0.6)	0	1 (0.6)	0	2 (0.6)	0
Respiratory, thoracic and mediastinal disorders	0	1 (0.6)	2 (1.3)	2 (1.3)	2 (0.6)	3 (1.0)
Pulmonary embolism	0	0	2 (1.3)[b]	0	2 (0.6)[b]	0
Metabolism and nutrition disorders	1 (0.6)	0	3 (1.9)	0	4 (1.3)	0
Dehydration	0	0	2 (1.3)	0	2 (0.6)	0
Nervous system disorders	1 (0.6)	0	3 (1.9)	0	4 (1.3)	0
Dizziness	1 (0.6)	0	1 (0.6)	0	2 (0.6)	0
Vascular disorders	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.7)	2 (0.6)	2 (0.6)
Musculoskeletal and connective tissue disorders	1 (0.6)	0	0	2 (1.3)	1 (0.3)	2 (0.6)
Musculoskeletal chest pain	0	0	0	2 (1.3)	0	2 (0.6)

n=number of patients with an event; N=number of patients in a treatment group; Neulasta EU=EU-authorized Neulasta; SAF set=safety analysis set; TEAE=treatment-emergent adverse event

[a] Only serious TEAEs with an incidence ≥ 2 patients in a treatment group of Pool 1

[b] Pulmonary embolism events were not suspected to be related to investigational medicinal product.

Patients could have events in more than one category.

Table 2-13 Serious TEAEs with suspected relationship to IMP in patients with breast cancer – Pool 1 (SAF set)

System Organ Class Preferred Term	LA-EP2006 (N=314) n (%)	Neulasta EU (N=310) n (%)
Total number of patients with serious TEAEs with suspected relationship to study drug	**7 (2.2)**	**1 (0.3)**
Blood and lymphatic system disorders	6 (1.9)	0
Febrile neutropenia	5 (1.6)	0
Neutropenia	1 (0.3)	0
Infections and infestations	2 (0.6)	1 (0.3)
Neutropenic sepsis	2 (0.6)	1 (0.3)
Investigations	1 (0.3)	0
Neutrophil count decreased	1 (0.3)	0
White blood cell count decreased	1 (0.3)	0

IMP=investigational medicinal product; n=number of patients with an event; N=number of patients in a treatment group; Neulasta EU=EU-authorized Neulasta; SAF set=safety analysis set; TEAE=treatment-emergent adverse event

Patients could have events in more than one category.

Source: [Module 5.3.5.3 Statistical Overview-Tables 5.8.1.1]

Laboratory findings

Healthy volunteers:

Hematology

In the three treatment groups, the mean number of leucocytes was markedly elevated due to the pharmacological effect of pegfilgrastim at the 48- and 72-hour post-dose assessments but was within normal ranges again at the Follow-up Visit, 14 days post-dose. The proportion of lymphocytes, monocytes, basophils and eosinophils tended to be reduced whereas the proportion of neutrophils was elevated at 48 and 72 hours post-dose. These observations were similar and in the same range for all pegfilgrastim products.

All other hematological parameters assessed showed no clinical significant abnormal hematological values in any subject.

Clinical chemistry and urinalysis

Values of LDH which were elevated at the 48- and 144-hour post-dose assessments decreased at follow-up. There were no differences among the treatment groups and no LDH elevations in any single subject which were categorized as CTCAE Grade 3 or 4.

All other clinical biochemistry and urinalysis parameters showed only incidental deviations from normal ranges which were of no clinical significance.

In summary, apart from changes of hematological parameters due to the effect of pegfilgrastim on neutrophils (absolute count of leukocytes, relative proportion of the subpopulations of white blood cells), there were no clinically significant deviations in hematology, clinical chemistry or urinalysis. The observations made were similar for all three pegfilgrastim products (ZIOXTENZO, Neulasta EU and Neulasta US).

No differences in hematology, chemistry and urinalysis were noted in healthy volunteers. Artificial hypoglycemia was observed in safety laboratory samples after the administration of pegfilgrastim in several subjects and when analyzing glucose from blood serum.

Patients with breast cancer

In studies LA-EP06-301 and LA-EP06-302, clinical laboratory evaluations for safety purposes were performed at screening, within 3 days before chemotherapy administration in each cycle and at end of treatment (EOT) (CBC for hematology efficacy parameters was measured more frequently in Cycle 1.) Clinically significant findings were reported as TEAEs.

Hematology

In both studies, numbers of patients in the SAF with clinically significant values in hematological parameters were similar between the treatment groups at all time points, and there were no considerable differences in absolute and relative changes from baseline between the treatment groups. Except for leukocytes, neutrophils and hemoglobin in study LA-EP06-301 and leukocytes, neutrophils and platelets in study LA-EP06-302, few patients (< 6%) in either treatment group had hematological parameters that were abnormal and clinically significant:

- study LA-EP06-301
- leukocytes (Cycle 1, Day 7): ZIOXTENZO: 19.6%; Neulasta EU: 22.4%
- neutrophils (Cycle 1, Day 7): ZIOXTENZO: 22.7%; Neulasta EU: 24.3%
- hemoglobin: ZIOXTENZO (Cycle 6, Day 1): 7.9%; Neulasta EU (Cycle 5, Day 1): 5.2%
- study LA-EP06-302 [Module 5.3.5.1 LA-EP06-302-Section 12.4.1]:
- leukocytes (Cycle 1, Day 7): ZIOXTENZO: 31.6%; Neulasta EU: 38.3%
- neutrophils (Cycle 1, Day 7): ZIOXTENZO: 37.0%; Neulasta EU: 42.4%
- platelets (Cycle 1, Day 8): ZIOXTENZO: 3.3%; Neulasta EU: 8.1%

Similar numbers of patients in the ZIOXTENZO and Neulasta EU treatment groups were observed with shifts from normal to abnormal values of hematological parameters. In study LA-EP06-301, differences > 4% were observed in hemoglobin, neutrophils, and monocytes.

In study LA-EP06-302, differences > 4% were observed in hemoglobin, erythrocytes, platelets, neutrophils, and eosinophils.

In Conclusion: incidences of abnormal and clinically relevant changes in hematologic parameters were similarly distributed between treatments, however a tendency for a lower number of deviations in leukocytes and consecutively in neutrophils for the biosimilar candidate is noted.

Clinical chemistry

Numbers of patients in the SAF with clinically significant values in clinical chemistry parameters were similar between the treatment groups and small in either treatment group (⩽ 2%) at any time point, and there were no considerable differences in absolute and relative changes from baseline between the treatment groups. Similar numbers of patients were observed with shifts to normal/abnormal values of clinical chemistry parameters.

Urinalysis

Numbers of patients in the SAF with clinically significant values in clinical chemistry parameters were similar between the treatment groups and small in either treatment group (< 2%) at any time point. Similar numbers of patients were observed with shifts to normal/abnormal values of urinalysis parameters.

In conclusion: no IMP related differences in Chemistry and urinalysis were noted.

