20 September 2018
EMA/724003/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Fulphila

International non-proprietary name: pegfilgrastim

Procedure No. EMEA/H/C/004915/0000

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

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List of abbreviations

a.u	Absorbance units
ADA	anti-drug antibodies
AE	adverse event
AET	Analytical evaluation threshold
AIEX	anionic exchange chromatography
ANC AUC0-t	above baseline levels of the subject's absolute neutrophil count
ANC Cmax	maximum absolute neutrophil count
ANC Tmax	time of maximum change from baseline of absolute neutrophil count
ANC	absolute neutrophil count
ANOVA	analysis of variance
ANS	1-anilinonaphthalene-8-sulfonate
APQR	Annual Product Quality Review
AR	Analytical Reagent
AS	active substance
ATR	Attenuated Total Reflectance
AUC	analytical ultracentrifugation
AUC0-∞	area under the curve extrapolated to infinity
AUC0-t	area under the curve from time zero to t
BPI	Brief Pain Inventory
BRL	Biocon Research Limited
BSC/LAF	Bio Safety Cabinet / Laminar Air Flow
CAS	Chemical Abstract Service
CD	Circular dichroism
CD34+ Cmax	maximum concentration change from baseline for CD34+ cell counts
CD34+ Tmax	time of maximum change from baseline for CD34+ cell counts
CD34+	hematopoietic progenitor antigen positive cells
CD34+AUC0-t	area under the curve from time zero to t from baseline for CD34+ cell counts
CDS	Coding DNA Sequence
CE-SDS	Capillary electrophoresis sodium dodecyl sulfate method
CHR	Chromatography
CI	confidence interval
cIEF	iso- electric focusing
CIEX	cation exchange chromatography
CIEX-HPLC	Cation-exchange-High performance liquid chromatography
Cmax	maximum concentration
CPP	Critical Process Parameter
CQA	Critical quality attribute
CSR	clinical study report
CSS	clinical summary of safety
CTCAE	common terminology criteria for adverse events
CV	coefficient of variation
Da	Daltons
DF	diafiltration
DLS	Dynamic light scattering
DP	Drug Product
DS	Drug Substance
DSC	Differential Scanning Calorimetry
DSN	duration of severe neutropenia
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EoPCB	End of Production Cell Bank
EP	Elution Pool
ESI	Electrospray Ionization
EU	European Union
EU	European Union
EU/ml	Endotoxin units/millileter
FDA	Food and Drug Administration

FMEA	Failure Modes Effect Analysis
FN	Febrile neutropenia
FTIR	Fourier Transform Infrared Spectroscopy FP finished product
GC	Gas Chromatography
GCSF	granulocyte colony-stimulating factor
GLM	general linear model
Glu-C	Endoproteinase Glu-C
GPP	cGMP Pilot Plant
HCDNA	Host Cell Deoxyribonucleic Acid
HCl	Hydrochloric acid
HCP	Host Cell Protein
HMW	high-molecular-weight
HMWP	high molecular weight proteins
HPLC	High Pressure Liquid Chromatography
IB	Inclusion Body
IEX	Ion Exchange Chromatography
INN	international non-proprietary name
IPC	In-Process Control
IPT	In-Process Test
IRS	Internal reference standard
ISR	Injection site reaction
ITT	intent-to-treat
JP	Japanese Pharmacopeia
kDa	kilo Dalton
Kel	terminal elimination rate constant
LB	Luria Bertani
LMW	low-molecular-weight
LMWP	low molecular weight proteins
LOD	Limit of detection
LOQ	Limit of quantitation
LS	least squares
MALDI-TOF	matrix-assisted laser desorption ionization time of flight mass spectrometry
MCB	Master Cell Bank
mdeg	Millidegree
MHRA	Medicines and Healthcare Products Regulatory Agency
MNFS-60	murine derived cells from a myelogenous leukemia
mPEG-AL	α-Methyl-ω-(3-oxopropoxy), polyoxyethylene
MS	Mass spectrometry
MYL-1401H	Pegylated Granulocyte Colony Stimulating Factor
N	number of patients in the sample
N	total number of patients with available data during Cycle 1
NA	Not Applicable
Nab	neutralizing antibodies
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NIBSC	National Institute for Biological Standards and Control
NLT	Not Less Than
NMR	Nuclear Magnetic Resonance;
NMT	Not More Than
NTU	Nephelometric Turbidity Units
OD	Optical Density
PAR	proven acceptable ranges
PC	process characterisation
pCPP	Potential Critical Process Parameter
PD	pharmacodynamic(s)
PEG	Polyethylene Glycol propionaldehyde
PEG	polyethylene glycol
PEG-GCSF	Pegylated Granulocyte Colony Stimulating Factor
PFS	single-use prefilled syringe
Ph. Eur.	European Pharmacopeia
PI	Principle Investigator (if not Product Information)
PK	pharmacokinetic(s)
PMF	Peptide mass fingerprinting
PP	per-protocol (population)

PPCB	Post Production Cell Bank
PPM	parts per million
PPs	process parameters
PT	preferred term
PV	process validation
QA	Quality Assurance
QC	Quality Control
RCB	Research Cell Bank
RFU	Relative fluorescence units
rhG-CSF	recombinant human granulocyte colony-stimulating factor
rINN	recommended International Non-proprietary Name
r-met-HuG-CSF	recombinant methionyl human granulocyte colony-stimulating factor
RP-HPLC	Reverse Phase High-Performance Liquid Chromatography
RRT	Relative Retention Time
RSD	Relative standard deviation
RT	Retention Time
SAP	statistical analysis plan
SBS	Side by side
SC	subcutaneous
SD	Standard deviation
SDS-PAGE	Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis
SE	standard error
SEC-HPLC	Size Exclusion High-Performance Liquid Chromatography
Sl.	Serial
SLS	Static light scattering
SmPC	summary of product characteristics
SPR	Surface plasmon resonance
t1/2	apparent terminal elimination half-life
TAC	docetaxel, doxorubicin, and cyclophosphamide; a chemotherapy combination ("Taxotere"+"Adriamycine"+Cyclophosphamide)
TEAE	treatment-emergent adverse event
TFF	Tangential Flow Filtration
Tmax	time to maximum concentration
TNM	tumor-node-metastasis (disease stage of cancer diagnosis)
TOF-MS	Time-of-Flight Mass Spectrometry
TSE	Transmissible Spongiform Encephalopathy
UF	ultrafiltration
ULT	Ultra Low Temperature
US	United States (of America)
USP	United States Pharmacopeia
USP	upstream processing
UV	Ultraviolet
VAS	Visual analogue scale
Vd/F	apparent volume of distribution
WCB	Working Cell Bank
WFI	Water for injections
λmax	Wavelength maxima

1. Background information on the procedure

1.1. Submission of the dossier

The applicant MYLAN S.A.S submitted on 3 November 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Fulphila, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 14 September 2017.

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

The legal basis for this application refers to:

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

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

The chosen reference product is:

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

- Product name, strength, pharmaceutical form: Neulasta, 6mg, solution for injection
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22/08/2002
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/02/227/001-002-004

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

- Product name, strength, pharmaceutical form: Neulasta, 6mg, solution for injection
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22/08/2002
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/02/227/001-002-004

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

- Product name, strength, pharmaceutical form: Neulasta, 6mg, solution for injection
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 22/08/2002
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/02/227/001-002-004

Information on Paediatric requirements

Not applicable

Information relating to orphan market exclusivity

Similarity

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

Scientific advice

The applicant did not seek Scientific advice at the CHMP.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Martina Weise Co-Rapporteur: Alexandre Moreau

The application was received by the EMA on	3 November 2017
The procedure started on	23 November 2017
The Rapporteur's first Assessment Report was circulated to all CHMP members on	16 February 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	14 February 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	19 February 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 March 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	22 August 2018
The following GMP inspection was requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GMP inspection at one manufacturing site responsible for primacy packaging, processing operations for the medicinal product, quality control testing of the medicinal product and secondary packaging in India on 16 March 2018. The outcome of the inspection carried out was issued on	18 July 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	29 August 2018 and 13 September 2018

The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	6 September 2018
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Fulphila on	20 September 2018

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Fulphila is indented to be used for the reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes).

The Applicant claims the authorisation for Fulphila (also referred to as MYL-1401H in this report) as a similar product to Neulasta (EU) which was granted a marketing authorisation in the EU on 22 of August 2002. The proposed indication for Fulphila is the same as for the reference product Neulasta (EU).

2.1.2. Epidemiology and risk factors, screening tools / prevention

Chemotherapy-induced neutropenia and its subsequent infectious complications represent the most common dose-limiting toxicity of cancer therapy. Febrile neutropenia, FN, develops in 25% to 40% of treatment-naïve patients during common chemotherapy regimens depending on the patient population; the dosage, timing and type of chemotherapy used[1]. The severity of febrile neutropenia depends on the dose intensity of the chemotherapy regimen, the patient's prior history of either radiation therapy or use of cytotoxic treatment, and comorbidities.

2.1.3. Biologic features, aetiology and pathogenesis

The principal regulator of physiological granulopoiesis human G-CSF is a glycoprotein that has been shown to regulate the production and release of neutrophils from the bone marrow, mediated via a single affinity extracellular receptor. By binding and signalling through granulocyte colony-stimulating factor receptor (G-CSFR), G-CSF has multiple effects on circulating neutrophils and on neutrophil precursors in bone marrow[2].

Stimulation of precursor cell proliferation in the bone marrow leads to an increase in the total mass of G-CSFR-expressing cells, which serves as a negative regulator of G-CSF levels through accelerated clearance of G-CSF[3].

[1] Dinan MA, Hirsch BR, Lyman GH. Management of chemotherapy-induced neutropenia: measuring quality, cost, and value. J Natl Compr Canc Netw. 2015 Jan;13(1):e1-7
[2] Roberts AW. G-CSF: a key regulator of neutrophil production, but that's not all! Growth Factors. 2005 Mar;23(1):33-41
[3] Anderlini P, Champlin RE. Biologic and molecular effects of granulocyte colony-stimulating factor in healthy individuals: recent findings and current challenges. Blood. 2008 Feb 15;111(4):1767-72

2.1.4. Clinical presentation, diagnosis and stage / prognosis

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

2.1.5. Management

Primary prophylaxis with colony-stimulating factors, CSFs, reduces the frequency of chemotherapy induced neutropenia, all-cause mortality during chemotherapy, and need for hospital care e.g. in breast cancer[4] . The administration of G-CSF can accelerate the development of neutrophils from committed progenitors, thereby reducing the incidence, duration, and severity of neutropenia[5]. Forms of G-CSF such as filgrastim and lenograstim including biosimilars, are administered by a course of daily injections, whereas pegfilgrastim allows once-per-cycle administration and may avoid suboptimal daily dosing.

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

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

Pegfilgrastim and filgrastim can accelerate neutrophil recovery, leading to a reduced duration of the neutropenic phase in patients receiving cytotoxic chemotherapy. Filgrastim was initially approved for the prevention of infection as manifested by febrile neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive chemotherapy. The pivotal study in patients with small cell lung carcinoma receiving cyclophosphamide, etoposide, and doxorubicin chemotherapy demonstrated an approximately 50% reduction in the incidence of febrile neutropenia and duration of Grade 4 neutropenia, as well as statistically significant reductions in the incidence of hospitalizations and IV antibiotic usage[6]. Subsequent indications for filgrastim included engraftment following bone marrow transplantation, mobilization of peripheral blood progenitor cells and engraftment following transplantation, induction or consolidation chemotherapy for acute myeloid leukemia, and severe chronic neutropenia. Because of its relatively short half-life of 3.5 hours, filgrastim is administered once daily by SC administration no less than 24 hours after chemotherapy and continuing until absolute neutrophil count (ANC) recovery within each cycle of treatment. Shortcomings of filgrastim include the requirement for either daily visits to the clinic or home injections by the patient during the period of administration, frequent ANC monitoring, the possibility of missed doses, and suboptimal duration of treatment (either too short or too long). Efforts to overcome these limitations led to the

[4] Renner P, Milazzo S, Liu JP, Zwahlen M, Birkmann J, Horneber M. Primary prophylactic colony-stimulating factors for the prevention of chemotherapy-induced febrile neutropenia in breast cancer patients. Cochrane Database Syst Rev. 2012 Oct 17;10

[5] Dale DC. Colony-stimulating factors for the management of neutropenia in cancer patients. Drugs. 2002;62 Suppl 1:1-15

[6] Crawford J, Ozer H, Stoller R, Johnson D, Lyman G, Tabbara I, Kris M, Grous J, Picozzi V, Rausch G, et al. Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. N Engl J Med. 1991 Jul 18;325(3):164-70

PEGylation of the G-CSF protein. The subsequent PEGylation of the G-CSF protein filgrastim altered the pharmacokinetic (PK) profile, resulting in slower clearance and a prolonged half-life (between 15 and 80 hours), thus permitting a single injection per cycle of chemotherapy[7]. Pegylation of filgrastim increases the size of filgrastim so that it becomes too large for renal clearance. Due to its high molecular weight, pegfilgrastim exhibits limited transport into the blood capillaries after SC administration and enters the systemic circulation via an indirect route, through the lymphatics.

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

About the product

The active substance is a recombinant human granulocyte colony-stimulating factor (G-CSF) with a single 20 kDa peg-filgrastim as active substance. This application is based on Article 10(4) of CD 2001/83/EC (similar to a reference biological product) claiming Fulphila being "biosimilar" to Neulasta EU sourced (EU/1/02/227/001-002+004). The reference product is a pegylated (ATC code pegfilgrastim: L03AA13) filgrastim (ATC code filgrastim: L03AA02), thus a colony stimulating factor (CSF; L03AA).

Type of Application and aspects on development

The current product specific (non-clinical/clinical) guidance document is the document "Biosimilar medicinal products containing recombinant granulocyte-colony stimulating factor (Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues), EMEA/CHMP/BMWP/31329/2005". This annex has been in effect since 01/07/2006 whereas the current overarching NfGs (i) Similar biological medicinal products, ii) Similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues, iii) Similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues) are in effect since 2014-15.

The applicant did not seek Scientific Advice from the CHMP but had interactions with the Medicines and Healthcare Products Regulatory Agency (MHRA) on the clinical requirements.

GMP

GMP compliance of all drug substance and drug product manufacturing sites was confirmed by either a valid GMP certificate or a manufacturing authorisation (sites located in the EEA). In addition, a confirmation was provided that the manufacturing authorisation (MIA) for the EU batch release site at McDermott Laboratories will be updated to add the new medicinal product and the testing sites by variation to the MIA.

2.2. Quality aspects

2.2.1. Introduction

The finished product (FP) is presented as a solution for injection containing 6 mg pegfilgrastim (INN) as active substance. Other ingredients are: D-sorbitol, polysorbate 20 and sodium acetate.

[7] Foley C, Mackey MC. Mathematical model for G-CSF administration after chemotherapy. J Theor Biol. 2009 Mar 7;257(1):27-44

The product is available in a pre-filled syringe (Type I glass), with a bromobutyl rubber stopper and a stainless steel needle with or without an automatic needle guard.

Fulphila has been developed as biosimilar medicinal product to the reference product Neulasta.

The name Pegfilgrastim (MYL-1401H) is used to describe the active substance in this application.

2.2.2. Active Substance

General information

Pegfilgrastim active substance (AS) is a conjugate of recombinant methionyl human granulocyte colony-stimulating factor (r-met-HuG-CSF; filgrastim), covalently linked to a 20 kDa mono-methoxypolyethylene glycol (mPEG).

Filgrastim is an *E.coli*-derived non-glycosylated rhG-CSF, consists of 175 amino acids and is identical to natural human G-CSF except for the presence of an additional methionine at the N-terminal end, which is covalently linked to a single 20 kDa PEG (overall relative molecular mass of approx. 40 kDa). Filgrastim has an α-helical structure and contains five cysteine residues, four of which form two intra-molecular disulphide bonds required to maintain the biologically active conformation of the protein.

Manufacture, characterisation and process controls

The active substance is manufactured at Biocon Limited, Electronics City, Bangalore, India.

Description of manufacturing process and process controls

The Pegfilgrastim active substance manufacturing process has been adequately described.

The manufacturing process is a convergent process of the two critical intermediates recombinant filgrastim and activated mPEG.

The upstream process of GCSF manufacture is a high density *E. coli* cell culture process. The process ends with the harvest and cell lysis to gain the inclusion bodies (IB) containing the protein of interest. One batch of IBs (corresponding to the harvest of one upstream processing (USP) run) is further processed downstream by the purification process starting with thawing and solubilisation of the IBs followed by a refolding step and additional chromatographic and filtration purification steps. The intermediate is formulated and stored until PEGylation.

The manufacture and control of the activated mPEG has been adequately described.

Batches of the intermediate are pooled for PEGylation. The PEGylated GCSF is purified by a series of chromatography and filtration steps, including sterile filtration into appropriate containers. A batch numbering system is in place and has been described.

No reprocessing is claimed for AS manufacture. The bulk AS is shipped from the AS manufacturing site to the finished product (FP) manufacturing site for processing to finished product. The process has been adequately defined and in-process controls (IPCs) described to control the process.

Control of materials

G-CSF is expressed in an *E.coli* expression system.

The generation of the expression plasmid and the production strain has been described. A synthetic G-CSF gene has been prepared in order to optimise the codon usage for expression in *E.coli*.

Characterisation data of the active substance show that the transcription of the synthetic gene results in the desired amino acid sequence. A standard two-tier cell banking system is used (master cell bank-MCB and working cell bank-WCB) and cell banks and appropriate stability testing criteria are established for cell bank testing. The criteria applied for testing of the current WCB will be applied for future testing upon establishment of a new WCB.

Stability of the expression construct was investigated by generating and testing an end-of-production cell bank (EoPCB) and a post-production cell bank (PPCB).

Information on the raw materials is considered satisfactory. Compendial raw materials are tested in accordance with the corresponding monograph. If no compendial monograph is available, in-house specifications have been set.

Some column resins/filters contain specified materials of animal origin. Respective TSE certificates have been provided.

The synthesis of mPEG aldehyde is adequately described. The PEGylation reagent, activated mPEG has been classified as an intermediate.

Control of critical steps and intermediates

The manufacturing process employs multiple controls to ensure consistent quality of the active substance. Critical process steps have been defined during development and process characterisation. Before initiation of the process characterisation experiments, a Failure Mode and Effect analysis (FMEA) risk assessment was conducted to identify which process parameters could have an impact on product quality. These parameters are termed potential critical process parameters (pCPP). Process characterisation experiments were performed to identify real CPPs from the list of pCPPs.

The manufacturing process description is very detailed. Critical and non-critical process parameters (PPs) are defined with their acceptable ranges. The classification of the PPs is considered conclusive and consistent.