Safety in special populations

Demonstration of safety in special populations is no integral part of a biosimilar exercise.

Immunological events

Healthy volunteers:

In study LA-EP06-101, serum samples for the assessment of immunogenicity were collected at 15 minutes pre-dose on Day 1 and on Days 15 and 28.

Subjects with a positive confirmatory result in the binding anti-pegfilgrastim antibody ELISA are summarized in Table 4-1. Neutralizing antibodies were not detected at any time point.

Table 4-1 Immunogenicity in healthy volunteers – study LA-EP06-101 (Safety population)

Binding ADA: confirmatory assay results	LA-EP2006 N=93 n (%)	Neulasta EU N=93 n (%)	Neulasta US N=93 n (%)
Pre-dose Visit 2 Day 1	0 (0.0)	3 (3.2)	3 (3.2)
Follow-up Visit Day 15	5 (5.4)	2 (2.2)	6 (6.5)
Follow-up Visit Day 28	2 (2.2)[a]	1 (1.1)[a]	3 (3.2)[a]

ADA=anti-drug antibody; n=number of subjects with an ADA positive sample; N=number of subjects in a treatment group; Neulasta EU=EU-authorized Neulasta; Neulasta US=US-licensed Neulasta

[a] Two subjects in the LA-EP2006 group, 1 subject in the EU-authorized Neulasta group and 1 subject in the US-licensed Neulasta group tested positive at Day 15 and Day 28.

The general incidence of ADAs was low in healthy volunteers. No neutralizing antibodies were detected.

3 patients in the Neulasta EU group had positive ADA titers at baseline, compared to none in the biosimilar group. 5 patients (5,4%) developed ADA at day 15 in the biosimilar group out of which 2 remained positive till day 28, whereas only one patient (1,1%) remained positive in the Neulasta EU group. The small numerical difference of non-neutralizing antibodies is not considered to be relevant.

Immunogenicity in patients with breast cancer

In studies LA-EP06-301 and LA-EP06-302, serum samples were collected prior to the first administration of the IMP (Cycle 1, Day1), on Day 15 of Cycle 6, on the EOS visit 4 weeks after the last administration of the IMP, and – in case of early termination – on the Early Termination visit. In study LA-EP06-301, an additional sample was collected on the 6-month SFU visit.

Numbers of patients with confirmed positive antibody results in the binding antibody ELISA at each sampling point are summarized for the individual studies and for Pool 1 in Table 4-2.

Table 4-2 Immunogenicity in patients with breast cancer: Number of patients with confirmed positive ADA results at each sampling point – studies LA-EP06-301 and LA-EP06-302 (SAF set)

	LA-EP06-301		LA-EP06-302		Pool 1	
Binding ADA: confirmatory assay results	LA-EP2006 N=159	Neulasta EU N=157	LA-EP2006 N=155	Neulasta EU N=153	LA-EP2006 N=314	Neulasta EU N=310
Cycle, Day	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Pegfilgrastim specific ADA						
Cycle 1, Day 1	15 (9.4)	19 (12.1)	8 (5.2)	10 (6.5)	23 (7.3)	29 (9.4)
End of study	1 (0.7)	0	0	0	1 (0.3)	0
6-month SFU	1 (0.8)	0	n.a.	n.a.	1 (0.8)	0
Combination positive samples[a]						
Cycle 1, Day 1						
Only Pegfilgrastim positive	0	1 (0.6)	0	0	0	1 (0.3)
Pegfilgrastim & PEG positive	7 (4.4)	13 (8.3)	8 (5.2)	10 (6.5)	15 (4.8)	23 (7.4)
Pegfilgrastim & Filgrastim & PEG positive	8 (5.0)	5 (3.2)	0	0	8 (2.5)	5 (1.6)
End of study						
Pegfilgrastim & PEG positive	1 (0.7)	0	0	0	1 (0.3)	0
6-month SFU						
Pegfilgrastim & PEG positive	1 (0.8)	0	n.a.	n.a.	1 (0.3)	0
Filgrastim specific ADA						
Cycle1, Day 1	8 (5.0)	5 (3.2)	0	1 (0.7)	8 (2.5)	6 (1.9)
Cycle 6, Day 15	0	0	0	1 (0.7)	0	1 (0.4)
End of treatment	1 (0.7)	0	0	1 (0.7)	1 (0.3)	1 (0.3)
End of study	0	0	0	1 (0.7)	0	1 (0.3)
6-month SFU	2 (1.7)	0	n.a.	n.a.	2 (1.7)	0
PEG specific ADA						
Cycle 1, Day 1	18 (11.3)	20 (12.7)	13 (8.4)	18 (11.8)	31 (9.9)	38 (12.3)
End of study	1 (0.7)	1 (0.7)	0	0	1 (0.3)	1 (0.3)
6-month SFU	1 (0.8)	0	n.a.	n.a.	1 (0.8)	0

ADA=anti-drug antibody; n=number of patients with an ADA positive sample; N=number of patients in a treatment group; n.a.=not applicable; Neulasta=EU-authorized Neulasta; PEG=polyethylene glycol; SAF set=safety analysis set; SFU=safety follow-up

Only time points with confirmed positive results in the confirmatory binding antibody enzyme-linked immunosorbent assay are shown.

Patients could have events in more than one category.

[a] Samples with pegfilgrastim specific ADA were further differentiated into: also filgrastim specific ADA; also PEG specific ADA; and only pegfilgrastim specific ADA

In study LA-EP06-301, one patient treated with ZIOXTENZO had combination positive anti-pegfilgrastim and anti-PEG antibody samples at EOS; as no pre-dose sample was taken, the immune status of the patient at the start of the study could not be evaluated. However, a developing immune response cannot be excluded. All samples were tested negative in the neutralizing antibody assay. Three patients in the ZIOXTENZO treatment group had positive ADA binding at 6-month SFU: One patient had combination positive anti-pegfilgrastim and anti-PEG antibody samples at the 6-month SFU. This patient was already tested positive for anti-pegfilgrastim and anti-PEG antibody at Cycle 1, Day 1, i.e. at pre-dose. Two patients had anti-filgrastim positive samples at the 6-month SFU visit. One of the patients was also tested positive at EOT, but not at Cycle 1, Day 1 and EOS. The second patient was tested negative at all other time points. However, all three patients were tested negative for anti-pegfilgrastim antibodies at the respective visits. As the anti-filgrastim test results for all positive samples were only slightly above the assay cut-points and the results were inconsistent to the anti-pegfilgrastim test, anti-filgrastim positivity was considered to be inconclusive; in addition, all samples were tested negative for neutralizing anti-pegfilgrastim antibodies.