In-process controls (IPC) and in-process tests (IPT) have been defined to ensure consistent quality of the active substance. Acceptance criteria, and relative ranges, have been adequately justified.

Overall, together with the non-critical PPs and the proposed IPCs and IPTs, the upstream process is considered adequately controlled. The composition of the media, feed solutions and buffers are stated. The downstream process is considered adequately described and controlled by the proposed in process controls and tests.

G-CSF is considered a critical intermediate. Appropriate tests for identity, purity, content and potency are included. Batch analysis and stability data of G-CSF are acceptable. The proposed storage condition and time for this intermediate in specified containers is accepted.

The activated PEG is declared as being manufactured under GMP conditions in compliance with ICH Q7. The QP declaration certificate confirming the GMP status is in order. The starting material has been defined. The manufacturing process has been elaborated in sufficient detail. All relevant information on mPEG-AL and the starting material is provided.

Release and stability specifications are provided.

Process validation

The pegfilgrastim active substance manufacturing process has been validated adequately. Consistency in production has been shown on an appropriate number of commercial batches. Appropriate protocols for the validation of i) the manufacture of the intermediate G-CSF and ii) PEGylation of G-CSF were

provided. All acceptance criteria for the critical operational parameters and likewise acceptance criteria for the in-process tests are fulfilled demonstrating that the purification process consistently produces active substance of reproducible quality that complies with the predetermined specification and in-process acceptance criteria. Hold periods for process intermediates have been qualified by data on physicochemical stability and bioburden for in-process stages and buffer solutions.

The clearance of process-related impurities (host cell proteins, DNA and other specified impurities) has been satisfactorily evaluated and supports the proposed control strategy. Chromatography resin and ultrafiltration cartridge lifetimes have been appropriately qualified. Validation also includes details of process plant cleaning validation, leachables and extractables evaluation for process plant contact materials and finished active substance shipping validation.

Column re-use is foreseen during the manufacture of G-CSF and the number of cycles is defined based on respective re-use validation studies included in the dossier which are considered acceptable. Specified membrane re-use is also suitably discussed.

Manufacturing process development

The manufacturing process development of pegfilgrastim active substance was initially based on a manufacturing process which was then optimised to the commercial process.

A comparability study has been carried on pre- and post-change batches, and data provided demonstrated that the change did not have a significant influence on the quality of the product.

Comprehensive process characterisation (PC) studies have been performed for the single process steps and based on the results the process parameters were classified with respect to their criticality. The scaled-down models used for these studies were representative of the at scale manufacturing process.

Characterisation

The active substance has been comprehensively characterised by orthogonal methods.

The Applicant has provided characterisation data on both pegfilgrastim and the protein backbone alone, G-CSF.

The intact molecular mass of the entire molecule was confirmed. The correct attachment of PEG to the primary PEGylation site was verified. The mass was within the expected range, substantiating the correct attachment of the PEG moiety. The disulphide bond structure of pegfilgrastim was shown to be consistent with the expected structure. Overall, the primary sequence of pegylated G-CSF was confirmed.

The apparent molecular weight was also analysed.The secondary and tertiary structure of pegfilgrastim was analysed. The size variants were analysed by various methods. Surface plasmon resonance (SPR) was used to determine the binding kinetics to the G-CSF receptor. The results were comparable within the batches of pegfilgrastim and to the reference product. The biological activity of pegfilgrastim was investigated using the compendial NFS-60 cell proliferation assay. The results were within the predefined acceptance criteria and confirm that pegfilgrastim possesses the correct three-dimensional structure and exhibits qualitatively and quantitatively the expected biological activity.

The G-CSF (before the PEGylation step) was characterised with respect to intact mass, primary structure, confirmation of the disulphide bonds, higher order structure, and biological activity. Qualification data for the potency assay were provided substantiating its suitability. Overall, the identity and the expected structure of the G-CSF could be confirmed.

The PEG moiety was characterised. These data confirm the expected molecular mass and distribution.

Orthogonal chromatographic methods were applied to analyse purity and impurities. Characterisation of the impurities was performed thoroughly with respect to identification of the impurities and their stability indicating properties. Size-related variants were identified. The main degradation pathways of Pegfilgrastim are dimerisation/ oligomerisation, truncation and Des-PEGylation and oxidation, as confirmed by stress studies. Overall, the characterisation of product-related impurities is considered comprehensive and the results are consistent across the orthogonal methods.

Process-related impurities were monitored during manufacture of the consistency batches. The small molecule impurities were consistently below the detection level. Data for HCP and DNA were below the detection levels. Free PEG was detectable at consistently low levels in the more concentrated AS solution. Bacterial endotoxin was below detection level in the finished AS.

In summary, the characterization is considered appropriate for this type of molecule.

Specification

The active substance specification includes test parameters on identity, potency and content, purity, impurities, excipients, microbiological safety. The list of parameters is considered comprehensive. The active substance release and shelf-life specifications are identical overall (and contain the same number of parameters) but differ in the acceptance limits for AS-related impurities.

Biological activity (potency) of the active substance is determined by parallel line assay using M-NFS-60 cells. The cells depend on the presence of growth factors like G-CSF for their viability and proliferation. The potency assay mimics the functioning of Pegfilgrastim (MYL-1401H) based on the purported mechanism of action in vivo. There is a defined concentration range of G-CSF in which a linear correlation between the proliferation of the cells when stimulated with growth factor is observed. Determination of proliferation is carried out by photometric measurement of absorption observed from the reduction of tetrazolium compound (formazan) which produces colour under assay conditions.

The release specification limits for post peaks by RP-HPLC and HMWP by SE-HPLC were established in consideration of the proposed shelf life limits and the rate of degradation observed for these species over the proposed shelf life.

Analytical methods

The descriptions of non-compendial analytical methods used in the control of the active substance have been provided and are found to be acceptable in the level of detail.

Residual DNA is an in-house method using commercial extraction and quantitative kits. Residual HCP is determined by a commercial ELISA kit. Overall, sufficiently detailed information has been provided with regard to the validation of the proposed in-house analytical procedures. The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch release results have been provided for AS batches, that were included in clinical studies, process validation and stability studies. All batches comply with the predefined specification acceptance criteria in place at the time of analysis.

Batch release results have been provided for several batches of AS, that were included in clinical studies, process validation and stability studies. The batches were produced at commercial scale. All batches comply with the predefined specification acceptance criteria in place at the time of analysis. In addition, batch data provided represent the early and final commercial processes.

Reference materials

Sufficient details have been provided on the reference standard system established for AS manufacture. In-house laboratory standards (internal reference standards, IRS) and certified reference materials are used. The currently used primary IRS used for PEG-GCSF potency measurement has been adequately qualified. Any secondary IRS will be qualified against the primary IRS in terms of potency which is considered adequate.

Stability

A suitable 24 months shelf life is proposed for active substance when stored at 2–8^{o}C in Type I glass bottles.

Stability data are provided for several commercial AS batches which have been stored for the proposed shelf-life at the proposed long-term storage condition and for a specified period at accelerated conditions which is in accordance with ICH requirements. The stability protocols comprise all AS release test parameters and are therefore considered appropriate. Stability-indicating methods have been used in investigations. The data provided show that the batches complied with limits in force at that time although the specifications have been updated during the study but also with the proposed AS specification containing tighter limits for the product-related substances.

The stability data provided is supportive of the proposed shelf life for active substance stored in the proposed packaging.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Fulphila finished product consists of MYL-1401H pegfilgrastim as active substance, D-sorbitol (tonicity agent), polysorbate 20 (stabilising agent) and sodium acetate buffer (Buffering agent) is obtained by titrating acetic acid and sodium hydroxide.

Fulphila is supplied in a single-use prefilled syringe (PFS) containing 0.6 mL of the solution at a protein concentration of 10 mg/mL resulting in 6 mg pegfilgrastim per syringe. A specified overfill is included to ensure a withdrawal of 0.60 mL. The qualitative composition of Fulphila is the same as that of the reference product Neulasta

All excipients comply with the specifications described in the respective Ph. Eur. monographs.

It has been confirmed that the excipients used during the production of the medicinal product are not of animal origin and all excipients are well known and widely used in pharmaceutical products.

The intended commercial formulation is the same as that used in clinical trials.Despite identical target concentrations with the reference product, various studies were performed during pharmaceutical development to further support the proposed final composition of Fulphila.

Taking all study results together the qualitative and quantitative composition of Fulphila is sufficiently justified with regard to finished product stability.

Adequate characterisation studies were performed on the FP manufacturing process. The acceptable ranges of the process parameters were appropriately evaluated with regard to product quality and stability Finally, compatibility of all materials of construct used for FP manufacture and product stability was confirmed.

The finished product is filled into a Ph. Eur. Type I glass PFS closed with a bromobutyl elastomer with a Fluorotec coating and fitted with a staked hypodermic needle. The PFS is presented with or without a needle guard. Appropriate compatibility studies were also conducted with Fulphila formulation and the selected primary packaging system including a thorough evaluation of extractables and leachables. Overall it can be concluded that there is no impact of the container closure materials on protein stability at the recommended storage conditions. The suitability of the selected container closure system and its compatibility with Fulphila FP is satisfactorily demonstrated. Container closure integrity test used during stability studies to replace sterility testing and during manufacturing process validation was appropriately validated.

A risk assessment on elemental impurities in Fulphila FP was conducted in line with ICH Q3D. Subsequent analysis of FP lots confirmed the absence of metal residues.

Manufacture of the product and process controls

Batch release for the finished product is performed at McDermott Laboratories, Malahide Road, Dublin, Ireland.

Batch formula for a representative FP batch is provided. The manufacturing process is depicted in detail. The entire manufacturing process is separated in three stages. Stage A includes all steps up to the final formulated FP. Stage B comprises filtration and filling. In stage C the filled syringes are visually inspected and then assembled with the plunger rod and a needle guard. In addition, the single process steps are additionally described along with the in-process controls (IPC)/tests (IPT) performed at this stage.

The process description is satisfactory. The final formulated bulk is controlled for bioburden and subsequently sterile filtered. A major objection was raised during the evaluation procedure with regard to the adequacy of some of the controls proposed during this step of the manufacturing process, as they would not provide sufficient assurance in the control of sterility of the finished product. The Applicant has satisfactorily addressed this point and revised the application accordingly.

All process parameters applied during manufacture are listed together with their target value and the proven acceptable ranges (PAR) as evaluated during pharmaceutical development or process validation. The Applicant's designation to critical and non-critical process parameters is acceptable.

The maximum hold times are supported by appropriate data generated in hold time studies.

Manufacturing process validation was performed by the manufacture of an adequate number of consecutive FP batches. The process parameters applied during the manufacture were kept within their PARs. Overall, the process validation program applied was adequate to evaluate process consistency. All parameters checked during manufacture or at release were within the pre-defined ranges and all results of the IPCs met the predefined acceptance criteria. The batch release results complied with the FP specification acceptance criteria. Hence, the FP manufacturing process can be considered validated.

Validation of the aseptic conditions during FP filling was demonstrated by media fill runs. Impact of shipping on Fulphila stability was adequately studied by various storage and shipping studies conducted with AS and FP samples. Evidence was provided that the routine conditions during shipment can maintain the desired temperature range.

Finally, it was demonstrated that the technical properties of the PFS and the product quality characteristics are not negatively affected by the assembly process of the PFS with the needle guard.

The container closure components are purchased pre-sterilised.

Product specification

The FP specification includes test parameters on identity, potency and content, purity and impurities, pharmaceutical properties, microbiological safety, pre-filled syringe functionality and safety device testing.

All acceptance limits are adequately justified.

The Applicant is recommended to revise the FP shelf-life specification for the parameter 'aggregates', as well as for the impurities quantified by RP-HPLC, when data from further batches are available (see recommendation).

Analytical methods

In-house analytical methods used in the control of finished product are common with those of the active substance with the exception of product-specific parameters. Methods are appropriately validated in accordance with ICH guidelines. The analytical methods are shown to be stability indicating. The protocol and the report on method transfer of the potency assay used at the site responsible for QC testing on importation into the EU has been provided.

Batch analysis

Batch release results of several FP batches are presented, manufactured at commercial scale and which were used in clinical studies/ process validation/ stability studies. All results comply with the specification acceptance criteria applicable at the time of testing but also comply with the currently proposed tighter limits for impurities and product-related substances, and confirm consistency of the manufacturing process. In addition, analysis of FP batches in comparison to Neulasta batches did not reveal any new unknown impurities.

Reference materials

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

Post-Approval Change Management Protocol (PACMP)

In preparation for Brexit, the applicant included a PACMP covering the addition of test sites for finished product release to ensure uninterrupted EU importation testing. No changes are being made to the analytical methods. The only change being made is to the location of the testing laboratories. The additional laboratories all hold GMP Certificates. The new sites will be qualified according to a pre-approved method transfer protocol. The data from the analytical method transfer will be submitted as a Type IB variation.

The proposed PACMP is deemed acceptable.

Stability of the product

The proposed FP shelf-life in the commercial container system is 36 months when stored at 5±3 °C.

Stability studies have been initiated in accordance to ICH requirements with Fulphila FP batches at commercial scale. Stability-indicating methods have been used in investigations. Stability data at recommended storage temperature have been presented for a suitable number of FP lots packaged in the proposed container closure system, as well as for process validation batches. Here, not only physicochemical parameters but also functional stability has been tested. No out-of-specification results have been reported. Under accelerated conditions, an increase in some of the impurities could be observed in the FP.

The parameters ‘extractable volume’ and ‘actuation of safety device’ were checked in a separate functional stability study. The results obtained so far do not show any impact on extractable volume and actuation of the safety device of the PFS.

Forced degradation studies were performed in the course of analytical comparability evaluation against Neulasta.

These data confirm that Fulphila FP is susceptible to degradation when subject to several stress agents (e.g. photo exposure, mechanical stress, acidic and alkaline pH).

For long-term storage, appropriate instructions are included in the SmPC section 6.4 (‘store in a refrigerator (2°C-8°C))’. Moreover, the warning to keep the container in the outer carton is supported by the results of the photo-stability study. The SmPC storage instruction that Fulphila may be exposed to not more than 30°C for a maximum of 72 hours is supported by stability data.

Fulphila PFS stability after freezing has been demonstrated with the applicants own data. However, in view of a potential impact on container closure integrity freezing of the PFS is not recommended.

In conclusion, appropriate stability studies on Fulphila FP have been conducted. The claimed FP shelf life of 36 months when stored at 2-8°C is supported by sufficient data and is approvable.

Adventitious agents

Contract vendors are stated as having been audited and only animal origin-free materials procured for cell banking and manufacture of bulk AS. Raw materials are confirmed free of Transmissible Spongiform Encephalopathies and Bovine Spongiform Encephalopathies and in compliance with the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01). All have been confirmed as being of yeast or vegetable origin.

TSE certificates provided for materials of biological and non-biological origin used throughout active substance and finished product manufacture have been provided

The control of microbial contamination has been evaluated elsewhere in the dossier.

Viral adventitious agents are not applicable for the *E.coli* cell line. Cell banks have been satisfactorily evaluated for presence of bacteriophage.

Biosimilarity

Overall, the analytical data package comprehensively covers the quality attributes that need to be compared for demonstration of analytical similarity. Orthogonal methods have been applied to assess the individual parameters.

The number of batches of EU-sourced Neulasta as well as of Fulphila were considered adequate for the analytical comparability analysis.

The Applicant executed a risk assessment of the critical quality attributes of Neulasta and ranked the CQAs according to their potential influence on efficacy and safety of the product. The Applicant consequently thoroughly investigated these attributes and the test and reference product were highly similar if not identical in this respect.

Analytical similarity was evaluated based on a straight-forward statistical approach.

The approach to evaluating comparability by comparing individual data from multiple batches of test product with individual data from multiple batches of US and EU reference product is acceptable

although the statistical approach proposed for acceptance criteria is not accepted as the primary measure of comparability – assessment has primarily based comparison on the actual data ranges observed.

The primary sequence has been confirmed by peptide mass fingerprinting (ESI MS) after either Glu-C or Trypsin digest. Intact mass and the N-terminal PEGylation were confirmed by MALDI-TOF MS. The results confirm the desired amino acid sequence, PEGylation at the N-terminal methionine and similarity of the intact molecular mass.

SEC-MALS was used as an orthogonal method to determine the molar mass and hydrodynamic radius and also polydispersity. The data show a high level of conformity of the products regarding polydispersity thus confirming analytical similarity.

Secondary and higher order structures were investigated by various orthogonal analytical methods. S-S-bonds could be confirmed by the peptide map, the secondary structure was analysed using far UV CD and fourier transformed infrared spectroscopy (FTIR). The levels of α-helix, β-sheet, β-turn and random coils were comparable between the products within a given method. Levels of free cysteine were determined and again, the results were within the predefined acceptance criteria. No considerable differences were detected when subjecting the biosimilar and the reference product to near UV CD, differential scanning calorimetry (DSC), intrinsic and extrinsic fluorescence spectrometry. The NMR spectroscopy was used for a comparative fingerprint analysis only which is considered appropriate taking into account the challenges associated with the PEG-moiety. Overall, it can be concluded that the biosimilar Fulphila is highly similar to the reference product in terms of primary, secondary and tertiary structure.

This was further confirmed by the data showing similarity with respect to relative potency as measured by the compendial NFS-60 cell proliferation assay and with respect to GCSF-receptor affinity as measured by surface plasmon resonance.

The protein content data show that Fulphila meets this requirement for analytical similarity to Neulasta.

Purity and impurities were investigated applying the chromatographic methods used for release, i.e. CIEX, RP-HPLC and SE-HPLC. The risk associated with aggregates and dimers was classified as “high” due to the potential impact on clinical safety (immunogenicity risk) whereas the Di-PEGylated species and deamidated species are considered having a “moderate risk”.

The aggregates and dimers were analysed by SE-HPLC (aggregates, dimers+Di-PEGylated GCSF), analytical ultracentrifugation- AUC (aggregates), SEC-MALS (aggregates) and RP-HPLC (dimers). Di-PEGylated species were also analysed by CIEX by which they can be separated from the dimers. The average level of aggregates is slightly higher in Fulphila compared to Neulasta with two single values outside the similarity range. As the amount of Di-PEGylated species is lower than in Neulasta, the average sum of HMWPs as measured by SE-HPLC is still below the average sum of HMWPs in Neulasta. Taking additionally into account the relatively low absolute level of aggregates found in Fulphila, the biosimilarity is not considered impaired by this issue. Aggregates were additionally analysed by CE-SDS and AUC and the results were consistent among batches showing similarity of biosimilar and reference product in this respect, too.

LMWP include Des-PEG pegfilgrastim and N-terminal truncated species without PEG. SE-HPLC data show that the LMWP content determined in the biosimilar product is well below the amount of LMWP in Neulasta.