In study LA-EP06-302, 1 patient in the Neulasta EU group was tested positive for anti-filgrastim binding antibodies at all sampling time points. However, neutralizing antibody results of this patient

were negative. One patient in the ZIOXTENZO treatment group had a positive neutralizing antibody result at Cycle 1, Day 1, i.e. at pre-dose This may be explained by pre-existing anti-PEG antibodies, which are frequently observed in the common population due to an improvement of the limit of detection of antibodies during the years and to greater exposure to PEG and PEG-containing compounds in cosmetics, pharmaceuticals and processed food products (Garay et al 2012). The characterization of the ADA response in the binding assay had demonstrated that ADAs were targeted against pegfilgrastim and/ or PEG, but not filgrastim. All post-dose sampling time points of this patient were determined negative for binding ADA. All neutralizing antibody results of the other patients with confirmed positive results for ADA binding were negative Hence, no neutralizing or clinically relevant antibodies were detected in studies LA-EP06-301 and LA-EP06-302 at post-dose time points.

In summary, the immunogenicity results evaluated by binding ADA and neutralizing antibody formation were similar in both treatment groups across both studies. In both treatment groups, varying amounts of patients tested positive for pegfilgrastim specific or peg specific ADAs. In the pooled data analysis from both studies, at baseline, 23 (7,3%) of patients were tested positive for pegfilgrastim specific antibodies in the biosimilar groups 29(9,4%) in the Neulasta EU group. 9,9% tested positive for PEG antibodies vs. 12,3 in the Neulasta EU group. The incidence antibodies present at baseline decreased or vanished during the treatment period. At end of study only one patient (0,7%) still tested positive for anti pegfilgrastim antibodies vs 0 in the Neulasta group. Similar numbers were detected when evaluating anti PEG antibodies. One patient in the biosimilar group tested positive for neutralizing antibodies at baseline, however all post dose samplings of this patient were negative for binding ADA. Apart from this not treatment related finding, no neutralizing antibodies could be detected. It can be concluded that the overall ADA incidence in both studies in breast cancer patients was low (higher at baseline, than end of treatment) and similar between treatments.

Safety related to drug-drug interactions and other interactions

n/a

Discontinuation due to AES

Table 2-14 TEAEs leading to discontinuation of treatment or study in patients with breast cancer – study LA-EP06-301 and LA-EP06-302 (SAF set)

	LA-EP06-301		LA-EP06-302		Pool 1	
System organ class Preferred term	LA-EP2006 N=159 n (%)	Neulasta EU N=157 n (%)	LA-EP2006 N=155 n (%)	Neulasta EU N=153 n (%)	LA-EP2006 (N=314) n (%)	Neulasta EU (N=310) n (%)
Total number of patients with TEAEs	**2 (1.3)**	**2 (1.3)**	**4 (2.6)**	**5 (3.3)**	**6 (1.9)**	**7 (2.3)**
Blood and lymphatic system disorders	1 (0.6)	0	1 (0.6)	0	2 (0.6)	0
Febrile neutropenia	1 (0.6)	0	1 (0.6)	0	2 (0.6)	0
Cardiac disorders	2 (1.3)	0	0	0	2 (0.6)	0
Cardio-respiratory arrest	2 (1.3)[a,b]	0	0	0	2 (0.6)[a]	0
Respiratory, thoracic and mediastinal disorders	0	0	1 (0.6)	2 (1.3)	1 (0.3)	2 (0.6)
Pulmonary embolism	0	0	1 (0.6)[c]	0	1 (0.3)	0
Allergic bronchitis	0	0	0	1 (0.7)	0	1 (0.3)
Organizing pneumonia	0	0	0	1 (0.7)	0	1 (0.3)
Nervous system disorders	0	1 (0.6)	1 (0.6)	0	1 (0.3)	1 (0.3)
Neuropathy peripheral	0	0	1 (0.6)	0	1 (0.3)	0
Peripheral sensory neuropathy	0	1 (0.6)	0	0	0	1 (0.3)
Infections and infestations	0	0	1 (0.6)	0	1 (0.3)	0
Clostridium difficile infection	0	0	1 (0.6)	0	1 (0.3)	0
Metabolism and nutrition disorders	1 (0.6)	0	0	0	1 (0.3)	0
Hypoglycemia	1 (0.6)[b]	0	0	0	1 (0.3)[a]	0
Gastrointestinal disorders	0	0	0	1 (0.7)	0	1 (0.3)
Peptic ulcer	0	0	0	1 (0.7)[d]	0	1 (0.3)[d]
Hepatobiliary disorders	0	1 (0.6)	0	0	0	1 (0.3)
Hepatotoxicity	0	1 (0.6)	0	0	0	1 (0.3)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	0	0	1 (0.7)	0	1 (0.3)
Breast cancer	0	0	0	1 (0.7)[d]	0	1 (0.3)[d]
Renal and urinary disorders	0	0	0	1 (0.7)	0	1 (0.3)
Renal impairment	0	0	0	1 (0.7)	0	1 (0.3)

No Adverse event leading to discontinuation occurred in healthy volunteers. In breast cancer patients discontinuations due to AEs happened sporadically in both treatment arms, after events which were mostly not considered related to the IMP. Of note two cases (0,6%) under ZIOXTENZO treatment in the pooled analysis discontinued, after events of febrile neutropenia, vs 0 under Neulasta treatment.

3.3.11. Discussion on clinical safety

In the Applicant's PK/PD study in healthy volunteers, the overall safety and tolerability of both products was good. There were no serious adverse events or adverse events which led to the premature withdrawal of subjects from the study. The most common adverse events were back pain, headache and myalgia. The majority of these AEs were suspected to be related to the IMP. There were no issues of clinical relevance with respect to clinical laboratory, vital signs, ECG recordings, local tolerability or immunogenicity (except for the PD effect on neutrophils).The cardiac safety profile assessed in a triplicate ECG sub study was similar in the 3 groups. There was an increase in heart rate (up to about 11 bpm) after 14 hours in all 3 treatment groups compared to baseline. The QTcF

intervals showed small changes which were mostly negative (QTcF shortening) for females and up to 4.2 ms prolongation for the males in the Neulasta US treatment group. For ZIOXTENZO treatment the largest QTcF prolongation was present in the male subgroup at 24 hours post-dose (1.8 ms). All 3 pegfilgrastim products showed good local tolerability, as judged by VAS subjective pain severity at the injection site and the Investigator injection site reaction (ISR) score. There was no bleeding, fluid loss or swelling at the injection site and a few cases of erythema. The immunogenicity evaluation confirmed the very low immunogenicity of pegfilgrastim. Overall, the majority of antibodies was detected pre-dose and directed against PEG; the presence of anti-PEG antibodies in normal subjects is known from the literature. A low anti-pegfilgrastim response was detected in a few healthy subjects (2.5%, equally distributed across treatment arms); none was neutralising. In the efficacy trials, no anti-pegfilgrastim antibodies were detected in the Neulasta arm while two patients (0.6%) exposed to ZIOXTENZO had anti-pegfilgrastim and anti-PEG antibodies at the end of the study/follow-up; however, one was already positive before treatment and the other had no pre-dose sample taken. These antibodies were not neutralising.

On the whole for most presumably study related TEAES in healthy volunteers the biosimilar candidate exhibits a similar to favourable pattern compared to Neulasta EU with the possible exception of gastrointestinal disorders (vomiting, diarrhea) and unspecific pain which were slightly more abundant in the ZIOXTENZO group.

The comparison of specific AEs in breast cancer patients is considered less straightforward, considering the heavy side effects inherent to the concomitant chemotherapy. However al lot of data has been generated in two confirmatory trials which, even if in terms of AEs it is hard to discriminate between study drug and chemotherapy related events, can provide deeper insight in the similarity of Zioxtenzo and Neulasta. Studies LA-EP06-301 and LA-EP06-302 independently showed comparable safety results: The overall incidences and pattern of TEAEs were widely similar in the ZIOXTENZO treatment groups compared with the Neulasta EU treatment groups in both studies. Expectably, TEAEs with the highest incidences were typical chemotherapy induced events (alopecia, nausea, asthenia, and vomiting). The most frequently affected SOC (i.e. >10% of patients in either treatment group) in Pool 1 was “gastrointestinal disorders” (primarily nausea, vomiting and diarrhea), followed by “general disorders and administration site conditions” (primarily asthenia, fatigue and pyrexia), “skin and subcutaneous tissue disorders” (primarily alopecia), “blood and lymphatic system disorders” (primarily neutropenia, leukopenia, anemia, and febrile neutropenia), “musculoskeletal and connective tissue disorders”, “infections and infestations”, “nervous system disorders”, “metabolism and nutrition disorders”, “respiratory, thoracic and mediastinal disorders”, and “investigations”. The remaining SOCs affected were reported by <10% of patients in either treatment group. Findings in the SAF-C set were similar to that of the SAF set. When trying to discriminate IMP related AEs, the most common, presumably treatment related AEs are musculoskeletal and connective tissue disorders (10,2% for ZIOXTENZO vs 9,7% in Neulasta EU). No clear pattern of dissimilarity can be deduced from this SOC. Incidences are either similar, more abundant in the Neulasta group (e.g. bone pain 4,1% vs. 9,7%) or more abundant in the ZIOXTENZO group (e.g. pain in extremity 2,9% vs. 1,6%).

Also other IMP related SOCs like GI disorders, General Disorders and Administration Site Conditions and Nervous system disorders, Investigations, Skin and sc. Disorders, as well as Respiratory, thoracic and mediastinal disorders display similar incidences.

Most “prominent” (>1%) differences in IMP related TEAEs occur in the SOC Blood and lymphatic tissue disorders (16/5,1% vs10/3,2%). There most notably, the incidence of the AE Neutropenia is about double the count for ZIOXTENZO (4/1,3% vs 2/0,6%) However, thorough evaluation of neutropenia has been closely linked to efficacy assessment (duration, incidence, febrile neutropenia, depth of nadir,

etc.) and no relevant differences could be detected. Hence, the meaningfulness of those differences are unclear. The “difference” stays the same when evaluating serious adverse events. The most frequently affected SOCs were “blood and lymphatic system disorders” (primarily febrile neutropenia and neutropenia), “gastrointestinal disorders” (primarily abdominal pain, diarrhea and vomiting), “infections and infestations” (primarily neutropenic sepsis), and “general disorders and administration site conditions” (primarily pyrexia).

Serious TEAEs with a suspected causal relationship to study drug as per investigator. assessment occurred with a low incidence in both treatment groups. Overall, febrile neutropenia was reported with similar frequencies in the two treatment groups (ZIOXTENZO: 8.0%; Neulasta EU: 10.0%;), however were considered more frequently to be related to IMP in the ZIOXTENZO treatment group as compared to the Neulasta EU treatment group (5 [1.6%] vs 0) (as per investigator assessment). All serious AEs were linked to neutropenia, and all were 0,3% to 1,6% more common in the Zioxtenzo group. It would be interesting to know, on what basis the same SAE was in some cases considered IMP related and in others not and how the different reporting system for AEs produced at least a small numerical difference, where non was seen in efficacy assessment.

In absolute numbers, there were 7 deaths in ZIOXTENZO treated patients and 4 deaths in Neulasta EU treated patients. One death case in the Zioxtenzo group had previously received commercial product.

Most deaths were due to cardiovascular events or infections, and were likely related to chronic underlying diseases or chemotherapy. Two deaths that occurred in the patients receiving ZIOXTENZO were reported as cardiac arrest and cardio-respiratory arrest, but no details on patients’ underlying diseases or conditions causing the death are available. By the investigators no death was judged as IMP related, however one patient died in the course of febrile neutropenia, which can, while probably caused by TAC regimen, at least be linked to the study drugs efficacy. Exposure adjusted mortality incidence rate was nearly double for ZIOXTENZO compared to Neulasta in Pool 1 (0.068 vs. 0.038). Three of the patients who died in the Neulasta arm had fatal chemotherapy or disease-related events of infection or disease progression. The last, a patient with a known history of reactive depression, committed suicide. Other than the 1 case of infection and 1 of disease progression, the cause of some of the deaths in the ZIOXTENZO arm is less clear. The patients had curable Stage II/ II disease and were receiving adjuvant treatment. Mortality should be relatively low and the majority did not have evidence of significant concomitant disease. Four patients suffered a cardiac/ cardio-respiratory arrest, attributed to PE (1), hypoglycaemia (1) and ‘unknown causes’ (2). It is unclear whether the PE was formally diagnosed by imaging and it is unusual to suffer fatal hypoglycaemia once hypoglycaemia has been diagnosed and is being managed. The case of hepatic necrosis is a concern, although ischaemic necrosis due to severe hypotension/ haemorrhagic shock likely contributed. The numbers of patients involved is small and may simply be due to chance.

It can be concluded, that while there is a small difference in death cases (7 vs. 4) none (or possibly only one) of them can be attributed to be caused by study drug. All death cases are most likely associated with the severity of the underlying disease and the concomitant chemotherapy.

The general incidence of ADAs was low in healthy volunteers. No neutralizing antibodies were detected.

The small numerical difference of non-neutralizing antibodies is not considered to be relevant, especially when considering the observed PK overexposure of the biosimilar candidate.

In breast cancer patients, the immunogenicity results evaluated by binding ADA and neutralizing antibody formation were similar in both treatment groups across both studies. In both treatment groups, varying amounts of patients tested positive for pegfilgrastim specific or peg specific ADAs. In the pooled data analysis from both studies, at baseline, 23 (7,3%) of patients were tested positive for

pegfilgrastim specific antibodies in the biosimilar groups 29(9,4%) in the Neulasta EU group. 9,9% tested positive for PEG antibodies vs. 12,3 in the Neulasta EU group. The incidence antibodies present at baseline decreased or vanished during the treatment period. At end of study only one patient (0,7%) still tested positive for anti pegfilgrastim antibodies vs 0 in the Neulasta group. Similar numbers were detected when evaluating anti PEG antibodies. One patient in the biosimilar group tested positive for neutralizing antibodies at baseline, however all post dose samplings of this patient were negative for binding ADA. Apart from this not treatment related finding, no neutralizing antibodies could be detected. Concerning immunogenicity it can be concluded that the overall ADA incidence in in breast cancer patients and healthy volunteers was low (higher at baseline, than end of study) and similar between treatments. No issues with local tolerability were identified with ZIOXTENZO or Neulasta EU. Changes in laboratory haematology parameters were similar between the 2 groups. Both products caused elevations in LFTs, with similar mean changes and proportion of results outside the normal range. As with the normal volunteers, elevation in ALT appeared more marked than in AST. More abnormal results were deemed to be clinically significant with ZIOXTENZO than Neulasta, particularly with regards to ALT levels in Study 301, although the numbers involved remained small. These were all recorded as TEAEs in the investigations SOC.