The RP-HPLC data show slight differences in the profiles of test and reference product. The amount of Q108 deamidated pegfilgrastim is slightly higher in Fulphila than in EU-Neulasta. However, the

absolute value is still rather low and no impact on safety is expected; thus, this difference is not considered precluding biosimilarity. The amount of M138 oxidised species is highly similar in biosimilar and reference products. The amount of dimer as measured by RP-HPLC is lower in Fulphila which is in agreement with the findings in the SE-HPLC analysis.

Comparability of biosimilar and reference product in terms of stability has been investigated by forced degradation studies under various stress conditions: acidic and alkaline conditions, oxidative stress, light exposure, accelerated, temperature stress conditions and mechanical stress by agitation. Even though slight differences in the degradation rates occurred, the degradation pathways were the same. The Applicant discussed the differences and postulates that the observed differences may be ascribed to the different age of the finished products when starting the stress studies and to the non-linear kinetics of degradation. Overall, the analytical similarity of MYL-1401H to Neulasta EU is considered proven.

The primary sequence has been confirmed as has the site of PEGylation. Secondary and higher order structures were investigated by various orthogonal analytical methods. Overall, it can be concluded that the biosimilar MYL-1401H is highly similar to the reference product in terms of primary, secondary and tertiary structure. This was further confirmed by the data showing similarity with respect to potency.

Purity and impurities were investigated. High and Low-molecular –weight species were analysed. Comparability of biosimilar and reference product in terms of stability has been investigated and no particular issues regarding the stability of MYL-1401H arose during DS and DP stability studies.

The similarity of EU to US-Neulasta is considered sufficiently demonstrated. This is of importance since several clinical studies were performed using the US-derived reference product. A summary of the biosimilarity studies is shown in **Table 1**.

Table 1: Summary of biosimilarity studies

Molecular parameter	Attribute	Methods for control and characterization	Key findings
Primary structure	Amino acid sequence	Peptide mass fingerprinting (Glu-C digest)	Identical primary sequence to reference product
		Peptide mass fingerprinting (Trypsin digest)	Identical primary sequence to reference product
		Intact MALDI TOF MS	Highly similar to reference product
	Pegylation site	N-terminal Pegylation by GluC digestion – MALDI-TOF MS	Highly similar to reference product
		N-terminal Pegylation by CNBr/trypsin digestion – ESI-TOF MS	Highly similar to reference product
		N-terminal Pegylation by Trypsin digestion – MALDI-TOF MS	Highly similar to reference product

Molecular parameter	Attribute	Methods for control and characterization	Key findings
	Polydispersity	MALDI-TOF	Highly similar to reference product
Higher order structure	Secondary and tertiary structure	Non-reduced peptide mass fingerprint Glu-C Digest (disulphide)	Identical to reference product
		Far UV CD spectroscopy	Highly similar to reference product
		FTIR	Highly similar to reference product
		Ellman's reagent (free Cysteine)	Highly similar to reference product
		Extrinsic Fluorescence	Highly similar to reference product
		Near UV CD spectroscopy	Highly similar to reference product
		Differential scanning calorimetry	Highly similar to reference product
		Intrinsic Fluorescence	Highly similar to reference product
		1D NMR	Highly similar to reference product
Biological Activity	Potency	MNFS-60 cell proliferation	Highly similar to reference product
	Receptor Binding	Surface Plasmon Resonance	Highly similar to reference product
Charge	Isoelectric point	cIEF	Highly similar to reference product
Purity/Impurities	HMWP-1 (Aggregates)	SEC-UV AUC SEC-MALS	Marginally higher than reference product Highly similar to reference product Highly similar to reference product
	Di-PEG-G-CSF	SEC-UV CIEX	Lower than reference product Lower than reference product

Molecular parameter	Attribute	Methods for control and characterization	Key findings
	Dimer	SEC-UV RP-HPLC	Lower than reference product Lower than reference product
	Des-PEG-G-CSF	RP-HPLC SEC-UV	Marginally higher than reference product Highly similar to reference product
	M138 Oxidation	RP-HPLC	Highly similar to reference product
	Q108 Deamidation	RP-HPLC	Marginally higher than reference product
	Total hydrophobic pre-peak	RP-HPLC	Highly similar to reference product
	Total hydrophobic post-peak	RP-HPLC	Marginally higher than reference product
	Purity by	RP-HPLC CIEX SEC-UV	Highly similar to reference product Marginally higher than reference product Marginally higher than reference product
Finished product attributes	Composition		Identical to reference product
	Protein content	UV280	Highly similar to reference product
	Subvisible particles	Micro-flow imaging	Lower than reference product

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The development, characterisation, manufacture and control of MYL-1401H active substance and finished product are adequately described, and questions raised during the procedure were adequately solved. Analytical similarity of MYL-1401H finished product to the reference product Neulasta (EU) has been satisfactorily demonstrated. Likewise, analytical similarity of Neulasta sourced from EU and US was proven.

At D120, a major objection has been raised as EU GMP compliance had not yet been confirmed for the finished product manufacturing site Biocon Limited, Plot No. 2-4, Phase IV, Bommasandra-Jigani Link

Road, Bangalore, India. A second major objection had been raised on the control of the FP sterile filtration step.

Both major objections have been satisfactorily solved.

As regards the finished product specifications, the Applicant is expected to revise the FP shelf-life specification for the parameter "aggregates", as well as for the impurities quantified by RP-HPLC, when data from further batches are available (see recommendation).

2.2.1. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data have been presented to give reassurance on TSE safety.

In conclusion, based on the review of the quality data provided, the CHMP considers that the marketing authorisation application for Fulphila is approvable from the quality point of view.

Analytical similarity of MYL-1401H finished product to the reference product Neulasta (EU) has been satisfactorily demonstrated. Likewise, analytical similarity of Neulasta sourced from EU and US was proven.

Based on the review of the data the CHMP considers that the active substance Pegfilgrastim contained in the medicinal product Fulphila is not to be qualified as a new active substance.

2.2.2. Recommendation for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended additional points for investigation:

- The Applicant is recommended to revise the FP shelf-life specification for the parameter "aggregates", as well as for the impurities quantified by RP-HPLC, when data from further batches are available. Revised specification acceptance criteria should then be introduced into the finished product shelf life specification via a variation application if appropriate.

2.3. Non-clinical aspects

2.3.1. Introduction

The functionality of MYL-1401H was compared to that of EU- and US-Neulasta with two *in vitro* assays, namely with the cell proliferation assay and the binding to target receptor by the SPR method. The binding affinity to the G-CSF receptor of MYL-1401H and EU- as well as US-Neulasta was also investigated using Surface Plasmon Resonance (SPR). The *in vitro* functionality assays were completed by a GLP-compliant *in vivo* study performed in chemically-induced neutropenic rats. A toxicokinetic study was included in the GLP compliant comparative 28 day repeat-dose toxicity study. The toxicological program included a single, GLP-compliant 28 day repeat-dose toxicity study in Sprague Dawley rats.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro

Receptor binding by SPR (Studies BDL/TR/BR.14.5003/15/001 and BDL/TR/BR.14.5003/16/001)

A summary of the kinetic data were provided in Table 2.

Table 2: Summary of Binding Kinetics of MYL-1401H and EU- and US-Neulasta to GCSF-R

Source	k_a (1/ms)			k_d (1/sec)			K_D (pM)		
	Min	Max	Avg (SD)	Min	Max	Avg (SD)	Min	Max	Avg (SD)
EU-Neulasta	4.23E+05	5.64E+05	4.91E+05 (5.36E+04)	1.53E-04	2.03E-04	1.70E-04 (1.58E-05)	302	393	350 (34)
MYL-1401H	3.98E+05	4.85E+05	4.33E+05 (2.90E+4)	1.54E-04	1.98E-04	1.69E-04 (1.38E-05)	324	440	394 (40)
US-Neulasta	3.83E+05	5.38E+05	4.26E+05 (5.58E+04)	1.25E-04	1.85E-04	1.49E-04 (1.97E-05)	287	488	356 (58)

EU: European Union; GCSF-R: granulocyte colony-stimulating factor receptor; k_a: association rate constant; k_d: dissociation rate constant; K_D: equilibrium dissociation constant; US: United States

Cell proliferation assay (Study DDL/TR/BR.14.5003/16/003)

M-NFS-60 cells were exposed to MYL-1401H, EU-Neulasta, or US-Neulasta for 44 to 50 hours. Proliferation was measured using a spectrophotometer after adding 3-(4,5- dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent, which is reduced to a soluble formazan product by living cells. The absorbance at 490 nm is proportional to the number of live cells. The geometric mean relative potencies ranged from 0.91 to 1.12 with a mean (± SD) of 1.05 ± 0.08 for MYL-1401H, from 0.91 to 1.07 with a mean of 1.00 ± 0.05 for EU-Neulasta, and from 1.03 to 1.16 with a mean of 1.07 ± 0.04 for US-Neulasta.

In vivo

Male CD / Crl:CD(SD) rats (10 animals per group) were treated once subcutaneously with MYL-1401H, EU- or US-Neulasta at dose levels of 100, 300, 1000 and 3000 mg/kg on 12 consecutive days. The results of the pharmacodynamics analysis of the neutrophilic granulocytes responses between the three products are given in Table 3 and of the ANC responses in Table 4:

Table 3: Pharmacodynamic Analysis of Neutrophilic Granulocyte Response Following Administration of MYL-1401H, EU-Neulasta, and US-Neulasta in Chemically-Induced Neutropenic Rats

Group	CPA dose on test day -1	Dose [µg/kg]	E_{max} [cells x 10^9/ L blood]	t_{max} [h]	$AUEC_{0-t\ last}$ [h*cells x 10^9/ L blood]	$AUEC_{eff\ 0-t\ last}$ [h*cells x 10^9/ L blood]*
1	None	0 (Vehicle)	2.598	103	483.840	-
2	CPA	0 (Vehicle)	2.588	226	305.928	-
3	CPA	100 (US Neulasta®)	8.124	38	929.640	623.712
4	CPA	300 (US Neulasta®)	7.978	72	971.772	665.844
5	CPA	1000 (US Neulasta®)	22.719	118	2358.324	2052.396
6	CPA	3000 (US Neulasta®)	39.344	122	3404.232	3098.304
7	CPA	100 (EU Neulasta®)	7.570	67	834.516	528.588
8	CPA	300 (EU Neulasta®)	7.468	89	866.292	560.364
9	CPA	1000 (EU Neulasta®)	19.574	113	1932.540	1626.612
10	CPA	3000 (EU Neulasta®)	40.164	122	3566.724	3260.796
11	CPA	100 (Biosimilar)	10.573	113	1097.292	791.364
12	CPA	300 (Biosimilar)	8.685	72	1008.468	702.540
13	CPA	1000 (Biosimilar)	21.172	132	2258.928	1953.000
14	CPA	3000 (Biosimilar)	48.573	125	4224.804	3918.876

#: effective AUEC of the dose level groups 3 to 14: $AUEC_{Gr.\ 3\ to\ 14} - AUEC_{Gr.2}$

Table 4: Pharmacodynamic Analysis of Leucocyte response Following Administration of MYL-1401H, EU-Neulasta, and US-Neulasta in Chemically-induced Neutropenic Rats

Group	CPA dose on test day -1	Dose [µg/kg]	E_{max} [cells x 10^9/ L blood]	t_{max} [h]	$AUEC_{0-t\ last}$ [h*cells x 10^9/ L blood]	$AUEC_{eff\ 0-t\ last}$ [h*cells x 10^9/ L blood]*
1	None	0 (Vehicle)	15.010	110	3451.680	-
2	CPA	0 (Vehicle)	10.050	264	1355.400	-
3	CPA	100 (US Neulasta®)	11.880	86	2057.760	702.360
4	CPA	300 (US Neulasta®)	12.450	118	2042.880	687.480
5	CPA	1000 (US Neulasta®)	30.350	118	3741.600	2386.200
6	CPA	3000 (US Neulasta®)	48.820	122	5005.080	3649.680
7	CPA	100 (EU Neulasta®)	11.040	96	1815.360	459.960
8	CPA	300 (EU Neulasta®)	11.130	120	1843.200	487.800
9	CPA	1000 (EU Neulasta®)	27.050	115	3334.320	1978.920
10	CPA	3000 (EU Neulasta®)	51.530	137	5235.000	3879.600
11	CPA	100 (Biosimilar)	15.120	113	1962.840	607.440
12	CPA	300 (Biosimilar)	12.770	86	2009.400	654.000
13	CPA	1000 (Biosimilar)	28.610	134	3640.080	2284.680
14	CPA	3000 (Biosimilar)	59.820	127	5961.840	4606.440

#: effective AUEC of the dose level groups 3 to 14: $AUEC_{Gr.\ 3\ to\ 14} - AUEC_{Gr.2}$

Secondary pharmacodynamic studies

The applicant did not submit secondary pharmacodynamic studies (see non-clinical discussion).

Safety pharmacology programme

The applicant did not submit safety pharmacology studies (see non-clinical discussion).

Pharmacodynamic drug interactions

The applicant did not submit pharmacodynamic drug interaction studies (see non-clinical discussion).

2.3.3. Pharmacokinetics

The applicant did not submit pharmacokinetic studies regarding absorption, distribution, metabolism, excretion, pharmacokinetic drug interaction and other pharmacokinetic studies conducted for MYL-1401H (see non-clinical discussion).

2.3.4. Toxicology

Single dose toxicity

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

Repeat dose toxicity

The applicant provided data from one comparative *in vivo* study in rats (Study TOX-071-001). MYL-1401H was administered subcutaneously once weekly at 0.15 mg/kg (low dose), 0.65 mg/kg (mid dose), and 1.5 mg/kg (high dose and high-dose recovery) to male and female Sprague Dawley rats for 28 days. These doses were equivalent to approximately 0.24, 1.05, and 2.43 times (respectively) the intended dose in humans (6 mg/chemotherapy cycle) based on body surface area. Animals in the recovery groups received no treatment for an additional 14 days after the 28-day treatment period.

Parameters	**Main findings**
Pharmacodynamic effect	Neutrophil counts increased in a dose-proportionate manner with both MYL-1401H and EU-Neulasta. Dose-proportionate changes to red blood cells, reticulocytes, lymphocytes, monocytes, eosinophils, and basophils were also seen for both MYL-1401H– and EU-Neulasta–treated animals. However, these changes were not of the magnitude of the neutrophil counts.
Injection site effects	At the injection sites, minimal fasciitis/fibrosis and occasional hemorrhage were recorded in animals administered MYL-1401H, animals administered EU-Neulasta, and in the controls.
Immunogenicity	No assessment of anti-drug antibody formation (binding and neutralizing capacity) of the test or reference product was performed. No significant differences in toxicokinetics were observed, anaphylactic reactions were absent, and the expected increases in pharmacodynamic markers were observed. Therefore, there was no indication that anti-drug antibody was present.
Mortality and clinical signs	There was 1 mortality during the study, at Day 28. A female in the high dose EU-Neulasta group was removed from the study due to clinical signs related to granulocytic leukemia characterized by a widespread, diffusely invasive proliferation of granulocytes (myeloid cells) in the spleen, bone marrow, and multiple other organs, including non-hematopoietic tissues. The following observations seen were across several dose groups in one or both sexes, with no apparent dose response. Several male animals across all dose groups (except Group 4) had hair loss, sores, or lesions to their neck, while several female animals across all dose groups showed signs of thinning neck fur and red staining to the neck.
Ophthalmological examination	There were no remarkable ophthalmic changes in animals given MYL-1401H or EU-Neulasta.
Body weights and cumulative net weight gains	All animals given MYL-1401H or EU-Neulasta had comparable weight gain during the dosing phase and treatment-free phase of the study.
Food consumption	Food consumption throughout the pre-dose, dosing, and treatment-free phases was

	comparable across all dose groups.
Hematology	Dose-proportional increases in white blood cell counts (in particular, absolute neutrophil counts) were seen in all MYL-1401H- and EU-Neulasta-treated animals. In males receiving 1.5 mg/kg MYL-1401H or EU-Neulasta, maximal neutrophil counts were 80.45 and 73.68 × 109/L, respectively, and occurred 48 hours post dose (Day 22 administration) on Day 24. In females receiving 1.5 mg/kg MYL-1401H or EU-Neulasta, maximal neutrophil counts of 50.96 and 53.54 × 109/L, respectively, were seen at 72 hours post dose (Day 22 administration) on Day 25. Additionally, there were smaller dose-proportional statistical changes (ANOVA and Dunnet' s; P≤ 0.001 to 0.05) in numbers of red blood cells, reticulocytes, lymphocytes, monocytes, eosinophils, and basophils in both MYL-1401H- and EU-Neulasta-treated animals. There were no other statistically significant (P≤ 0.05) treatment-related changes in hemoglobin, packed cell volume, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, red cell distribution width, hemoglobin distribution width, total and differential white cell count, platelet count, plateletcrit, mean platelet volume, platelet distribution width, or coagulation (prothrombin time).
Clinical chemistry	Alkaline phosphatase levels increased in treated males and females in a dose-dependent manner. Similar increases were seen with MYL-1401H and EU-Neulasta. Males given 0.15 mg/kg MYL-1401H or EU-Neulasta showed comparable levels of alkaline phosphatase elevation above control; approximately 2.4 and 2.3 times higher, respectively. Similarly, males given 1.5 mg/kg MYL-1401H or EU-Neulasta showed elevation of alkaline phosphatase approximately 5 times higher than males given placebo (controls). Females showed a comparable trend, with elevation of alkaline phosphatase in animals given 0.15 mg/kg or 1.5 mg/kg MYL-1401H or EU-Neulasta (3 and 5 times, respectively, the level of controls). No statistically significant (P≤ 0.05) changes in other clinical chemistry parameters were observed following treatment with either MYL-1401H or EU-Neulasta, including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total cholesterol, total bilirubin, total protein, albumin, globulin, albumin/globulin ratio, sodium, potassium, chloride, calcium, inorganic phosphate, creatinine, urea, and glucose.
Urinalysis	No treatment-related differences from control were seen for volume, color, turbidity, specific gravity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, and microscopic observation of sediment.
Organ weights and organ weight ratios	Group mean spleen weights (adjusted for terminal body weight) were increased by 46%, 80%, and 115% in males and 33%, 54%, and 77% in females given 0.15, 0.65, or 1.5 mg/kg MYL-1401H, respectively, compared with those in males and females given placebo (controls). Similar increases were observed in animals given EU-Neulasta; group mean spleen weights (adjusted for terminal body weight) were increased by 44% and 116% in males and 8% and 67% in females given 0.15 or 1.5 mg/kg EU-Neulasta.
Gross pathology	Large spleen was recorded for most animals given 0.65 or 1.5 mg/kg MYL-1401H and for most animals given 1.5 mg/kg EU-Neulasta. These findings correlated with increased spleen weight at necropsy. There were no other macroscopic findings considered related to test article administration. Other tissues were either macroscopically unremarkable or the findings were typical for animals of this strain and age.
Histopathology	Histopathological changes attributable to test article administration were observed in the

	bone marrow, spleen, and liver. Microscopic findings in other tissues and at the injection site were generally infrequent, minor, and typical for animals of this strain and age. Increased granulopoiesis was seen in the bone marrow of the femur and sternum, in animals given 1.5 mg/kg MYL-1401H or EU-Neulasta. This was characterized by increased cellularity and decreased basophilia of the bone marrow due to an increased proportion of white cell precursors. This was also associated with a reduction in marrow fat spaces. In the spleen, an increase in the severity of hematopoiesis was recorded for animals given 1.5 mg/kg MYL-1401H or EU-Neulasta. This was characterized by increased hematopoietic cells in the red pulp and correlated with large spleen visible macroscopically. In the liver, an increase in the incidence and severity of hematopoiesis was recorded for animals given 1.5 mg/kg MYL-1401H or EU-Neulasta. Hematopoiesis was characterized by foci of predominantly myeloid cells in the periportal areas and in the liver parenchyma with small foci of hyperchromatic erythroid cells and occasional megakaryocytes also in the liver parenchyma.