3.3.12. Conclusions on clinical safety

Overall safety as assessed in the PK/PD study in healthy volunteers and efficacy studies in the target population was similar between ZIOXTENZO and Neulasta. There were more deaths with ZIOXTENZO (7) than Neulasta (4) and the cause of death in some of the ZIOXTENZO cases, labelled only as cardiac arrest, has not been adequately evaluated. However, it is unlikely that further information will become available to adequately determine the cause of death. This numerical difference is likely a chance finding. A question remains regarding liver function tests. There were more LFT abnormalities, particularly ALT, considered to be clinically significant with ZIOXTENZO than Neulasta, a difference that should be discussed. Otherwise, it is considered that the safety data support biosimilarity of the test and reference products.

3.4. Risk management plan

Safety concerns

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

Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	• Severe splenomegaly/splenic rupture • Cutaneous vasculitis • Sweet's syndrome • Anaphylactic reaction • Capillary leak syndrome • Serious pulmonary adverse events (including Interstitial pneumonia and ARDS)

Summary of safety concerns	
	• Sickle cell crisis in patients with sickle cell disease • Musculoskeletal pain-related symptoms • Leukocytosis • Thrombocytopenia • Glomerulonephritis
Important potential risks	• AML/MDS • Cytokine release syndrome • Medication errors including overdose • Drug interaction with lithium • Off-label use • Immunogenicity (incidence and clinical implications of anti-pegfilgrastim antibodies) • Extramedullary hematopoiesis
Missing information	• Risks in children <18 years of age • Risks during pregnancy and lactation

Conclusions of the PRAC rapporteur on the safety specification

Having considered the data in the safety specification, the CHMP agrees that given that there is no need for additional pharmacovigilance activities or additional risk minimisation measures and, that this safety concern is unlikely to be specific to Zioxtenzo, the applicant should remove glomerulonephritis from the list of safety concerns in the RMP for Zioxtenzo. The PRAC Rapporteur agrees with this request.

Pharmacovigilance plan

The applicant proposes to monitor the majority of safety concerns via routine pharmacovigilance activities that include a targeted follow up questionnaires for the safety concerns: drug interaction with lithium and immunogenicity. The questionnaires are provided at Annex7 of the RMP.

Following the review of the follow up questionnaire for reports indicative of a drug interaction between pegfilgrastim and lithium, the form is designed as a check listed aimed at gathering further information on the adverse effect, diagnostic tests, past medical history and concomitant medications. The form assumes that patient details will be provided in the original report and this may not necessarily be the case. The applicant is asked to revise this questionnaire to include a request for patient details, white blood cell count at start of treatment and date, drug rechallenge and dechallenge and, include free text for medical history and diagnostic tests.

As there is a potential for all biologicals/biosimilars to lead to immunogenicity including pegfilgrastim and in line with the reference product, the applicant should give consideration to the feasibility of offering antibody testing for anti-pegfilgrastim antibodies for patients who are reported to have experienced adverse effects indicative of immunogenicity as part of routine pharmacovigilance activities. A flow diagram describing the process for testing should be provided at Annex 12 of the RMP. The flow diagram should describe the steps from identifying a report of pegfilgrastim associated adverse effects that may be indicative of immunogenicity, sending a request for a blood sample to the healthcare professional, shipment of the sample to the applicant and reporting back the result to the healthcare professional. The applicant should give consideration to revising the follow up questionnaire for the important potential risk of immunogenicity (incidence and clinical implications of anti-GCSF antibodies) in line with the request to offer an antibody test.

In line with the reference product, as part of routine pharmacovigilance activities the applicant should produce short follow up questionnaires for the safety concerns: capillary leak syndrome, cytokine release syndrome, medication errors, off-label use and pregnancy and lactation. The questionnaires should be submitted for consideration at Annex 7 of the RMP.

Summary of planned additional PhV activities from RMP

No additional pharmacovigilance activities are proposed by the applicants such as category 1-3 studies. No category 4 studies are planned or ongoing.

Additional pharmacovigilance activities to assess the effectiveness of risk minimisation measures

No additional pharmacovigilance activities to assess the effectiveness of risk minimisation measures are proposed by the applicant.

Overall conclusions on the PhV Plan

The applicant proposes to monitor the safety concerns for Zioxtenzo via routine pharmacovigilance activities and, this is considered to be acceptable. This is in line with the reference product for which routine pharmacovigilance activities are in place and no post-authorisation safety studies (Category 1-3) are required.

The proposed targeted follow up questionnaire for the safety concern of drug interaction between pegfilgrastim and lithium requires revision to include requests for patient details, white blood cell count at start of treatment and date, drug rechallenge and dechallenge and, include free text for medical history and diagnostic tests.

In line with the reference product, the applicant should develop follow up questionnaire for the safety concerns: capillary leak syndrome, cytokine release syndrome, medication errors, off-label use and pregnancy and lactation. The questionnaires should be submitted for consideration at Annex 7 of the RMP.

In line with the reference product, the applicant is asked to consider offering antibody testing for anti-pegfilgrastim antibodies for patients who are reported to have experienced adverse effects indicative of immunogenicity as part of routine pharmacovigilance activities. A flow diagram describing the process for testing should be provided at Annex 12 of the RMP. The flow diagram should describe the steps from identifying a report of pegfilgrastim associated adverse effects that may be indicative of immunogenicity, sending a request for a blood sample to the healthcare professional, shipment of the sample to the applicant and reporting back the result to the healthcare professional. The applicant

should give consideration to revising the follow up questionnaire for the important potential risk of immunogenicity (incidence and clinical implications of anti-GCSF antibodies) in line with the request to offer an antibody test.

The PRAC Rapporteur, having considered the data submitted, is of the opinion that routine pharmacovigilance is sufficient to identify and characterise the risks of the product.

The PRAC Rapporteur also considered that routine PhV are sufficient to monitor the effectiveness of the risk minimisation measures.

The proposed post-authorisation PhV development plans are sufficient to identify and characterise the risks of the products and are in line with that for the reference product.

Plans for post-authorisation efficacy studies

No post-authorisation efficacy studies are proposed by the applicants. This is acceptable.

Risk minimisation measures

Summary of risk minimisation measures from the RMP

The applicant proposes the following risk minimisation measures for Zioxtenzo (pegfilgrastim).

Table 1 Proposal from applicant for risk minimisation measures

Safety concern	**Routine risk minimisation measures**	**Additional risk minimisation measures**
Important identified risks		
Severe splenomegaly/splenic rupture	Guidance is provided in sections 4.4 Special warnings and precautions for use, 4.8 Undesirable effects, and 5.3 Preclinical safety data of the SmPC.	None
Cutaneous vasculitis	Guidance is provided in section 4.8 Undesirable effects of the SmPC.	None
Sweet's syndrome	Guidance is provided in section 4.8 Undesirable effects of the SmPC.	None
Anaphylactic reaction	Guidance is provided in sections 4.3 Contraindications, 4.4 Special warnings and precautions for use, 4.8 Undesirable effects and 6.6 Special precautions for disposal and other handling of the SmPC.	None
Capillary leak syndrome	Guidance is provided in sections 4.4 Special warnings and precautions for use and 4.8 Undesirable effects of the SmPC.	None
Serious pulmonary adverse events (including Interstitial pneumonia and ARDS)	Guidance is provided in sections 4.4 Special warnings and precautions for use and 4.8 Undesirable effects of the SmPC.	None
Sickle cell crisis in patients with sickle cell disease	Guidance is provided in sections 4.4 Special warnings and precautions for use	None

	and 4.8 Undesirable effects of the SmPC.	
Musculoskeletal pain-related symptoms	Guidance is provided in section 4.8 Undesirable effects of the SmPC.	None
Leukocytosis	Guidance is provided in sections 4.4 Special warnings and precautions for use and 4.8 Undesirable effects of the SmPC.	None
Thrombocytopenia	Guidance is provided in sections 4.4 Special warnings and precautions for use and 4.8 Undesirable effects of the SmPC.	None
Glomerulonephritis	Guidance is provided in sections 4.4 Special warnings and precautions for use and 4.8 Undesirable effects of the SmPC.	None
Important potential risk		
AML/MDS	Guidance is provided in section 4.4 Special warnings and precautions for use of the SmPC.	None
Cytokine release syndrome	Currently available data do not support the need for risk minimization.	None
Medication errors including overdose	Guidance regarding overdose is provided in sections 4.9 Overdose and 5.3 Preclinical Safety Data of the SmPC.	None
Drug interaction with lithium	Guidance is provided in section 4.5 Interaction with other medicinal products and other forms of interaction of the SmPC.	None
Off-label use	Currently available data do not support the need for risk minimization.	None
Immunogenicity (incidence and clinical implications of anti-pegfilgrastim antibodies)	Guidance is provided in section 4.4 Special warnings and precautions for use of the SmPC.	None
Extramedullary hematopoiesis	Currently available data do not support the need for risk minimization.	None
Missing information		
Risks in children <18 years of age	Guidance is provided in sections 4.2 Posology and method of administration, 4.8 Undesirable effects and 5.1 Pharmacodynamic properties and 5.2 Pharmacokinetic properties of the SmPC.	None
Risks during pregnancy and lactation	Guidance is provided in sections 4.6 Fertility, pregnancy and lactation and 5.3 Preclinical Safety Data of the SmPC.	None

Part V.3 and VI.1.4 (Summary table of risk minimisation measures), second column titled “routine risk minimisation measures” should be revised to include a summary of the relevant SmPC and PIL wording. Furthermore, in line with GVP Module V, the legal status should be mentioned as a part of a routine risk minimisation measure.

Additional risk minimisation measures

No additional risk minimisation measures are proposed by the applicant. This is considered to be acceptable.

Overall conclusions on risk minimisation measures

The PRAC Rapporteur having considered the data submitted was of the opinion that in line with the reference product the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication. However Parts V.3 and VI.1.4 of the summary table of risk minimisation measures should be revised to include a summary of the relevant SmPC/PIL wording and the legal status of the product.

Summary of activities in the risk management plan by medicinal product

Part VI.2.2 Summary of treatment benefits: the last paragraph referring to two studies demonstrating benefit of Zioxtenzo (pegfilgrastim) should be revised to briefly describe the number of subjects studied and their ages. Any differences in target populations where experience is limited and potential impact on efficacy due to factors such as age, gender, race, and organ impairment should also be described.

Part VI.2.4 Unknowns relating to treatment benefits: the second paragraph regarding limited evidence available in children should be revised to "…in children less than 18 years old."

Part VI.2.4 Summary of safety concerns: the risk of "severe allergic reactions" should be changed to "rapidly progressing life-threatening allergic reaction" to better explain the adverse effect to the lay reader. The use of the drug code ZIOXTENZO is unlikely to be recognised by the lay reader and therefore should be replaced with the active substance pegfilgrastim.

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.1 could be acceptable if the applicant implements the changes to the RMP as detailed in the endorsed Rapporteur assessment report and in the list of questions in section 6.3.

3.5. Pharmacovigilance system

The CHMP considers that the Pharmacovigilance system as described by the applicant fulfils the requirements and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

4. Orphan medicinal products

N/A

5. Benefit risk assessment

A biosimilar product refers to the demonstrated beneficial effects of the reference product and – besides a comprehensive comparability program - the benefit per se does not have to be established. Benefits and risks have been established for the reference product and can be deduced by

demonstrating similarity of the test product to the reference product in terms of quality, preclinical aspects, clinical pharmacology, efficacy and safety.

Benefits

Beneficial effects

Regarding the quality documentation, the relevant physicochemical and biological quality attributes of the proposed biosimilar have been characterised and compared with those of the reference medicinal product. In a first step similarity at quality level between the biosimilar candidate and Neulasta EU was demonstrated in a risk based, tiered statistical evaluation and then confirmed in a final head-to-head comparability study. Comparative stability data indicating similar stability behaviour under long-term, accelerated temperature and stress storage conditions support the biosimilar claim.

Furthermore, the Applicant has demonstrated that the overall manufacturing process for ZIOXTENZO, operated within established parameters, can perform effectively and reproducibly to produce material meeting its predetermined specifications and quality attributes.

The submitted non-clinical dossier included relevant comparative in vitro and well powered in vivo studies in naïve and neutropenic animals and did not indicate any differences regarding biosimilarity.

From a clinical perspective:

Pharmacodynamic comparability between ZIOXTENZO and Neulasta EU has been robustly demonstrated both in healthy volunteers and in the target population of neutropenic patients.

In healthy volunteers, the comparison of ANC parameters (AUEC0→last and Emax) showed geometric mean ratios very close to 100% (101 and 102%, respectively) and the 95% CIs contained within [-6%; +10%] are sufficiently narrow to rule out clinically meaningful differences between the two products. PD similarity could be demonstrated in the whole study population as well as in the gender specific subgroups. Furthermore, the CD34+ cell count curves appeared superimposable. In both confirmatory efficacy trials comparable performance of ZIOXTENZO and Neulasta could be demonstrated in terms of the mean duration of severe neutropenia (primary endpoint); the 95% confidence interval of the difference in group means was clearly within the pre-defined margin of ±1 day.