Genotoxicity

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

Carcinogenicity

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

Reproduction Toxicity

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

Toxicokinetic data

Toxicokinetic and statistical analysis for MYL-1401H and EU-Neulasta were performed and included:

AUC_{0-t}: Area under the concentration-time curve from hour 0 to the last measurable concentration, estimated by the log/linear trapezoidal rule

C_{max}: Maximum observed concentration from the concentration/time graphs

t_{max}: Time of maximum concentration from the concentration/time profile

Other parameters, including dose normalized C_{max} (C_{max}/D) and AUC_{0-t} ($AUC_{0-t/D}$), area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$), terminal plasma half-life (t1/2), and accumulation ratios based on C_{max} (RA_{Cmax}) and AUC (RA_{AUC}).

Blood samples for toxicokinetics (1 mL nominal) were taken from all toxicokinetic animals on Day 1 and Week 4 at pre-dose, 4, 8, 12, 24, 48, 72, 96, 120 and 144 hours after dosing.

Table 5: Summary of Toxicokinetic Parameters of MYL-1401H in Rat Plasma

Day	Parameter	0.15 mg/kg/week		0.65 mg/kg/week		1.5 mg/kg/week	
		Male	Female	Male	Female	Male	Female
Day 1	C_{max} (pg/mL)	86900	258000	1010000	1590000	2340000	4200000
	t_{max} (h)	12.0	12.0	24.0	12.0	12.0	12.0
	$t_{1/2}$ (h)	6.98	4.93	4.91	4.83	4.74	4.73
	$AUC_{(0-t)}$ (pg.h/mL)	1700000	5190000	33000000	48600000	113000000	170000000
	$AUC_{(0-\infty)}$ (pg.h/mL)	1710000	5190000	33000000	48600000	113000000	170000000
	C_{max}/D	580000	1720000	1550000	2450000	1560000	2800000
	$AUC_{(0-t)}$/D	11300000	34600000	50800000	74700000	75200000	113000000
Day 22	C_{max} (pg/mL)	98900	315000	565000	1380000	2410000	4260000
	t_{max} (h)	12.0	12.0	24.0	12.0	24.0	12.0
	$t_{1/2}$ (h)	NR	NR	10.1	5.32	8.31	6.00
	$AUC_{(0-t)}$ (pg.h/mL)	1570000	4860000	13200000	38600000	83600000	148000000
	$AUC_{(0-\infty)}$ (pg.h/mL)	NR	NR	13200000	38600000	83600000	148000000
	C_{max}/D	660000	2100000	870000	2120000	1600000	2840000
	$AUC_{(0-t)}$/D	10500000	32400000	20300000	59300000	55700000	98600000
	RAC_{max}	1.14	1.22	0.562	0.865	1.03	1.01
	RA_{AUC}	0.922	0.938	0.399	0.794	0.741	0.869

AUC_{0-t}: area under the concentration-time curve until the last measurable concentration; $AUC_{(0-t)}$/D: dose-normalized $AUC_{(0-t)}$, calculated by dividing the $AUC_{(0-t)}$ value by the nominal dose; $AUC_{0-\infty}$: area under the concentration-time curve extrapolated to infinity; C_{max}: peak plasma concentration; C_{max}/D: dose-normalized C_{max}, calculated by dividing the C_{max} by the nominal dose; NR: not reported; RA_{AUC}: accumulation ratio based on $AUC_{(0-t)}$; RAC_{max}: accumulation ratio based on C_{max}; $t_{1/2}$: terminal plasma half-life; t_{max}: time to reach peak plasma concentration.
All reported values are individual values derived from composite data (ie, arithmetic mean of concentrations from 3 rats at each time point).

Table 6: Summary of Toxicokinetic Parameters of EU-Neulasta in Rat Plasma

Day	Parameter	0.15 mg/kg/week		1.5 mg/kg/week	
		Male	Female	Male	Female
Day 1	C_{max} (pg/mL)	140000	268000	2280000	4030000
	t_{max} (h)	12.0	24.0	24.0	12.0
	$t_{1/2}$ (h)	6.20	NR	NR	4.13
	$AUC_{(0-t)}$ (pg.h/mL)	2640000	6020000	106000000	158000000
	$AUC_{(0-\infty)}$ (pg.h/mL)	2640000	NR	NR	158000000
	C_{max}/D	931000	1780000	1520000	2690000
	$AUC_{(0-t)}$/D	17600000	40100000	70500000	106000000
	C_{max} ratio[a]	0.623	0.964	1.03	1.04
	$AUC_{(0-t)}$ ratio[a]	0.645	0.862	1.07	1.07

Day	Parameter	0.15 mg/kg/week		1.5 mg/kg/week	
		Male	Female	Male	Female
	C_{max} (pg/mL)	269000	437000	2500000	2900000
	t_{max} (h)	12.0	12.0	12.0	24.0
	$t_{1/2}$ (h)	8.29	4.99	NR	6.18
	$AUC_{(0-t)}$ (pg.h/mL)	4080000	6860000	79500000	124000000
	$AUC_{(0-\infty)}$ (pg.h/mL)	4080000	6860000	NR	124000000
Day 22	C_{max}/D	1800000	2920000	1670000	1930000
	$AUC_{(0-t)}$/D	27200000	45700000	53000000	82400000
	RAC_{max}	1.93	1.63	1.10	0.719
	RA_{AUC}	1.55	1.14	0.752	0.780
	C_{max} ratio[a]	0.367	0.721	0.963	1.47
	$AUC_{(0-t)}$ ratio[a]	0.384	0.709	1.05	1.20

AUC_{0-t}: area under the concentration-time curve until the last measurable concentration; $AUC_{(0-t)}$/D: dose normalized $AUC_{(0-t)}$, calculated by dividing the $AUC_{(0-t)}$ value by the nominal dose; $AUC_{0-\infty}$: area under the concentration-time curve extrapolated to infinity; C_{max}: peak plasma concentration; C_{max}/D: dose normalized C_{max}, calculated by dividing the C_{max} by the nominal dose; EU: European Union; NR: not reported; RA_{AUC}: accumulation ratio based on $AUC_{(0-t)}$; RAC_{max}: accumulation ratio based on C_{max}; $t_{1/2}$: terminal plasma half-life; t_{max}: time to reach peak plasma concentration.
a MYL-1401H/Neulasta ratio
All reported parameters are individual values derived from composite data (ie, arithmetic mean of concentrations from 3 rats at each time point).

The NOAEL was considered to be 1.5 mg/kg in male and female rats for both MYL-1401H and EU-Neulasta in this study. This dose is equivalent to approximately 2.4 times the intended therapeutic dose in humans (6 mg/chemotherapy cycle) based on body surface area.

Local Tolerance

Comparative local tolerance assessments between MYL-1401H, EU-Neulasta, and US-Neulasta were made in the *in vivo* pharmacodynamic study and in the repeat-dose toxicity study. No injection site reactions were observed in the pharmacodynamic study. In the toxicity study, minimal fasciitis/fibrosis and occasional hemorrhage were observed.

Other toxicity studies

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

2.3.5. Ecotoxicity / environmental risk assessment

The applicant provided a justification for waiving ERA studies in accordance with the guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 Corr 2).

It is considered that Fulphila will not pose any greater risk to the environment than Neulasta. Pegfilgrastim is extensively metabolised in man and predicted to be rapidly biodegraded in the environment. Furthermore, it is considered that Fulphila will replace other similar pegfilgrastim products on the market. Hence, it is expected that the total amount of pegfilgrastim will not be substantially increased and no additional environmental burden is envisaged. Furthermore, proteins

and peptides are excluded from the need for an environmental risk assessment in accordance with the respective guideline.

2.3.6. Discussion on non-clinical aspects

MYL-1401H was developed as a biosimilar biological medicine to Neulasta. As such, according to the guideline EMEA/CHMP/42832/05, a reduced preclinical program is acceptable in order to show comparability of test and reference product. Mylan provided comparative *in vitro* and *in vivo* pharmacodynamic studies as well as one comparative 28 day repeat dose toxicity study in neutropenic rats.

The *in vitro* cell based proliferation assay using the murine NFS 60 cell line demonstrated similar relative potencies. The binding activity using receptor-binding Surface Plasmon Resonance showed comparable binding characteristics for Association rate constant, Dissociation rate constant and Equilibrium dissociation constant. Pharmacodynamic responses of the *in vivo* study showed comparable number of leucocytes and neutrophilic granulocytes with higher values of MYL-1401H compared to the reference product (EU-Neulasta).

The toxicological profile of MYL-1401H in comparison to EU-Neulasta was evaluated in a 28-day repeat dose toxicity study in rats. This study included toxicokinetic analysis. Dose-proportional increases in mean maximal concentration exposures and comparable half-lives were seen with both products. Minor gender differences were observed, with males having higher exposure levels than females. As expected, neutrophil counts increased in a dose-proportional manner across all treated groups. Expected and treatment related increases in spleen weight as well as elevated and comparable alkaline phosphatase levels were observed. Splenomegaly is an expected finding of pegfilgrastim therapy and therefore a known adverse effect. The toxicokinetic data from this study showed comparability between doses of 0.15 and 1.5 mg/kg Myl-1401H and EU-Neulasta with the exception of the low dose in males, where systemic exposure to MYL-1401H was notably lower than EU-Neulasta, suggesting that there may be a gender difference. However, the gender difference may be a chance findings and is not considered relevant.

According to the guideline EMEA/CHMP/BMWP/31329/2005 as well as the concept paper published on 27/07/2015, studies on single dose toxicity, reproduction, genotoxic, carcinogenic potential are not required for the development of biosimilar G-CSF products.

A justification for waiving ERA studies had been provided. This is considered acceptable, given that the active substance pegfilgrastim is a polypeptide which is exempted from an ERA as per guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 Corr 2). Pegfilgrastim is not expected to pose a risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

The non-clinical aspects of pharmacology, pharmacokinetic and toxicology for Fulphila have been well characterised and are considered acceptable. There were no further changes to the SmPC and the product information is aligned with the reference product Neulasta.

2.4. Clinical aspects

2.4.1. Introduction

GCP

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

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

- Tabular overview of clinical studies

Type of Study	**Study Number**	**Study Objective(s)**	**Study Design**	**Test Product(s), Dosage, Regimen, Route of Administration**	**Number of Subjects/ Diagnosis**	**Duration of Treatment**
PK/PD, safety	MYL-1401H-1001	To compare the PK, PD, safety, and tolerability of MYL-1401H and NEULASTA	Single-center, randomized, double-blind, 3-period, 3-treatment, 3-way crossover study	MYL-1401H or NEULASTA (EU-approved NEULASTA or US-licensed NEULASTA) 2-mg SC injection	216 healthy adult subjects	Single dose
Immuno-genicity, safety	MYL-1401H-1002	To descriptively compare immunogenicity between 2 SC injections of MYL-1401H and NEULASTA To evaluate the safety and tolerability of MYL-1401H and NEULASTA after 2 injections (6 mg each)	Single-center, randomized, open-label, 2-dose, parallel study	MYL-1401H or NEULASTA (US-licensed NEULASTA) 6-mg SC injection	50 healthy adult subjects	2 doses
Efficacy, safety, immuno-genicity	MYL-1401H-3001	To compare the efficacy, safety, and immunogenicity of MYL-1401H and NEULASTA	Multicenter, randomized, double-blind, therapeutic-equivalence study Subjects were randomly assigned (2:1) to either MYL-	MYL-1401H or EU-approved NEULASTA 6-mg SC injection	194 adult patients with Stage II/III invasive breast cancer in the adjuvant/neo-adjuvant setting who were receiving TAC	Single dose of MYL-1401H or EU-approved NEULASTA on Day 2 of each chemotherap y cycle. Each cycle was approximatel

			1401H or EU-NEULASTA and were stratified based on their age and country.		chemotherapy	y 3 weeks (from the first day of chemotherapy [Day 1 Cycle 1] to the last scheduled assessment in Cycle 1). Study treatment duration was up to 6 cycles of chemotherapy.

Abbreviations: EU = European Union; PD = pharmacodynamics; PK = pharmacokinetics; SC = subcutaneous; TAC = docetaxel, doxorubicin, and cyclophosphamide; US = United States.

2.4.2. Pharmacokinetics

Absorption

The pivotal cross-over PK/PD study MYL-1401H-1001 investigated single 2 mg doses of MYL-1401H, EU- and US-Neulasta (0.2 mL of 10 mg/mL dose strengths based on protein content with all 3 drug products being transferred into identical 0.3-mL syringes), whereas the parallel-design study 1002 used single 6 mg doses and primarily investigated immunogenicity in healthy subjects.

Also in trial MYL-1401H-1002 concentrations of the analyte PEG GCSF were determined but analysed only descriptively (for the means of the primary objective of this trial immunogenicity).

Thus, trial MYL-1401H- 1001 is the pivotal BE (and equivalent PD) study of this application:

Trial ***MYL-1401H-1001*** was a single centre, randomized, double-blind, 3-period, 3 treatments, 3-way crossover trial to evaluate the PD, PK, safety and tolerability of pegfilgrastim from a test product (MYL-1401H) compared to reference products EU- and US-Neulasta in 216 healthy volunteers intended to be in accordance with EU and US biosimilar guidelines.

After randomization to one of six possible treatment sequences, subjects were administered MYL-1401H or one of two reference products in Period 1. After the 1st crossover, subjects received one of the remaining alternate treatments in Period 2.

After the 2nd crossover, subjects received the other alternate treatment in Period 3. The washout between drug administrations was at least 4 weeks.

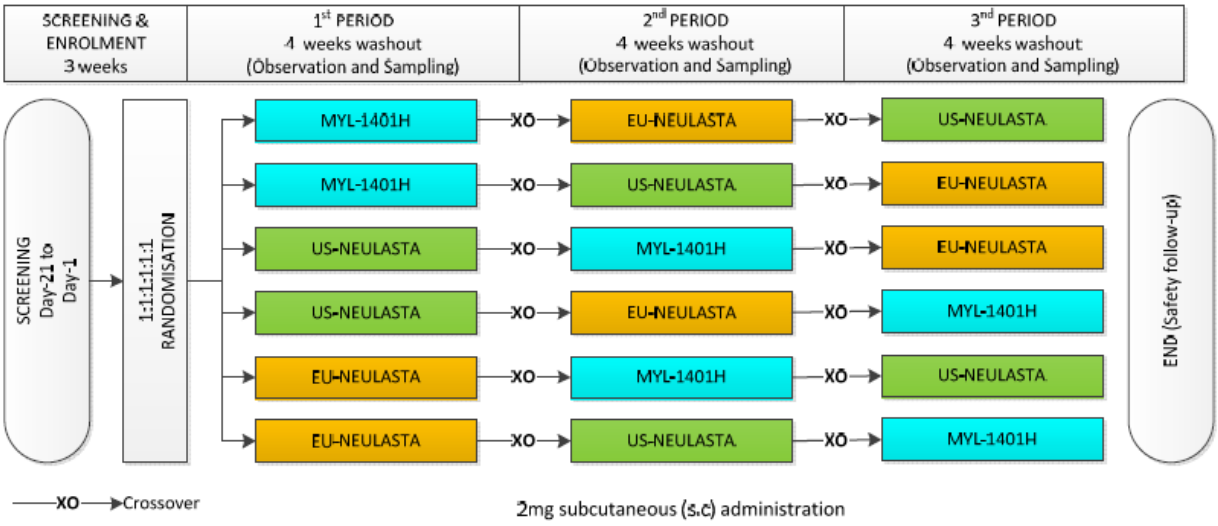


Figure 1: Overview of Study Design MYL-1401H-1001

The inclusion and exclusion criteria can be briefly summarized as selecting for healthy adults (18-65 years of age) of both genders. Specific for the scope of the trial are only the two exclusion criteria:

- Known history of previous exposure to filgrastim, pegfilgrastim, GCSF or any analogue of these.
- Hypersensitivity to the constituents of Neulasta (sorbitol E420, polysorbate 20 and acetate or acetic acid) or hypersensitivity to E. coli derived proteins.

Methods

- ELISA Assay for the Quantitation of MYL-1401H/Neulasta in Human Serum (has been satisfactorily validated and considered suitable for its intended use)
- Neutrophils and CD34+ cells were counted via flow cytometry (Validation reports have not been submitted. Flow cytometry is considered a standard approach so that validation of this method is not needed for this application)
- Analysis of Normal Human Serum Samples for detection of Anti-Drug Antibodies against MYL-1401H and Neulasta (EU and US) to support Phase 1 Clinical Study (MYL-1401H-1001) using the Mesoscale Discovery Platform
- Cell-Based Assay to Detect Neutralizing Antibodies (NAb) Against MYL-1401H and Neulasta (EU and US) in Normal Human Serum.

Pharmacokinetic Measurements

At the time points defined blood samples of 2.5 m each were taken for the analysis of PEG-GCSF concentration in serum samples.

Pharmacodynamic Measurements

At the time points defined, blood samples of 3 mL each were taken for the analysis of ANC and CD34+.

Both ANC and CD34+ cell count was determined with flow cytometry by the clinical safety laboratory of the centre.

Safety and Tolerability Measurements

Safety and tolerability assessments consisted of AEs, clinical laboratory, vital signs, 12-lead ECG, local tolerability, physical examination and immunogenicity and were performed as scheduled.

Primary PK Parameters

The primary PK parameters to be determined or calculated from the serum-concentration time data for PEG-GCSF were:

- Cmax = Observed maximum serum concentration

- AUC0-inf = Area under the serum concentration-time curve (time 0 to infinity)

Bioequivalence was to be concluded if the 90% CI for the ratio of geometric means of two treatments falls completely within the limits of 0.8000-1.2500 for the primary PK parameters.

Secondary PK Parameters

The secondary PK parameters to be determined or calculated from the serum-concentration time data for PEG-GCSF were:

- AUC0-t = Area under the concentration-time curve (time 0 to time of last quantifiable concentration)

- Tmax = Time to attain maximum serum concentration

- kel = Terminal elimination rate constant

- t1/2 = Apparent terminal elimination half-life

- Vd/F = Apparent volume of distribution

An additional PK parameter to be determined or calculated from the serum-concentration time data for PEG-GCSF was:

- %AUCextra = Percentage of estimated part for the calculation of AUC0-inf of serum PEG-GCSF: ([AUC0-inf - AUC0-t]/AUC0-inf)*100%.

The chosen PK parameters are standard parameters for BE trials and accepted for demonstration of similar PK profiles of two pegfilgrastims.

Primary PD Parameters

The primary PD parameters to be determined or calculated from the cell count-time data for ANC were:

- ANC AUC0-t = Area under the ANC above baseline values versus time curve (time 0 to time of last data collection point)

– ANC Cmax = Maximum change from baseline for ANC*

* ANC Cmax was changed from secondary PD parameter to primary PD parameter after completion of the study, as documented in CSP Version 3.0.

Equivalence was to be concluded if the 95% CI for the ratio of geometric means of two treatments fell completely within the limits of 0.8500-1.1765 for the primary PD parameters.

Secondary PD Parameters

The secondary PD parameter to be determined or calculated from the cell count-time data for ANC was:

– ANC Tmax = Time of maximum change from baseline for ANC

The secondary PD parameters to be determined or calculated from the cell count-time data for CD34+ cell counts were:

- CD34+ AUC0-t = Area under the CD34+ cell counts above baseline versus time curve

– CD34+ Cmax = Maximum change from baseline for CD34+ cell counts

– CD34+ Tmax = Time of maximum change from baseline

The choice of the primary and secondary PD parameters is in line with the respective guideline and acceptable.

Determination of Sample Size

The actual sample size of 216 healthy volunteers was based on the following assumptions laid down in the protocol:

- Intrasubject variability from the MYL-PER-0001 pilot study11[8]:
    - ANC AUC0-t = 14%
    - PEG-GCSF AUC0-t and AUC0-inf = 36%
    - PEG-GCSF Cmax = 50%
- ANC AUC0-t:
    - 95% CI
    - equivalence range [0.8500-1.1765]
    - ratio of geometric means in interval [0.95-1.05]
- PEG-GCSF AUC0-t, AUC0-inf and Cmax
    - 90% CI
    - equivalence range [0.8000-1.2500]
    - ratio of geometric means in interval [0.95-1.05]

It was estimated that with 180 evaluable subjects the study will have a combined power for PD and PK of over 90% to establish equivalence for each of the 3 pairwise comparisons.

According to the applicant, the sample size estimation was not literature derived but was based on a pilot study with Neulasta.

Results

- *Disposition of Subjects and Data Sets analysed*

[8] A pilot phase 1, repeated single dose study evaluating the variability of pharmacokinetics and pharmacodynamics of long acting filgrastim following subcutaneous administration to healthy volunteers. Myl-Per0001/MYB262EC-122621. Final Clinical Study Report. 18 June 2013.

372 subjects were screened and 216 subjects were included in the study. All of these 216 subjects were randomized and received at least one dose of 2 mg pegfilgrastim. The doses of pegfilgrastim were administered at least 4 weeks apart. All 216 subjects were included in the safety analysis set.

Twenty subjects discontinued the study for the following reasons:

8 subjects were withdrawn because of a protocol violation (tested positive for cannabinoids and cocaine; inability to follow protocol instructions).

8 subjects withdrew consent for personal reasons.

3 subjects were withdrawn due to AEs (1 SAE).

1 subject dropped out after dosing in Period 2 because he missed too many visits due to illness.

A total of 196 subjects completed the study as per protocol. All of these subjects were part of the 208 subjects who were included in both the PK and PD analysis sets.

Number and reasons for withdrawal were as expected. The about 10% withdrawal rate seems to equally distribute over the 6 sequences. As to the subjects withdrawn due to AEs or SAEs see safety assessment.

- *Baseline characteristics*

Table 7: Summary of Demographic Characteristics (MYL-1401H-1001)

Parameter		Statistic / Category	Safety Set (N = 216)	PK and PD Set (N = 208)
Gender	– Male	n (%)	170 (79%)	163 (78%)
	– Female	n (%)	46 (21%)	45 (22%)
Ethnicity	– Hispanic or Latino	n (%)	3 (1%)	3 (1%)
	– Not Hispanic or Latino	n (%)	213 (99%)	205 (99%)
Race	– American Indian Or Alaska Native	n (%)	2 (1%)	2 (1%)
	– Asian	n (%)	5 (2%)	5 (2%)
	– Black	n (%)	6 (3%)	5 (2%)
	– White	n (%)	196 (91%)	189 (91%)
	– Multiple	n (%)	7 (3%)	7 (3%)
Age (years)		mean (SD)	37 (14)	37 (14)
		median	33	33
		min - max	18 - 65	18 – 65
Weight (kg)		mean (SD)	78.5 (10.7)	78.4 (10.8)
		median	78.3	78.0
		min-max	59.4 - 106.5	59.4 - 106.5
Height (cm)		mean (SD)	178 (9)	178 (9)
		median	179	179
		min-max	156 - 201	156 - 201
Body Mass Index (kg/m^2)		mean (SD)	24.6 (2.6)	24.6 (2.6)
		median	24.4	24.4
		min-max	19.5 - 30.4	19.5 - 30.4

max = maximum; min = minimum; N (n) = number of subjects; PD = pharmacodynamics; PK = pharmacokinetics; SD = standard deviation

Source: Table 15.1-2, Table 15.1-3 and Table 15.1-4

- *Pharmacokinetic Results*

Concentration Data of PEG-GCSF in Serum

After administration of a single sc injection of 2 mg pegfilgrastim, PEG-GCSF (analyte) appeared in serum within 2 to 4 hours post-dose. Only for 2 of the 216 subjects, PEG-GCSF concentrations were first observed at 6 hours after dosing with EU-Neulasta.

The concentrations of PEG-GCSF in serum increased slowly, with maximum mean concentrations reached at approximately 12 hours post-dose.

Mean PEG-GCSF concentrations could be determined in serum up to 144 hours post-dose for all 3 treatments.

Pharmacokinetic Parameters of PEG-GCSF in Serum

The exposure to PEG-GCSF (in terms of Cmax, AUC0-inf and AUC0-t) was most similar between MYL-1401H and US-Neulasta, whereas the exposure of EU-Neulasta appeared to be slightly lower than the other 2 treatments (Table 8).

The median Tmax of PEG-GCSF in serum was 12 hours for all 3 treatments.

The geometric mean t1/2 of PEG-GCSF varied minimally between 49.3 and 51.1 hours across treatments.

The %AUCextra, Vd/F and kel were comparable between the 3 treatments.

Considerable inter-subject variability was observed for the primary PK parameters Cmax and AUC0-inf of PEG-GCSF (CV% ~70%).

Table 8: Summary of PK Parameters for PEG-GCSF in Serum (Geometric Mean [CV%]) (MYL-1401H-1001)

Parameter	MYL-1401H N=204	EU-Neulasta® N=203	US-Neulasta® N=207
C_{max} (pg/mL)	36.7 (72.1%)	34.2 (72.1%)	37.3 (67.6%)
AUC_{0-inf} (h·ng/mL)	869 (69.1%)	833 (70.1%)	876 (66.3%)
AUC_{0-t} (h·ng/mL)	827 (71.4%)	787 (72.7%)	832 (68.6%)
$\%AUC_{extra}$ (%)	3.2 (97.2%)	3.6 (97.9%)	3.2 (105.9%)
T_{max} (h)	12.00 (6.00 - 24.02)	12.00 (6.00 - 48.00)	12.00 (4.02 - 24.02)
k_{el} (1/h)	0.014 (31.0%)	0.014 (39.1%)	0.014 (40.1%)
V_d/F (L)	164 (100%)	177 (101%)	168 (113%)
$t_{1/2}$ (h)	49.3 (36.5%)	51.1 (48.9%)	51.0 (42.5%)

CV% = coefficient of variation; PK = pharmacokinetic
For T_{max} the median (range) is presented.

Statistical Analysis of Pharmacokinetic equivalence

When comparing the primary PK parameters Cmax and AUC0-inf of PEG-GCSF between MYL-1401H, EU-Neulasta and US-Neulasta, GLM ANOVA results showed that the 90% CIs of the ratios of geometric means for these PK parameters ranged between 0.907 and 1.18. The 90% CIs were therefore well contained within the standard bioequivalence interval of 0.8000 - 1.2500 for each of the comparisons.

These results demonstrate similar PK profiles of MYL-1401H, EU-Neulasta and US-Neulasta. The intra-subject CV% (within-subject variability) for the primary PK parameters Cmax and AUC0-inf was 54.8% and 41.8%, respectively, across the 3 treatments.

Table 9: Summary of Bioequivalence Analysis on Primary PK Parameters of PEG-GCSF in Serum (Geometric Mean [CV%]) (MYL-1401H-1001)

Treatment Comparison (Test versus Reference)	PK Parameter	Geometric LS means		Ratio Test/Reference			Intra CV%
					90% CI #		
		Test	Reference	Estimate	Lower	Upper	
MYL-1401H / EU-Neulasta®	C_{max} (pg/mL)	36.6	34.2	1.07	0.984	1.16	54.8*
	AUC_{0-inf} (h·ng/mL)	871	835	1.04	0.977	1.11	41.8*
MYL-1401H / US-Neulasta®	C_{max} (pg/mL)	36.6	37.2	0.986	0.907	1.07	
	AUC_{0-inf} (h·ng/mL)	871	873	0.998	0.935	1.07	
US-Neulasta® / EU-Neulasta®	C_{max} (pg/mL)	37.2	34.2	1.09	0.998	1.18	
	AUC_{0-inf} (h·ng/mL)	873	835	1.05	0.979	1.12	

CI = confidence interval; intra CV% = intra-subject coefficient of variation; LS = least squares; PK = pharmacokinetic

Natural log transformation of C_{max} and AUC_{0-inf} was used for analysis. Using PROC general linear model (GLM) analysis of variance (ANOVA) with treatment, sequence and period as fixed effects, and subject within sequence as a random effect.

Bioequivalence is established if the 90% CI of the ratio is contained completely within acceptance range (0.800 - 1.2500).

* The intra CV% (within-subject variability) is displayed only once for each parameter, as it is equal for each comparison.

Source: Table 15.2-5

Relationship between Pharmacokinetics and Anti-Drug Antibodies

Descriptive statistics were used to summarize the serum PEG-GCSF concentrations and PK parameters by treatment and ADA status as defined as follows.

- ADA positive: Subjects with any confirmed positive ADA response against PEG G-CSF at any point during the study
- ADA negative: Subjects with no confirmed positive ADA response against PEG G-CSF at any point during the study

In addition, geometric mean ratios and corresponding 90% CIs for the 3 pairwise comparisons between 2 treatments were repeated by ADA status for the primary and secondary PK parameters.

Minimal differences (≤10%) in the exposure to PEG-GCSF were observed between ADA positive and negative subjects. For MYL-1401H treatment the geometric mean AUC0-inf was approximately 1.1-fold higher in ADA positive subjects (932 h·ng/mL; n=62) than in ADA negative subjects (843 h·ng/mL; n=142), whereas for EU-Neulasta the AUC0-inf was approximately 1.1-fold lower in ADA positive (775 h·ng/mL; n=62) than in ADA negative subjects (860 h·ng/mL; n=141). For US-Neulasta the differences in exposure were less than 5% between ADA positive (857 h·ng/mL; n=64) and negative subjects (885 h·ng/mL; n=143).

When excluding the ADA positive subjects from the comparison of the primary PK parameters Cmax and AUC0-inf of PEG-GCSF between the 3 treatments, results showed that the upper limit of the 90% CIs of the geometric means ratios were still contained within 0.8000 - 1.2500 bioequivalence interval for each comparison.

Distribution

The applicant did not submit studies in distribution (see pharmacology discussion)

Elimination

The applicant did not submit studies in elimination (see pharmacology discussion)

Dose proportionality and time dependencies

The applicant did not submit studies in dose proportionality and time dependencies (see pharmacology discussion)

Special populations

The applicant did not submit studies in special populations (see pharmacology discussion)

Pharmacokinetic interaction studies

The applicant did not submit studies in pharmacokinetic interaction studies (see pharmacology discussion)

Pharmacokinetics using human biomaterials

The applicant did not submit studies in pharmacokinetic using human biomaterials (see pharmacology discussion)

2.4.3. Pharmacodynamics

Mechanism of action

The applicant did not submit studies in mechanism of action (see pharmacology discussion)

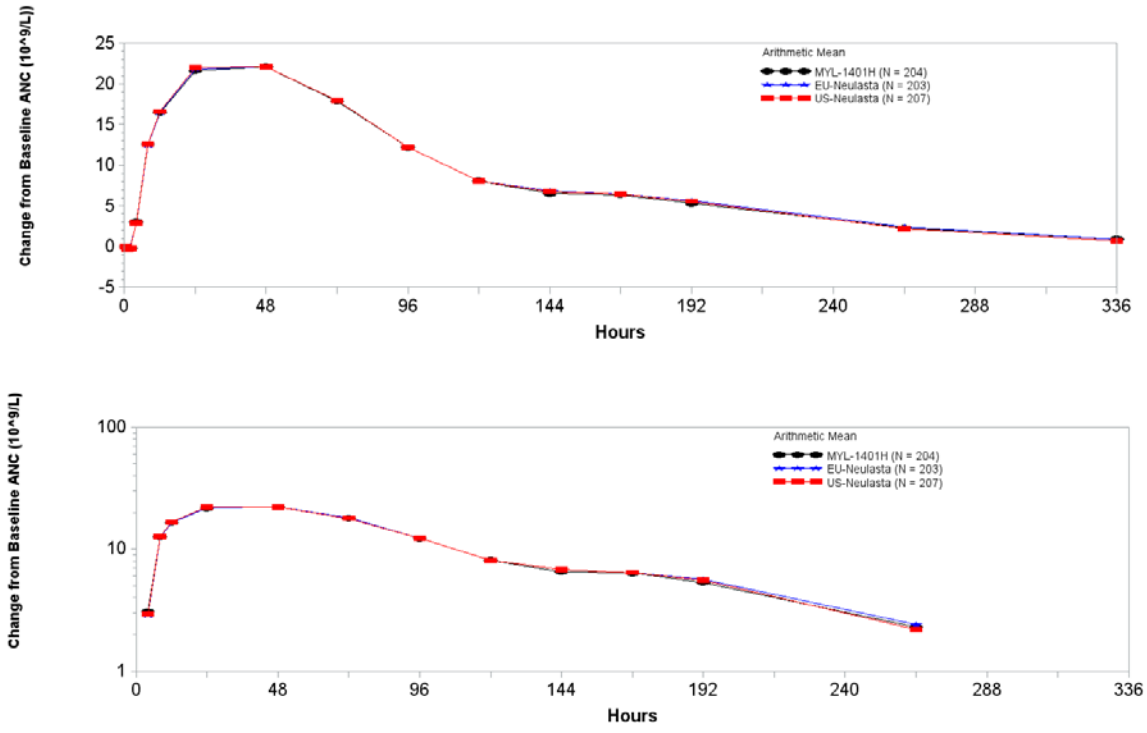
Primary and Secondary pharmacology

Study MYL-1401H-1001

Concentration Data of ANC and CD34+ in Serum ANC

After administration of a single sc injection of 2 mg MYL-1401H, EU-Neulasta or US-Neulasta, mean ANC levels above baseline were first observed at 4 hours post-dose on Day 1. For all 3 treatments, a similar peak increase of approximately 8-fold compared to baseline was observed between Day 2 and Day 3 (24-48 hours post-dose; see Figure 2).

Figure 2: Arithmetic Mean Change from Baseline ANC Serum Concentration-Time Profiles (MYL-1401H-1001)



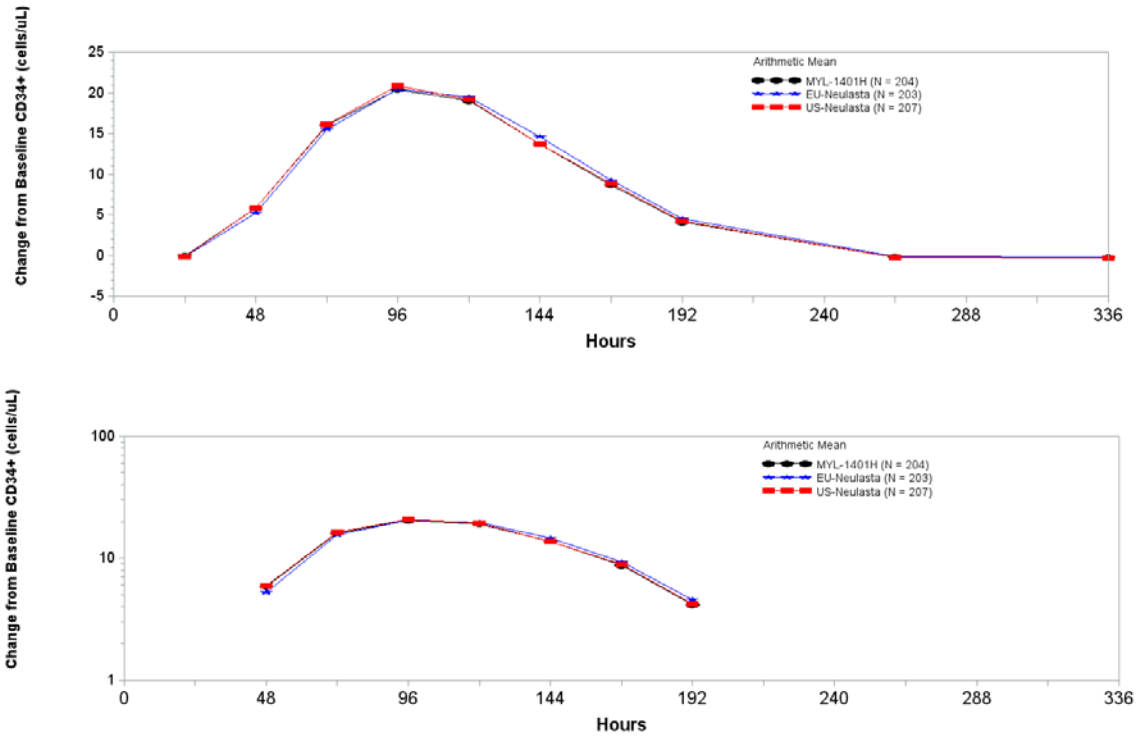
Thereafter the mean ANC appeared to decrease in a multiphasic manner, with a relatively slow elimination phase between Day 6 and Day 9 (between 120 and 192 hours post-dose), before the ANC returned to values near baseline on Day 12 (264 hours post-dose). The mean ANC versus time profiles were very similar between the 3 treatments.