Comparable (numerical) results between Neulasta and ZIOXTENZO were seen in all secondary efficacy endpoints of both efficacy trials.

No relevant difference between Neulasta and ZIOXTENZO in the incidence of ADAs was observed in healthy volunteers and breast cancer patients.

Uncertainty in the knowledge about the beneficial effects

On the quality level some concerns which add to the uncertainty in the knowledge about the beneficial effects are related to short-comings in the description of the statistical methodology used for assessment of similarity, sampling and selection of batches included in the comparisons, and the size variability of the PEG moiety. A somewhat lower level of impurities has been observed for the biosimilar candidate which leaves residual uncertainty on biosimilarity. In addition, the shortcomings in process validation and process control lead to a minor doubt concerning process consistency and reliability.

Nonclinical in vivo studies suffered from high inter-individual variability regarding PK and PD readouts. This frequently limits their usefulness in a biosimilarity exercise, but seems especially intensified for pegylated modalities administered subcutaneously as the lymphatic system is heavily involved while PK measurement needs to rely on serum analysis. Additionally, bioanalysis of pegylated filgrastim is likely less sensitive if compared to non-pegylated filgrastim (as PEG may to a certain degree mask the binding site) and not discriminatory for non-pegylated variants likely occurring in the vascular system due to enzymatic digestion.

On the clinical level, uncertainties are identified with regard to the PK/PD study ZIOXTENZO-101and the clinical studies:

Similarity could not be demonstrated for the PK endpoints AUC0-t, Cmax and AUC0-inf in the PK/PD study. The variability turned out to be substantially larger than anticipated in study planning, which certainly can be considered a major reason for failing to demonstrate PK similarity. Furthermore, the exposure seems to be larger in ZIOXTENZO treated subjects when compared to Neulasta, a trend observed in two independent models. The observation of a larger exposure (after a single dose of ZIOXTENZO compared with Neulasta) was replicated in the PK profiles generated in breast cancer patients, where the point estimate of the geometric mean difference in AUC0-last of ZIOXTENZO versus Neulasta was around 120%.To determine whether there is a molecular difference between the study drugs or difference regarding clinical parameters that could account for the observed differences in PK, molecular attributes (including size, charge, hydrophobicity, pegylation, and higher order structure) as well as clinical baseline characteristics, study conduct, and immunogenicity results were evaluated for their potential contribution to the PK observations. The applicant concluded that small differences that do exist between the products based on structural and functional characterization, cannot individually account for the observed differences in PK.

In the pivotal PK/PD trial, the 90%CIs of the AUC and Cmax ratios were not contained within the standard equivalence interval but the study was clearly underpowered given that the assumed inter-subject variability used to calculate the sample size (CV 35%) was much lower than the observed variability (CV 73-89% for the test and EU reference product). Importantly, the EU and US reference products were not shown to be bioequivalent either, while they are highly similar at the quality level as would be expected. In the failed PK/PD study the observed coefficient of variation was higher than expected and ranged approximately between 73% and 92%.

Underestimation of the variability in the planning stage and preference of a parallel-group design might therefore be one reason that biosimilarity could not be demonstrated. However, on the basis of the upper limit of the 90% CI, a higher exposure to pegfilgrastim with ZIOXTENZO (up to 36%) cannot be ruled out compared to EU Neulasta. Overall, the range of true ZIOXTENZO/Neulasta EU ratios that are compatible with the study results is too large to make firm conclusions: the 90% CI of the ratio of geometric means of AUC0-last (and also AUC0-∞, which is very similar to AUC0-last), ranges from 89 to 136% in the healthy volunteer study, and from 82 to 180% in patients with breast cancer.

Furthermore considering the more than dose-proportional exposure and non-linear pharmacokinetics of pegfilgrastim (Yang, 2011), although no single causative factor could be detected in the physicochemical analysis, minor product differences may cumulatively translate into distinct PK differences. It should be further evaluated by the applicant, if relevant differences in baseline absolute neutrophil count between the treatment arms exist in study La-EP06-101, and to which extent this could influence the exposure, since the elimination of pegfilgrastim is predominantly neutrophil-mediated.

According to the post-hoc performed analysis, the exposure in the PK/PD study differs clearly between men and women: In females the PK response was lower, whereas the CV and difference in exposure (up to 80% higher exposure with ZIOXTENZO in the very limited subgroup of female subjects) between test and reference was higher than in male subjects. In the male subgroup the mean ratios for AUC0-last were close to 100%, whereas the 90% CIs remained outside the 80-125% range, explained by the applicant mainly due to the high variability and the smaller number of subjects included in this subgroup analysis. The Applicant's explanation of some gender differences being the root cause of the pivotal study failure is not convincing and likely to be an artefact of a post-hoc sub-group analysis. The applicant is asked to further analyse, which of these variables cause the gender-specific difference, explain their plausibility and how the PK parameters might be influenced by these factors.

Current literature is inconclusive concerning the question whether the used standard dose (6mg) is most sensitive (i.e. in the steep part of the dose response curve) to detect differences in PD. The PD results are assessed bearing in mind that, although ANC being a widely accepted PD surrogate efficacy endpoint, the sensitivity to show potential differences between test and reference product might be lower compared to pharmacokinetic comparison. In the pivotal PK/PD trial, a descriptive comparative analysis of the CD34+cells response is lacking.

The primary and secondary efficacy endpoints of both efficacy trials are, in terms of sensitivity, rather capable of confirming results observed in more sensitive (PK/PD) models than to prove similarity of therapeutic performance by themselves.

It is unclear how strongly safety and immunogenicity results were influenced by the occasional use of commercial G-CSF products.

One secondary endpoint "days of fever" was retrospectively adapted since the assessment of a fever episode contained a self-reported component, probably resulting in underreporting. Hence, it was decided to modify the endpoint "number of days of fever" to "number of patients with at-least one fever episode", which is considered acceptable, although the categorization may lead to information loss and the modified variable may be less sensitive to detect differences. If available, the information on the initial endpoint should be provided. Additionally, more information is needed about the instructions given to the patient about self-medication with antipyretics, analgesics or anti-inflammatories.

Efficacy analyses were performed to demonstrate therapeutic equivalence for duration of severe neutropenia (DSN) during Cycle 1, with the 95% CIs for the differences in mean DSN within a margin of ± 1 day. Both the endpoint and equivalence margin are considered acceptable and in line with CHMP Scientific Advice. The primary efficacy endpoint is calculated based on ANC, which was determined in local laboratories. It remains unclear whether the participating labs used validated techniques (e.g., certification, participation in quality control scheme) and how it was ensured that the methods were standardised across the laboratories. The Applicant is asked to elaborate on this further.

As the pre-filled syringes (including the colour of the products) were different, blinding of the trial was performed through the injection of the study products by an unblinded administrator. More details are requested about the procedures in place to ensure effective blinding in practice, in particular in the out-patient setting

Risks

Unfavourable effects

From the quality perspective the missing confirmation of the GMP compliance status of a manufacturing site is considered as a major risk.