The combined individual change from baseline ANC versus time profiles showed minimal inter-individual variability. However, for 2 subjects the increase in the ANC was very low (approximately 2-fold compared to baseline) after administration of US-Neulasta in Period 2 compared to the other subjects. These minimal ANC increases were consistent with the relatively low PEG-GCSF concentrations observed for these subjects.

Mean CD34+ counts above baseline were first observed on Day 3. A maximum increase of approximately 9.5-fold compared to baseline was observed on Day 5 (96 hours post-dose; see Figure 3). Thereafter the mean CD34+ counts decreased to values near baseline on Day 12. The mean CD34+ versus time profiles were very similar between the 3 treatments.

The combined individual change from baseline CD34+ counts versus time profiles showed considerable inter-individual variability in the extent of increase in CD34+ counts over time.

Figure 3: Arithmetic Mean Change from Baseline CD34+ Serum Concentration-Time Profiles (MYL-1401H-1001)



The primary PD parameters ANC Cmax and ANC AUC0-t were very similar across treatments. Also the secondary PD parameters CD34+ Cmax and CD34+ AUC0-t were comparable between these treatments (Table 10).

For MYL-1401H and EU-Neulasta the median ANC Tmax was 48 hours and for US-Neulasta the median ANC Tmax was 24 hours. For the CD34+ counts, the median CD34+ Tmax was 96 hours for all 3 treatments.

The inter-subject variability was much higher for the secondary CD34+ PD parameters (CV% up to ~80%) than for the primary ANC PD parameters (CV% up to ~30%).

Table 10: Summary of PD Parameters for ANC and CD34+ Count Data (Geometric Mean [CV%]) (MYL-1401H-1001)

Parameter	MYL-1401H N=204	EU-Neulasta® N=203	US-Neulasta® N=207
ANC PD Parameters			
ANC AUC_{0-t} ($h \cdot 10^9/L$)	2784.356 (29.0%)	2792.623 (30.7%)	2744.700 (30.8%)
ANC C_{max} ($10^9/L$)	22.507 (25.7%)	22.686 (25.9%)	22.546 (26.4%)
ANC T_{max} (h)	47.98 (12.00 - 96.00)	48.00 (12.00 - 96.00)	24.05 (8.00 - 72.03)
CD34+ PD Parameters			
CD34+ AUC_{0-t} ($h \cdot cells/\mu L$)	1652.305 (79.7%)	1633.532 (81.0%)	1598.443 (81.2%)
CD34+ C_{max} (cells/µL)	17.469 (76.5%)	17.681 (77.0%)	17.445 (77.1%)
CD34+ T_{max} (h)	96.00 (71.97 - 168.00)	96.02 (72.00 - 192.00)	96.00 (48.00 - 192.00)

ANC = absolute neutrophil count; CV% = coefficient of variation; PD = pharmacodynamic
For T_{max} the median (range) is presented.

Source: Table 15.3-3 and Table 15.3-4

- *Statistical Analysis of Pharmacodynamic Equivalence*

Primary PD Parameters

When comparing the primary PD parameters ANC AUC0-t and ANC Cmax between the 3 treatments (MYL-1401H, EU-Neulasta and US-Neulasta), GLM ANOVA results showed that the 95% CIs of the ratios of geometric means for these PD parameters ranged between 0.943 and 1.061 for each of the comparisons. The 95% CIs were therefore well contained within the predefined equivalence interval of 0.8500 - 1.1765 for each of the comparisons (Table 11). Likewise, the 90% CIs ranging between 0.950 and 1.054 were well contained within the 0.80 – 1.25 similarity range which was conducted as additional analysis. The intra-subject CV% was low for the primary PD parameters and comparable between ANC AUC0-t (22.3%) and ANC Cmax (17.7%).

Table 11: Summary of Equivalence Analysis for the Primary PD Parameters for ANC (MYL-1401H-1001)

Treatment Comparison (Test versus Reference)	PD Parameter	Geometric LS means		Ratio Test/Reference			Intra CV%
					95% CI #		
		Test	Reference	Estimate	Lower	Upper	
MYL-1401H / EU-Neulasta®	ANC AUC_{0-t} ($h \cdot 10^9$/mL)	2794.628	2791.608	1.001	0.959	1.045	22.3*
	ANC C_{max} (10^9/mL)	22.539	22.687	0.993	0.960	1.028	17.7*
MYL-1401H / US-Neulasta®	ANC AUC_{0-t} ($h \cdot 10^9$/mL)	2794.628	2747.813	1.017	0.974	1.061	
	ANC C_{max} (10^9/mL)	22.539	22.542	1.000	0.966	1.035	
US-Neulasta® / EU-Neulasta®	ANC AUC_{0-t} ($h \cdot 10^9$/mL)	2747.813	2791.608	0.984	0.943	1.027	
	ANC C_{max} (10^9/mL)	22.542	22.687	0.994	0.960	1.028	

ANC = absolute neutrophil count; CI = confidence interval; intra CV% = intra-subject coefficient of variation; LS = least squares; PD = pharmacodynamic

Natural log transformation of C_{max} and AUC_{0-t} was used for analysis. PROC general linear model (GLM) analysis of variance (ANOVA) with treatment sequence and period as fixed effects, and subject within sequence as a random effect was performed for these parameters.

Equivalence is established if the 95% CI of the ratio is contained completely within acceptance range (0.8500 - 1.1765).

* The intra CV% (within-subject variability) is displayed only once for each parameter, as it is equal for each comparison.

Secondary PD Parameters

The estimates and corresponding 95% CIs of the geometric mean ratios were close to 1 for the secondary PD parameters CD34+ Cmax and CD34+ AUC0-t, with 95% CIs ranging between 0.915 and 1.104 for each of the comparisons (Table 12). The intra-subject variability was comparable between CD34+ Cmax (33.7%) and CD34+ AUC0-t (34.1%) across the 3 treatments. For the secondary PD parameters ANC Tmax and CD34+ Tmax, all estimates and corresponding 95% CIs were zero (0.000).

Table 12: Summary of Statistical Analysis on Secondary PD Parameters for ANC and CD34+ Count Data (MYL-1401H-1001)

Treatment Comparison (Test versus Reference)	PD Parameter	Median		Test Minus Reference		
					95% CI	
		Test	Reference	Estimate	Lower	Upper
Secondary ANC PD Parameter						
MYL-1401H / EU-Neulasta®	ANC T_{max} (h)	47.975	48.000	0.000	0.000	0.000
MYL-1401H / US-Neulasta®	ANC T_{max} (h)	47.975	24.050	0.000	0.000	0.000
US-Neulasta® / EU-Neulasta®	ANC T_{max} (h)	24.050	48.000	0.000	0.000	0.000
Secondary CD34+ Parameter						
MYL-1401H / EU-Neulasta®	CD34+ T_{max} (h)	96.000	96.020	0.000	0.000	0.000
MYL-1401H / US-Neulasta®	CD34+ T_{max} (h)	96.000	96.000	0.000	0.000	0.000
US-Neulasta® / EU-Neulasta®	CD34+ T_{max} (h)	96.000	96.020	0.000	0.000	0.000

Treatment Comparison (Test versus Reference)	PD Parameter	Geometric LS means		Ratio Test/Reference			Intra CV%
					95% CI		
		Test	Reference	Estimate	Lower	Upper	
Secondary CD34+ PD Parameters							
MYL-1401H / EU-Neulasta®	CD34+ C_{max} (cells/µL)	17.670	17.701	0.998	0.936	1.065	33.7*
	CD34+ AUC_{0-t} (h·cells/µL)	1655.336	1638.707	1.010	0.946	1.078	34.1*
MYL-1401H / US-Neulasta®	CD34+ C_{max} (cells/µL)	17.670	17.428	1.014	0.951	1.081	
	CD34+ AUC_{0-t} (h·cells/µL)	1655.336	1600.001	1.035	0.970	1.104	
US-Neulasta® / EU-Neulasta®	CD34+ C_{max} (cells/µL)	17.428	17.701	0.985	0.924	1.050	
	CD34+ AUC_{0-t} (h·cells/µL)	1600.001	1638.707	0.976	0.915	1.042	

ANC = absolute neutrophil count; CI = confidence interval; intra CV% = intra-subject coefficient of variation; LS = least squares; PD = pharmacodynamic

Natural log transformation of C_{max} and AUC_{0-t} was used for analysis. PROC general linear model (GLM) analysis of variance (ANOVA) with treatment sequence and period as fixed effects, and subject within sequence as a random effect was performed for these parameters.

For T_{max} a non-parametric Hodges-Lehmann method was performed on the non-transformed values.

* The intra CV% (within-subject variability) is displayed only once for each parameter, as it is equal for each comparison.

Source: Table 15.3-6, and Table 15.3-7

- *Relationship between Pharmacodynamics and Anti-Drug Antibodies*

Descriptive statistics were used to summarise the PD parameters for ANC and CD34+ count by treatment and ADA status.

In addition, geometric mean ratios and corresponding 95% CIs for the 3 pairwise comparisons between 2 treatments were repeated by ADA status for the primary PD parameters for ANC and secondary PD parameters for ANC and CD34+ count data.

Minimal differences in the PD response were observed between ADA positive and negative subjects. For all 3 treatments, the primary PD response in terms of ANC AUC0-t appeared to be approximately 10% lower in ADA positive subjects compared to in ADA negative subjects.

When excluding the ADA positive subjects from the comparison of the primary PD parameters in terms of ANC Cmax and ANC AUC0-t between the 3 treatments, results showed that the upper limit of the 95% CIs of the geometric means ratios were still contained within 0.8500 - 1.1765 equivalence interval for each comparison (Table 13).

Still in the smaller ADA positive subgroup the equivalence margin was met for the primary PD parameters. Also for the secondary PD parameters in this ADA negative subgroup, the estimates and corresponding 95% CIs of the geometric mean ratios were close to 1 for CD34+ Cmax and CD34+ AUC0-t, and the median difference was zero (0.000) for ANC Tmax and CD34+ Tmax.

Table 13: Summary of Equivalence Analysis for the Primary PD Parameters for ANC in ADA Negative Subjects (MYL-1401H-1001)

Treatment Comparison (Test versus Reference)	PD Parameter	Geometric LS means		Ratio Test/Reference			Intra CV%*
		Test	Reference	Estimate	95% CI # Lower	95% CI # Upper	
MYL-1401H / EU-Neulasta®	ANC AUC_{0-t} ($h \cdot 10^9$/mL)	2849.073	2892.386	0.985	0.945	1.027	17.9
	ANC C_{max} (10^9/mL)	22.675	22.945	0.988	0.958	1.020	13.4
MYL-1401H / US-Neulasta®	ANC AUC_{0-t} ($h \cdot 10^9$/mL)	2849.073	2840.594	1.003	0.962	1.046	
	ANC C_{max} (10^9/mL)	22.675	23.090	0.982	0.952	1.013	
US-Neulasta® / EU-Neulasta®	ANC AUC_{0-t} ($h \cdot 10^9$/mL)	2840.594	2892.386	0.982	0.942	1.024	
	ANC C_{max} (10^9/mL)	23.090	22.945	1.006	0.975	1.038	

ADA = anti-drug antibodies; ANC = absolute neutrophil count; CI = confidence interval; intra CV% = intra-subject coefficient of variation; LS = least squares; PD = pharmacodynamic

Natural log transformation of C_{max} and AUC_{0-t} was used for analysis. PROC GLM (general linear model) analysis of variance (ANOVA) with treatment sequence and period as fixed effects, and subject within sequence as a random effect was performed for these parameters.

ADA status is defined as negative for subjects without any positive ADA response at any point during the study.

Equivalence is established if the 95% CI of the ratio is contained completely within acceptance range (0.8500 - 1.1765).

* The intra CV% (within-subject variability) is displayed only once for each parameter, as it is equal for each comparison.

Study MYL-1401H-1002

Trial MYL-1401H-1002 was a single-centre, randomised, open-label, repeated dose, parallel group trial intended to evaluate immunogenicity, PD, safety, and tolerability of the test product, MYL-1401H, compared with the reference product, US-licensed Neulasta, in healthy subjects.

Methods

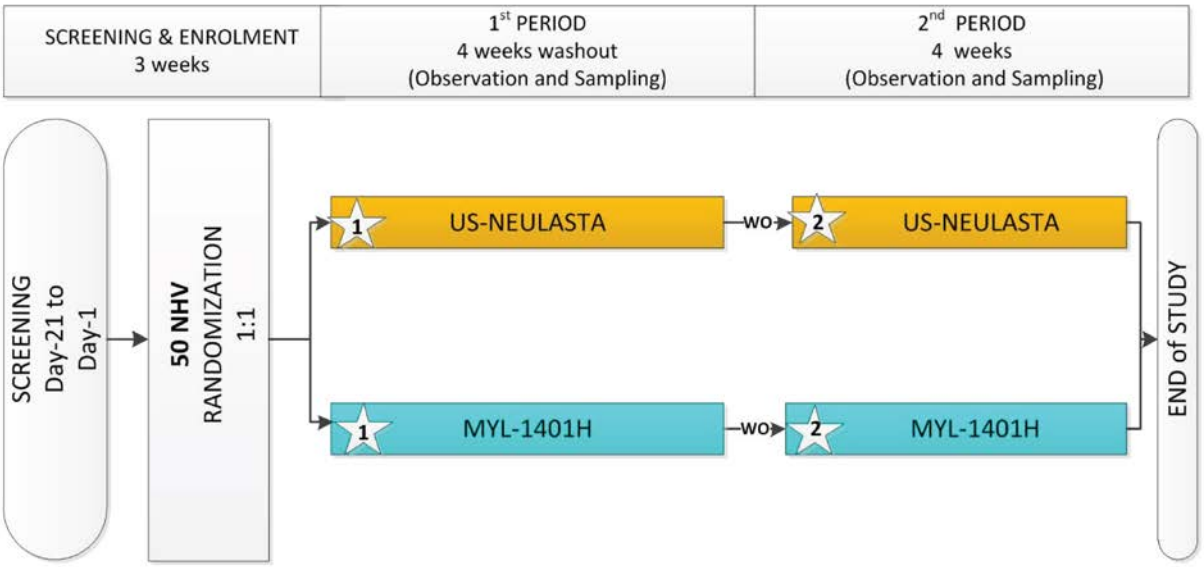


Figure 4: Study design (MYL-1401-1002)

Each subject received 2 single sc. injections of 6 mg of either the test product, MYL-1401H, or the reference product, US-Neulasta, in 2 separate periods with a washout period of 4 weeks between study drug administrations. The sc. injections were given using a prefilled syringe.

As primary objective the immunogenicity between two sc injections of MYL-1401H and US Neulasta was descriptively compared. As secondary objective the safety and tolerability of MYL-1401H and US Neulasta after two sc injections (6 mg) in healthy volunteers was evaluated.

The sample size estimation is based on an “expected immunogenicity event rate” as follows:

The expected immunogenicity event rate in this study was 13%. A total sample size of 44 normal healthy volunteers (22 per group) would provide 95% confidence to rule out an immunogenicity event rate of 13% or more in each treatment group if no events are observed.

Results

- *Disposition of Subjects and Data Sets Analysed*

Of the 85 subjects who were screened, 50 subjects were included in the study. Of these, 25 subjects received 6 mg MYL-1401H and 25 subjects received 6 mg US-Neulasta in the first treatment period. After dosing in Period 1, 6 subjects were withdrawn due to non-serious TEAEs. As a result, 23 of 25 subjects who received 6 mg MYL-1401H in the first treatment period received the same dose in the second treatment period, and 21 of 25 subjects who received 6 mg US-Neulasta in the first treatment period received the same dose in the second treatment period. In addition, Subject 004 withdrew consent in the second treatment period after receiving the second dose of US-Neulasta. A total 43 subjects completed the study and all were included in the PP set. The subject who withdrew consent after completion of dosing in Period 2 was included in the PP set as well, which consisted of 44 subjects in total. All 50 dosed subjects were included in the SAF set.

There were a few protocol deviations that were considered minor and not having affected the outcome of the study.

- *Demographic and Other Baseline Characteristics*

Based on Table 14, demographic characteristics are comparable between treatment groups.

Table 14: Summary of Demographic Characteristics (Safety Set)

Parameter		Statistic / Category	MYL-1401H (N=25)	US-Neulasta® (N=25)	Total (N=50)
Gender	– Male	n (%)	13 (52.0)	11 (44.0)	24 (48.0)
	– Female	n (%)	12 (48.0)	14 (56.0)	26 (52.0)
Race	– American Indian or Alaska Native	n (%)	0 (0.0)	1 (4.0)	1 (2.0)
	– Asian	n (%)	1 (4.0)	0 (0.0)	1 (2.0)
	– Black or African American	n (%)	1 (4.0)	0 (0.0)	1 (2.0)
	– White	n (%)	20 (80.0)	22 (88.0)	42 (84.0)
	– Multiple	n (%)	3 (12.0)	2 (8.0)	5 (10.0)
Ethnicity	– Hispanic or Latino	n (%)	0 (0.0)	1 (4.0)	1 (2.0)
	– Not Hispanic or Latino	n (%)	25 (100.0)	24 (96.0)	49 (98.0)
Age (years)		mean (SD)	34.7 (14.64)	41.4 (15.76)	38.0 (15.42)
		min - max	19 - 65	19 - 64	19 - 65
Height (cm)		mean (SD)	178.4 (11.36)	173.4 (8.47)	175.9 (10.23)
		min-max	153 - 200	157 - 193	153 - 200
Weight (kg)		mean (SD)	75.9 (11.85)	74.4 (11.62)	75.2 (11.64)
		min-max	62 - 113	60 - 106	60 - 113
Body Mass Index (kg/m^2)		mean (SD)	23.82 (2.40)	24.66 (2.61)	24.24 (2.52)
		min-max	20.60 - 28.50	19.60 - 29.00	19.60 - 29.00

max = maximum; min = minimum; N (n) = number of subjects; SD = standard deviation

- *Pharmacodynamic Results*

Samples for determination of ANC were taken each period on Day -1 (as part of the clinical laboratory assessments), on Days 2 (as part of the clinical laboratory assessments), 3, 8, 15, and 22, and at follow-up (as part of the clinical laboratory assessments). The Day 3 assessment was expected to be close to the time of maximum potential drug effect on ANC.

The mean ANC versus time profiles were relatively similar between the 2 treatments. An ANC elevation was observed at the first sampling time point of 24 hours after dosing of either the test product, MYL-1401H, or the reference product, US-Neulasta. The strongest ANC response was observed 48 hours after the second dose; ANC levels were approximately 9-fold higher for both treatments compared with baseline. On subsequent days, ANC levels decreased and had returned to normal by 14 days after dosing. CD34+ counting was not performed.

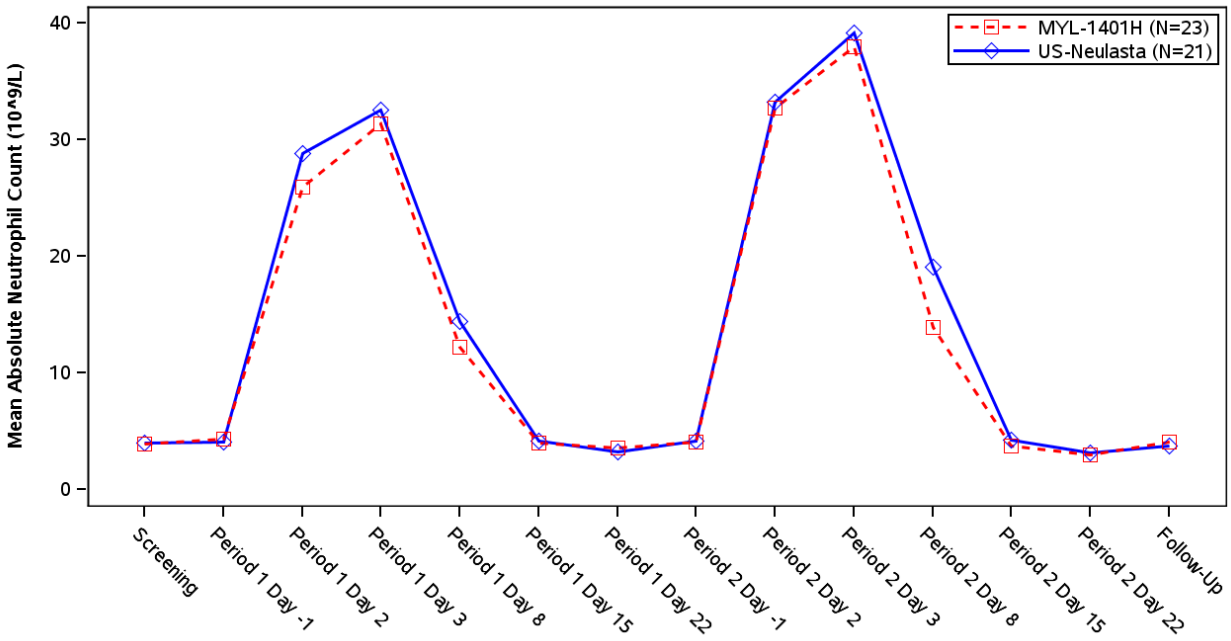


Figure 5: Mean Absolute Neutrophil Count Versus Time by Treatment (Per-Protocol Set)

2.4.4. Discussion on clinical pharmacology

Study MYL-1401H-1001 demonstrated in an appropriate and sensitive model in a confirmatory way that 2 mg MYL-1401H and 2 mg reference MP (Neulasta EU sourced) were equivalent in terms of PK profiles and the co-primary PD endpoints ANC AUC0-t and ANC Cmax. This study also showed PD equivalence as to CD34+ count as a secondary parameter.

There were small differences in the PD response observed between ADA positive and negative subjects where responses in terms of ANC AUC0-t appeared to be approximately 10% lower in ADA positive subjects compared to in ADA negative subjects. Although the study MYL-1401H-1001 was not powered to evaluate equivalence of the primary PD parameters for ANC in a smaller subgroup of ADA negative subjects, these results indicate that the primary PD parameters continued to be equivalent between MYL-1401H and the reference treatments EU-Neulasta and US-Neulasta in a subgroup of subjects without any ADA positive response at any time point. Also the secondary PD parameters appeared to be similar between MYL-1401H and the reference treatments in this subgroup.

PD was also descriptively analysed in trial MYL-1401H-1002. The results reasonably support those of study 1001. In this study US-sourced Neulasta was used. The study results are relevant for the current application because an analytical bridge between US- and EU-sourced reference product has been established.

The applicant did not submit studies on distribution, elimination, dose-proportionality and time dependencies, special populations, pharmacokinetics interaction studies, pharmacokinetics using biomaterials and mechanism of action. This is acceptable as according to the guideline EMEA/CHMP/BMWP/31329/2005, these studies are not required.

Taken together, these results support the claim of biosimilarity between Fulphila and the reference product Neulasta.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology has been well described for Fulphila and the claim of biosimilarity is supported by the primary and secondary PK parameters which were fully contained within the acceptance interval of 80.00-125.00% in the study MYL-1401H-1001 as well as the secondary PD parameters where the GMR were close to 1.

Study MYL-1401H-1002 was supportive of the claim for biosimilarity.

Therefore, overall PK/PD data from the two studies show that similarity between Fulphila and the reference product Neulasta could be demonstrated.

2.5. Clinical efficacy

2.5.1. Dose response study(ies)

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

2.5.2. Main study(ies)

Study MYL-1401H-3001: A Multicenter, Double-Blind, Randomized, Comparative Efficacy and Safety Study of MYL-1401H and European Sourced Neulasta in Stage II/III Breast Cancer Patients Receiving Neoadjuvant or Adjuvant Chemotherapy

Methods

Study Participants

Inclusion criteria

1. Patients aged ≥18 years.
2. Women of child-bearing potential agreed to use effective methods of birth control during the treatment period from the first dose of study drug until 6 months following the last dose of study drug. Acceptable methods of contraception included nonhormonal intrauterine device and barrier methods (male condom, female condom, diaphragm, or cervical cap) with spermicide. Female patients who normally abstained from sexual activity were recruited, provided that they remained abstinent during the study or if they became sexually active, they agreed to use effective methods of birth control as described above.
3. Male patients without a vasectomy agreed to use a condom and their female partners of child-bearing potential agreed to use another form of contraception (hormonal contraceptives, intrauterine device, diaphragm with spermicide, or cervical cap with spermicide) during the treatment period from the first dose of study drug until 6 months following the last dose of study drug.

4. Newly diagnosed, pathologically confirmed breast cancer. Stage II or III breast cancer with adequate staging workup (National Comprehensive Cancer Network guidelines; Version 1.2014 and adequate surgery if receiving adjuvant therapy.
5. Patients planned/eligible to receive neoadjuvant or adjuvant treatment with TAC for their breast cancer. Cancer chemotherapy and radiotherapy naïve.
6. Eastern Cooperative Oncology Group (ECOG) performance status ≤1.
7. Absolute neutrophil count ≥1.5 × 10^9/L.
8. Platelet count ≥100 × 10^9/L.
9. Hemoglobin >10 g/dL without blood transfusions or cytokine support during the 2 weeks previous to the hemoglobin level.
10. Adequate cardiac function (including left ventricular ejection fraction ≥50% as assessed by echocardiography) within 4 weeks prior to start of chemotherapy.
11. Adequate renal function, ie, creatinine <1.5 × upper limit of normal (ULN).

Exclusion criteria

1. Participation in a clinical trial in which they received an investigational drug within 28 days before randomization.
2. Previous exposure to filgrastim, pegfilgrastim, lenograstim, lipegfilgrastim, or other filgrastims on the market or in clinical development.
3. Received blood transfusions or erythroid growth factors within 2 weeks prior to first dose of chemotherapy.
4. Known hypersensitivity to any drugs or excipients that patients received during the study.
5. Known hypersensitivity to *E. coli*-derived products.
6. Known fructose intolerance (related with sorbitol excipient).
7. Underlying neuropathy of Grade 2 or higher.
8. Active infectious disease or any other medical condition which might have put the patient at significant risk to tolerate 6 courses of TAC chemotherapy (eg, recent myocardial infarction).
9. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2.5 × ULN, ALT and/or AST >1.5 × ULN with alkaline phosphatase (ALP) >2.5 × ULN; any bilirubin >ULN. Any alteration of liver function and/or ALP elevation, even within acceptance limits, was investigated before randomization to exclude any Stage IV disease.
10. Treatment with systemically active antibiotics within 5 days before first dose of chemotherapy.
11. Patients under treatment with lithium.
12. Chronic use of oral corticosteroids.
13. Splenomegaly of unknown origin by physical examination and/or computerized tomography scan or ultrasound and any condition which can cause splenomegaly, eg, thalassemia, glandular fever, hemolytic anemias, and malaria.
14. Myeloproliferative or myelodysplastic disorders, sickle cell disorders, and any illness or condition that in the opinion of the investigator might affect the safety of the patient or the evaluation of any study endpoint.

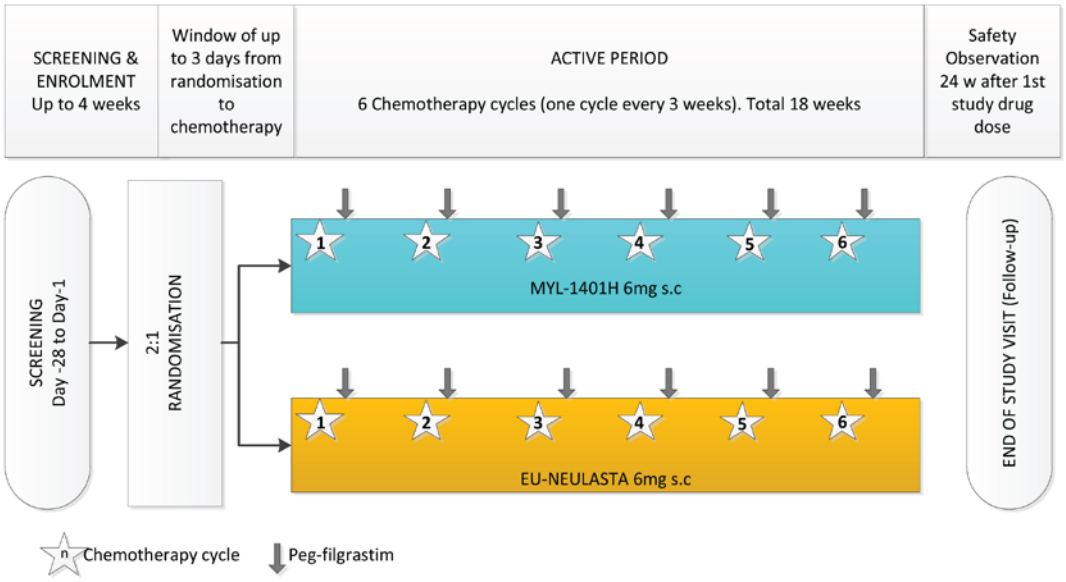
15. Increased potential risk of Adult Respiratory Distress Syndrome.
16. Pregnant or nursing women.
17. Patients known to be seropositive for human immunodeficiency virus, or having an acquired immunodeficiency syndrome defining illness or a known immunodeficiency disorder.
18. A known active abuse of drugs or alcohol precluded patient participation and evaluation in the study.
19. Any known psychiatric conditions.
20. Any disease or physical condition that would have interfered with adequate performance of study assessments, such as lack of access to patient's domiciliary, and distance of patient's domiciliary from clinic site.

Treatments

MYL-1401H 6 mg injection administered as a single sc dose, on Day 2 of each cycle, ie, 24 h (+ 2 h after) after the end of chemotherapy.

The planned duration for the entire study was approximately 28 weeks (from Screening to follow-up [24 weeks from the first dose of study drug]), assuming no delays in dosing.

The planned duration of patient treatment during the entire study was approximately 18 weeks (from the first day of chemotherapy [Day 1 Cycle 1] to the last scheduled assessment in Cycle 6), assuming no delays in dosing.



Abbreviations: CTX = cytotoxic chemotherapy; mg = milligram; n = chemotherapy cycle; sc = subcutaneous; w = weeks

Figure 6: Overview of Study Design MYL-1401H-3001

Objectives

The primary objective of this clinical trial was to compare the efficacy of MYL-1401H versus European-sourced Neulasta (EU-Neulasta) for the prophylactic treatment of chemotherapy-induced neutropenia

in patients with Stage II/III breast cancer receiving docetaxel, doxorubicin, and cyclophosphamide (TAC) anti-cancer chemotherapy.

The secondary objectives of this clinical trial were as follows:

- to assess the safety of MYL-1401H and EU-Neulasta when administered through 6 cycles of TAC anti-cancer chemotherapy.
- to assess the potential immunogenicity of MYL-1401H and EU-Neulasta during chemotherapy and up to 24 weeks following the first administration.

Outcomes/endpoints

Primary Efficacy Endpoint:

The primary efficacy endpoint was the duration of severe neutropenia (DSN) in Cycle 1, defined as days with ANC $<0.5 \times 10^9$/L.

Secondary Efficacy Endpoints:

☐ The frequency of the worst grade (Grade 3 or 4) neutropenia by cycle (Grade 3 defined as ANC $<1.0 \times 10^9$/L and Grade 4 as ANC $<0.5 \times 10^9$/L).

☐ The depth of the ANC nadir in Cycle 1.

☐ The time to the post-nadir ANC recovery (ANC $\geq 1.5 \times 10^9$/L) in Cycle 1.

☐ The ANC-time to nadir in Cycle 1 (ie, time from the beginning of chemotherapy to the occurrence of the ANC nadir).

☐ The rate of febrile neutropenia (FN) defined by the European Society of Medical Oncology Clinical Practice Guidelines as ANC <0.5 × 109/L, or expected to fall below 0.5 × 109/L, with a single oral temperature >38.5°C or 2 consecutive readings of an oral temperature >38.0°C for 2 h, by cycle and across all cycles.

☐ The percentage of scheduled chemotherapy doses that were delivered.

☐ The proportion of chemotherapy doses reduced, omitted, or delayed related to neutropenia, FN, or documented infections.

☐ The number of days of delay of chemotherapy related to neutropenia, FN, or documented infection.

Safety:

The following safety endpoints were evaluated:

☐ The incidence, nature, and severity of adverse events (AEs) including adverse drug reactions.

☐ The incidence, severity, and distribution of bone pain by brief pain inventory (BPI) form (Short Form) in Cycle 1 and Cycle 2 only.

☐ The incidence, severity, and distribution of infections.

☐ Injection site tolerance.

☐ Incidence, titer, and neutralizing capacity of antibodies against MYL-1401H and EU-Neulasta.

Sample size

Approximately 189 patients were planned for enrolment into the study in a 2:1 ratio of the 2 treatment groups (126:63 in the MYL-1401H and EU-Neulasta arm, respectively).

A total sample size of 135 patients allocated in a 2:1 ratio (90 and 45 patients treated with MYL-1401H and Neulasta, respectively) is required to provide 90% power to declare that MYL-1401H is comparable to Neulasta in the analysis of DSN in cycle 1. This sample size assumes that the mean DSN will be 1.70 days in cycle 1 for both MYL-1401H and Neulasta.

The common SD is assumed to be 1.5 days. Equivalence will be declared if the two-sided 95% confidence interval (CI) of the difference between the mean DSNs falls wholly within a region defined as [-1, +1 day].

The region of [-1, +1 day] was established by analyzing historical Neulasta data and estimating a 50% retention of the Neulasta mean treatment benefit over placebo.

Randomisation

Patients were randomised to receive either MYL-1401H or EU-Neulasta (in a 2:1 ratio, respectively), and were stratified based on their age and country.

Blinding (masking)

The oncology pharmacist who prepared the doses and the person administering the drug (eg, study nurse, physician [other than the principal investigator or sub-principal investigator]) were the only individuals who had access or knowledge of the actual drug delivered. When administering the drug, the application syringes were covered in order to make them indistinguishable to the patient.

Statistical methods

The ITT Population (ITT) consisted of all patients who were randomized into the study. Patients in the ITT population were categorized to the treatment as-randomized.

The per protocol (PP) population was defined at the end of Cycle 1 and was a subset of the ITT, including patients receiving treatment to which they were randomized and had no major protocol deviations.

The primary efficacy analysis was based on the PP population, an in the ITT as a sensitivity analysis.

An ANOVA model with treatment as independent variable, and country and age-group as factors, was used to produce a 2-sided 95% CI for the difference in least squares means DSNs. Equivalence was declared if the CI was completely within the range of ± 1 day.

The difference in mean DSN in Cycle 1 within the PP population was statistically compared with the following hypotheses:

H0: (μ MYL-1401H – μNeulasta ≤-1) or (μ MYL-1401H - μNeulasta ≥1)

H1: -1 day <(μ MYL-1401H – μNeulasta) <1 day,

where μ MYL-1401H and μNeulasta are the mean DSN for MYL-1401H and EU-Neulasta, respectively; calculated in days.

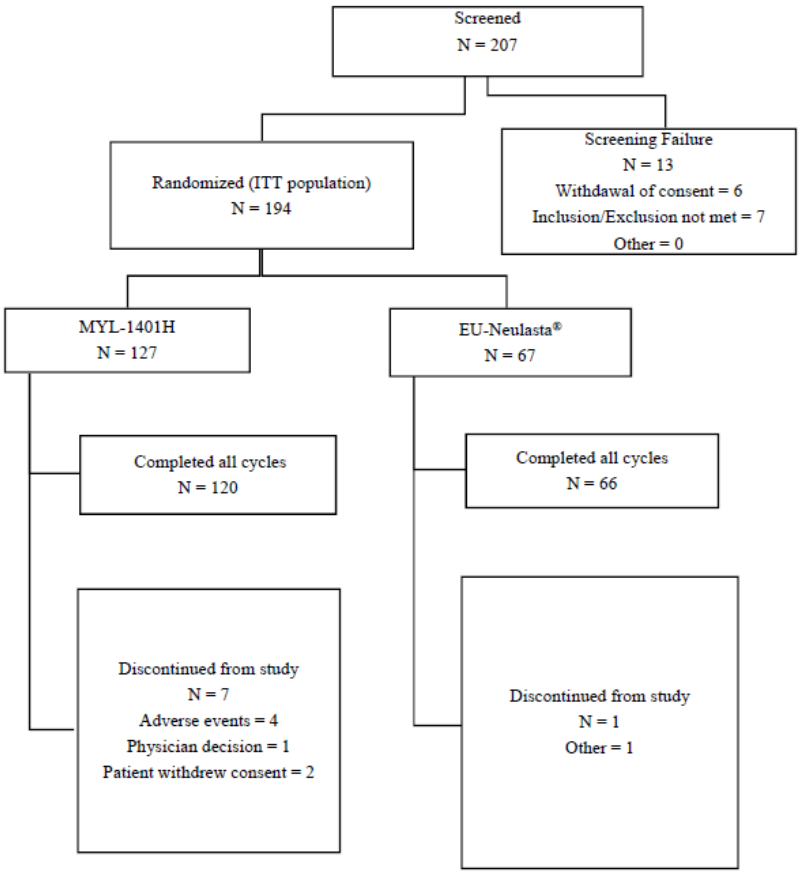
An analysis of variance (ANOVA) model with treatment as an independent variable, and country and age-group as factors, was used to produce a 2-sided 95% confidence interval (CI) for the difference in least squares mean (LS Mean) DSNs. The 2-sided 95% CI is equivalent to two 1-sided tests at the 2.5% level. Equivalence was declared if the CI was completely within the range of ± 1 day.