From the clinical perspective:

Due to shortages in IMP throughout both clinical trials, patients were occasionally treated with different commercial G-CSF products. The Applicant provided sets (FAS-C and SAF-C) where only patients who never received commercial products were included. Differences or similarity between the FAS and SAF set on the one hand and the FAS-C and the SAF-C on the other hand, were discussed for the primary endpoint and overall TEAES, but not for e.g. pooled immunogenicity data and serious treatment related AEs.

A lower incidence of drug-related TEAEs was reported in study ZIOXTENZO-301 compared to study ZIOXTENZO-302. In addition, the main ADR of pegfilgrastim, i.e. musculoskeletal pain, was reported overall at a much lower rate than in the studies of the reference product (Neulasta EPAR). The Applicant should discuss the possible reasons for these observations.

The incidence of the AE neutropenia is about twice as high for ZIOXTENZO (4/1.3% vs 2/0.6%) than for Neulasta. The “difference” stays the same when evaluating serious adverse events. Also all serious IMP related AEs were linked to neutropenia, and all were 0.3% to 1.6% more common in the ZIOXTENZO group. Since reporting of AEs in the case of neutropenia, can be perceived as a “second perspective” on efficacy assessment, where different aspects of neutropenia were assessed as similar, it should be discussed whether small differences in safety reporting can “challenge” the similarity of efficacy.

A question remains regarding liver function tests. There were more LFT abnormalities, particularly ALT, considered to be clinically significant with ZIOXTENZO than Neulasta, a difference that should be discussed

Uncertainty in the knowledge about the unfavourable effects

As mentioned above from the quality perspective the unconfirmed GMP compliance status has been identified as major risk. In addition, a number of concerns related to various parts of Module 3 have been raised and add to the uncertainty in the knowledge about possible unfavourable effects. These concerns address primarily the control of certain raw materials, discrepancies in process control section, short-comings in the process validation, setting of specification limits, validation of analytical methods, and qualification of reference materials.

From a clinical perspective:

Across both trials, the company did not provide data of 14 patients which were in the FAS but not included in the tables of the primary efficacy measure due to “Missing due to blind data review meeting decision (absolute neutrophil count not available)” The applicant should justify, why those ANC profiles were not available and included in the analysis and present the patients in tabulated form.

Benefit-risk balance

Importance of favourable and unfavourable effects

Overall similarity of ZIOXTENZO to the reference product has been demonstrated at the quality- and the non-clinical level, even if a few minor issues still need to be clarified before reaching a definitive conclusion. So far clinical similarity of ZIOXTENZO to Neulasta has been shown as regards PD and clinical efficacy. Safety and immunogenicity also seem widely comparable, while the PK profiles, which are considered the most sensitive part of the clinical comparability exercise, are markedly different. (See discussion on B/R).

Benefit-risk balance

For a biosimilar, the benefit-risk balance is derived from the reference product provided the totality of evidence collected from the quality, non-clinical, and clinical data package supports the comparability of both products. Similarity has to be demonstrated throughout the development program and cannot be outbalanced by other factors.

Discussion on the benefit-risk balance

From a quality perspective the applicant conducted a robust and extensive overall biosimilarity exercise including a panel of highly sophisticated and state-of-the art methods, which compare the relevant physicochemical and biological quality attributes of the pegfilgrastim molecule. The data derived from these studies demonstrate that for most of the quality attributes similarity to the reference medicinal product has been shown. It should be noted that the biosimilar candidate has a slightly higher purity profile respective lower content of certain impurities than its reference product. However, from a quality point of view the absence of the GMP certificate for a manufacturing site is seen as major risk which currently precludes a recommendation for a positive opinion.

Results of several clinical studies were presented in order to provide evidence of biosimilarity. While two confirmatory efficacy trials reveal no substantial difference between biosimilar candidate and Neulasta (EU), similarity could not be demonstrated for the PK endpoints AUC0-t, Cmax and AUC0-inf in the PK/PD study. The variability turned out to be substantially larger than anticipated in study planning, which can be considered a major reason for failing to demonstrate PK similarity. Furthermore, the exposure seems to be larger in ZIOXTENZO treated subjects when compared to Neulasta, reflected by a point estimate of the GMRs of AUC0-t of 110%. The trend towards larger exposure has also been observed in breast cancer patients (point estimate of the GMR in AUC0-last around 120%).

The applicant's argumentation is mostly based on the suggestion that observed PK differences do not translate into relevant differences on the clinical level, underlining the similarity in efficacy between ZIOXTENZO and Neulasta as shown in two clinical trials of identical design. However, such statements deserve a closer look: The chosen models for both efficacy trials, study drug on top of TAC-chemotherapy in female breast cancer patients, with duration of severe neutropenia during cycle one, as primary efficacy measure, are meant to confirm results observed in earlier, more sensitive stages, rather than to counterbalance dissimilarity.

Efficacy endpoints, although chosen in line with current EMA guidance documents, are only supportive when judging clinical relevance due to their limited sensitivity. In terms of safety, clinical data is hard to compare in patients who suffer from a life threatening underlying disease and are heavily co-

treated. However it is acknowledged that in both trials no relevant differences regarding efficacy and safety were noted. So rather than stating that observed differences on PK level are not “clinically relevant” one should argue that observed PK differences do not seem to translate into relevant differences in efficacy and safety in a model of limited sensitivity.

Also a lot of emphasis is put on the fact that ZIOXTENZO and Neulasta are comparable in healthy volunteers with respect to the primary pharmacodynamic endpoint (ANC $AUEC_{0\text{-tlast}}$ and secondary PD endpoints (ANC E_{max}, ANC Tmax,E). However, current literature is inconclusive concerning the question whether the used standard dose (6mg) is sensitive (i.e. in the steep part of the dose response curve) to detect differences in PD.

In general a failed PK study cannot be compensated by results in less sensitive models even if they are suggestive of similarity. To determine whether there is a molecular difference between the study drugs or difference regarding clinical parameters that could account for the observed differences in PK, molecular attributes (including size, charge, hydrophobicity, pegylation, and higher order structure) as well as clinical baseline characteristics, study conduct, and immunogenicity results were evaluated for their potential contribution to the PK difference. Of note, some data indicate a slightly lower impurity profile of the biosimilar candidate. A critical discussion of these structural differences has been performed by the Company. Cumulatively, the applicant concluded that small differences that do exist between the products based on structural and functional characterization, cannot individually account for the observed differences in PK.

In forming an opinion on the biosimilarity of ZIOXTENZO the identification and discussion of quality differences (beside an already thorough comparability exercise on this level) potentially adding up to clinically meaningful differences is not of primary importance since knowing the underlying reason for dissimilarity cannot disparage its presence. Therefore, further elaboration on how to demonstrate biosimilarity is needed on a clinical level (see MO).

5.1. Conclusions

The overall B/R of ZIOXTENZO is negative.