Secondary endpoints were analysed descriptively.

A blinded interim analysis was conducted when 50% of the required patients had completed cycle 1. If the common SD had been estimated to be > 1.5 days, then the sample size would have been adjusted accordingly. Since this evaluation is blinded, there was no impact on the overall type 1 error rate and no adjustment of the final analysis of the primary objective was required according to the applicant. Because the SD was less than 1.5 days, no adjustment to the sample size was made.

Results

Participant flow



Abbreviations: EU = European Union; ITT = intent to treat; N = number of patients

Recruitment

Actual: 194 patients were randomized and received study treatment; 127 patients were randomized to receive MYL-1401H and 67 patients were randomized to receive EU-Neulasta.

Completed: 186 patients completed the study.

Analysed: 194 patients were included in the data analysis.

Conduct of the study

Version 3.0 of the SAP (dated 25 September 2015) included information on additional immunogenicity assessments to be performed in anticipation of a protocol change. However, due to operational reasons, the protocol change was not initiated and as a consequence the additional immunogenicity assessments were not performed.

A summary of the major protocol deviations is presented in Table 15.

Table 15: Major protocol deviations (ITT population)

Protocol Deviations	MYL-1401H (N=127)	EU-Neulasta® (N=67)
	n (%)	
Number of Subjects Who Had Major Protocol Deviations	34 (26.8%)	15 (22.4%)
Protocol Deviation Description		
Inclusion/Exclusion Criteria	2 (1.6%)	0 (0.0%)
Prohibited Concomitant Medication	1 (0.8%)	0 (0.0%)
Additional Protocol Deviations	33 (26.0%)	15 (22.4%)
Lab Testing Deviations	15 (11.8%)	10 (14.9%)
Special Testing Deviation	11 (8.7%)	1 (1.5%)
Other	6 (4.7%)	4 (6.0%)
Dosing-Time Deviation	3 (2.4%)	0 (0.0%)
Subject Visit Window Deviation	2 (1.6%)	0 (0.0%)
Missed Subject Visit(s)	1 (0.8%)	2 (3.0%)

Abbreviations: ITT = intent-to-treat; N = number of patients; n = number of patients in the sample

Baseline data

Out of the 194 (100.0%) patients with newly diagnosed, pathologically confirmed breast cancer 117 (60.3%) had undergone prior breast cancer surgery, 5 (2.6%) had undergone a lumpectomy, 43 (22.2%) had undergone partial or segmented mastectomy, 3 (1.5%) had undergone a simple or total mastectomy, 52 (26.8%) had undergone radical mastectomy, and 21 (10.8%) had undergone modified radical mastectomy.

Table 16: Patient Demographics (ITT Population)

Parameter	MYL-1401H (N=127)	EU-Neulasta® (N=67)	Overall (N=194)
Age (years)			
Mean ± SD	49.5 ± 10.61	50.1 ± 9.85	49.7 ± 10.33
Median (min, max)	49.0 (25, 79)	50.0 (29, 68)	50.0 (25, 79)
Age group (years), n (%)			
<50	64 (50.4%)	32 (47.8%)	96 (49.5%)
50-65	56 (44.1%)	30 (44.8%)	86 (44.3%)
>65	7 (5.5%)	5 (7.5%)	12 (6.2%)
Sex, n (%)			
Male	1 (0.8%)	0 (0.0%)	1 (0.5%)
Female	126 (99.2%)	67 (100.0%)	193 (99.5%)
Ethnicity, n (%)			
Hispanic or Latino	0 (0.0%)	0 (0.0%)	0 (0.0%)
Not Hispanic or Latino	127 (100.0%)	67 (100.0%)	194 (100.0%)
Race, n (%)			
White	127 (100.0%)	67 (100.0%)	194 (100.0%)
Black or African American	0 (0.0%)	0 (0.0%)	0 (0.0%)
Asian	0 (0.0%)	0 (0.0%)	0 (0.0%)
American Indian/Alaska Native	0 (0.0%)	0 (0.0%)	0 (0.0%)
Native Hawaiian/Other Pacific Islander	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other	0 (0.0%)	0 (0.0%)	0 (0.0%)

Abbreviations: N = number of patients; n = number of patients in the sample; SD = standard deviation
Source: Table 14.1.3.1

Numbers analysed

ITT Population:

The ITT population consisted of all patients who were randomized into the study. The ITT population consisted of a total of 194 (100%) patients.

Safety Population:

The safety population included all patients who received at least 1 dose of study drug and consisted of 194 (100%) patients.

Per Protocol Population:

The PP population was defined at the end of Cycle 1 and included a subset of the ITT population who started treatment without major protocol deviations and consisted of 193 (99.5%) patients.

Outcomes and estimation

Primary Efficacy Endpoint: Duration of Severe Neutropenia: Cycle 1 (PP population)

The mean (± SD) DSN in the MYL-1401H group was 1.2 (± 0.93), the median DSN was 1.0, and the DSN ranged from 0 to 5 days. In the EU-Neulasta group, the mean (± SD) DSN was 1.2 (± 1.10), the median DSN was 1.0, and the DSN ranged from 0 to 4 days. The DSN was 1 day for 51 (40.5%) patients in the MYL-1401H group and 17 (25.4%) patients in the EU-Neulasta group. The DSN was 0 days for 32 (25.4%) patients in the MYL-1401H group and for 24 [35.8%] patients in the EU-Neulasta

group. The DSN was 2 days for 25 (27.8%) patients in the MYL-1401H group and for 17 (25.4.%) patients in the EU-Neulasta group (Table 17).

Table 17: Duration of Severe Neutropenia in Cycle 1 (PP Population)

Parameter	MYL-1401H (N=126)	EU-Neulasta® (N=67)
Duration of severe neutropenia (days)		
Mean ± SD	1.2 ± 0.93	1.2 ± 1.10
Median, (range)	1.0, (0-5)	1.0, (0-4)
LS Mean (SE)	1.31 (0.139)	1.30 (0.154)
LS Mean difference from Neulasta® (SE)	0.01 (0.148)	
95% CI[a]	(-0.285, 0.298)	
Duration (days), n (%)		
0	32 (25.4%)	24 (35.8%)
1	51 (40.5%)	17 (25.4%)
2	35 (27.8%)	17 (25.4%)
3	7 (5.6%)	8 (11.9%)
4	0 (0.0%)	1 (1.5%)
5	1 (0.8%)	0 (0.0%)

Abbreviations: ANOVA = analysis of variance ; CI = confidence interval; N = total number of patients with available data in Cycle 1; n = number of patients in the sample; LS Mean = least squares mean; SD = standard deviation; SE = standard error

Source: Table 14.2.1.1

a: The 95% CI for the difference in least square means is based on the result of an ANOVA model with treatment group, country, and age group as factors. Comparable efficacy was declared if the 95% CI was completely within this range of (-1 day, +1 day)

The 95% CI (-0.285, 0.298) for the difference in least square mean DSN of MYL-1401H and EU-Neulasta was found to be within the pre-specified equivalence range of [-1 day, +1 day] based on the ANOVA model with treatment group, country, and age group as factors. Therefore comparable efficacy of MYL-1401H and EU-Neulasta can be declared (null hypothesis that mean DSN in Cycle 1 on MYL-1401H differs from mean DSN on EU-Neulasta by 1 day or more can be rejected).

In summary, trial MYL-1401H-3001 met its primary objective.

There were 19 out of 126 (15%) patients in the MYL-1401H group and 13 out of 67 (19.4%) in the EU-Neulasta group who tested positive for anti-drug antibody (ADA) and 107 out of 126 (85%) patients in the MYL-1401H group and 54 out of 67 (80.6%) in the EU-Neulasta group who tested positive negative for anti-drug antibody (ADA). Results of DSN in this subgroup are presented in

Table 18: Duration of severe neutropenia in cycle 1 in patients positive for antibody (based on assay with MYL-1401H) (PP population)

Parameter	MYL-1401H (N=126)	EU-Neulasta® (N=67)
Duration of severe neutropenia (days)		
n, Mean ± SD	19, 1.5 ± 0.77	13, 0.9 ± 0.95
Median, (range)	2.0, (0-3)	1.0, (0-3)
LS Mean (SE)	1.45 (0.311)	0.81 (0.314)
LS Mean difference from EU-Neulasta® (SE)	0.64 (0.289)	
95% CI[a]	(0.039, 1.231)	
Duration (days), n (%)		
0	2 (1.6%)	5 (7.5%)
1	7 (5.6%)	5 (7.5%)
2	9 (7.1%)	2 (3.0%)
3	1 (0.8%)	1 (1.5%)

Abbreviations: ANOVA = analysis of variance ; CI = confidence interval; N = total number of patients with available data in Cycle 1; n = number of patients in the sample; LS Mean = least squares mean; SD = standard deviation; SE = standard error

a: The 95% CI for the difference in LS Mean is based on the result of an ANOVA model with treatment group, country, and age group as factors.

Table 19: Duration of severe neutropenia in Cycle 1 in patients negative for antibody (based on assay with MYL-1401H) (PP population)

Parameter	MYL-1401H (N=126)	EU-Neulasta® (N=67)
Duration of severe neutropenia (days)		
n, Mean ± SD	107, 1.1 ± 0.94	54, 1.2 ± 1.13
Median, (range)	1.0, (0-5)	1.0, (0-4)
LS Mean (SE)	1.20 (0.154)	1.32 (0.175)
LS Mean difference from EU-Neulasta® (SE)	-0.12 (0.165)	
95% CI[a]	(-0.442, 0.210)	
Duration (days), n (%)		
0	30 (23.8%)	19 (28.4%)
1	44 (34.9%)	12 (17.9%)
2	26 (20.6%)	15 (22.4%)
3	6 (4.8%)	7 (10.4%)
4	0 (0.0%)	1 (1.5%)
5	1 (0.8%)	0 (0.0%)

Abbreviations: ANOVA = analysis of variance ; CI = confidence interval; N = total number of patients with available data in Cycle 1; n = number of patients in the sample; LS Mean = least squares mean; SD = standard deviation; SE = standard error

a: The 95% CI for the difference in LS Mean is based on the result of an ANOVA model with treatment group, country, and age group as factors.

Secondary (efficacy) endpoints

There were small numerical differences for secondary efficacy endpoints (depths of nadir, frequency of severe neutropenia, frequency of febrile neutropenia) not precluding a conclusion of biosimilarity.

Table 20: Frequency, depth, and time of neutropenia in cycle 1 (PP population)

Parameter	MYL-1401H (N=126)	EU-Neulasta® (N=67)
Frequency of the worst Grade 3 or 4 neutropenia, n (%)*		
Grade 3 or 4 neutropenia	114 (90.5%)	55 (82.1%)
Grade 4 neutropenia	94 (74.6%)	43 (64.2%)
Grade 3 neutropenia	20 (15.9%)	12 (17.9%)
ANC nadir (10^9/L)		
Mean (SD)	0.40 (± 0.47)	0.78 (± 1.43)
Median (range)	0.21 (0.0-2.5)	0.27 (0.0-6.7)
ANC-time to nadir (days)		
Mean (SD)	6.2 (± 0.98)	6.3 (± 1.57)
Median (range)	6.0 (0-12)	6.0 (1-14)
Post-nadir ANC recovery, n (%)		
No	0 (0.0%)	0 (0.0%)
Yes	125 (99.2%)	67 (100.0%)
Not evaluable	1 (0.8%)	0 (0.0%)
Time to post-nadir ANC recovery		
n, Mean (SD)	125, 1.9 (± 0.85)	67, 1.7 (± 0.91)
Median (range)	2.0 (0-4)	2.0 (0-3)
Time (day), n (%)		
0	6 (4.8%)	9 (13.4%)
≤1	38 (30.4%)	24 (35.8%)
≤2	101 (80.8%)	56 (83.6%)
≤3	121 (96.8%)	67 (100.0%)
≤4	125 (100.0%)	67 (100.0%)

Abbreviations: ANC = absolute neutrophil count; L = liter; N = total number of patients with available data in Cycle 1; n = number of patients in the sample; PP = per protocol; SD = standard deviation

Table 21: Frequency of neutropenia by cycle (ITT population)

Parameter Cycle	MYL-1401H (N=127)	EU-Neulasta® (N=67)
Frequency of the worst Grade 3 or 4 neutropenia, n (%)*		
Grade 3 or 4 neutropenia	120 (94.5%)	56 (83.6%)
Grade 4 neutropenia	103 (81.1%)	49 (73.1%)
Grade 3 neutropenia	17 (13.4%)	7 (10.4%)
Cycle 2		
Grade 3 or 4 neutropenia	53 (42.4%)	29 (43.3%)
Grade 4 neutropenia	19 (15.2%)	15 (22.4%)
Grade 3 neutropenia	34 (27.2%)	14 (20.9%)
Cycle 3		
Grade 3 or 4 neutropenia	51 (41.1%)	28 (41.8%)
Grade 4 neutropenia	34 (27.4%)	16 (23.9%)
Grade 3 neutropenia	17 (13.7%)	12 (17.9%)
Cycle 4		
Grade 3 or 4 neutropenia	66 (53.2%)	30 (44.8%)
Grade 4 neutropenia	30 (24.2%)	18 (23.9%)
Grade 3 neutropenia	36 (29.0%)	12 (17.9%)
Cycle 5		
Grade 3 or 4 neutropenia	60 (48.8%)	26 (39.4%)
Grade 4 neutropenia	38 (30.9%)	13 (19.7%)
Grade 3 neutropenia	22 (17.9%)	13 (19.7%)
Cycle 6		
Grade 3 or 4 neutropenia	59 (49.2%)	28 (42.4%)
Grade 4 neutropenia	34 (28.3%)	20 (30.3%)
Grade 3 neutropenia	25 (20.8%)	8 (12.1%)

Abbreviations: n = number of patients in the sample; N = total number of patients with available data
Source: Table 14.2.3.2
*If a patient experienced more than 1 grade of neutropenia, only the highest grade was counted.

Table 22: Rate of febrile neutropenia (cycle 1) (ITT population)

Parameter	MYL-1401H (N=127)	EU-Neulasta® (N=67)	P-value
Rate of febrile neutropenia, n (%)	5 (3.9%)	1 (1.5%)	0.35

Febrile neutropenia is defined as febrile neutropenia reported as an AE.
Abbreviations: AE = adverse event; ITT = intent to treat; N = total number of patients with available data in Cycle 1; n = number of patients in the sample

Table 23: Rate of febrile neutropenia by cycle (ITT population)

Parameter	MYL-1401H (N=127)	EU-Neulasta® (N=67)
	n (%)	
Rate of febrile neutropenia for all cycles	7 (5.5%)	1 (1.5%)
Rate of febrile neutropenia in Cycle 1	5 (3.5%)	1 (1.5%)
Rate of febrile neutropenia in Cycle 2	1 (0.8%)	0 (0.0%)
Rate of febrile neutropenia in Cycle 3	1 (0.8%)	0 (0.0%)
Rate of febrile neutropenia in Cycle 4	0 (0.0%)	0 (0.0%)
Rate of febrile neutropenia in Cycle 5	0 (0.0%)	0 (0.0%)
Rate of febrile neutropenia in Cycle 6	0 (0.0%)	0 (0.0%)

Febrile neutropenia is defined as febrile neutropenia reported as an AE.
Abbreviations: AE = adverse event; ITT = intent to treat; n = number of patients in the sample

Ancillary analyses

Not applicable.

Summary of main study(ies)

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

Title: A Multicenter, Double-Blind, Randomized, Comparative Efficacy and Safety Study of MYL-1401H and European Sourced Neulasta in Stage II/III Breast Cancer Patients Receiving Neoadjuvant or Adjuvant Chemotherapy		
Study identifier	Study MYL-1401H-3001 EudraCT Number: 2014-002324-27	
Design	25 Mar 2015 to 09 Feb 2016	
	Duration of main phase:	Patient treatment during the entire study was approximately 18 weeks (from the first day of chemotherapy [Day 1 Cycle 1] to the last scheduled assessment in Cycle 6)
	Duration of Run-in phase:	Not applicable
	Duration of study:	28 weeks (from Screening to follow-up [24 weeks from the first dose of study drug])
Hypothesis	Equivalence, Non-inferiority	
Treatments groups	Group 1: MYL-1401H	MYL-1401H 6 mg injection administered as a single sc dose, on Day 2 of each cycle, ie, 24 h (+ 2 h after) after the end of chemotherapy
	Group 2: Neulasta	EU-Neulasta 6 mg injection administered as a single sc dose, on Day 2 of each cycle, ie, 24 h (+ 2 h) after the end of chemotherapy.

Endpoints and definitions	Primary endpoint	mean duration of severe neutropenia (DSN) during Cycle 1	The primary efficacy endpoint was the duration of severe neutropenia (DSN) in Cycle 1, defined as days with ANC $<0.5 \times 10^9$/L.
	Secondary endpoint	Grade 3/4 Neutropenia	The frequency of the worst grade (Grade 3 or 4) neutropenia by cycle (Grade 3 defined as ANC $<1.0 \times 10^9$/L and Grade 4 as ANC $<0.5 \times 10^9$/L).
	Secondary endpoint	Time to ANC recovery	The time to the post-nadir ANC recovery (ANC $\geq 1.5 \times 10^9$/L) in Cycle 1.
	Secondary endpoint	Depth of ANC nadir	The depth of the ANC nadir in Cycle 1
	Secondary endpoint	ANC- time to nadir	The ANC-time to nadir in Cycle 1 (ie, time from the beginning of chemotherapy to the occurrence of the ANC nadir).
	Secondary endpoint	Rate of Febrile Neutropenia	The rate of febrile neutropenia (FN) defined by the European Society of Medical Oncology Clinical Practice Guidelines as ANC <0.5 × 109/L, or expected to fall below 0.5 × 109/L, with a single oral temperature >38.5°C or 2 consecutive readings of an oral temperature >38.0°C for 2 h, by cycle and across all cycles.
	Secondary endpoint	The incidence, nature, and severity of adverse events (AEs) including adverse drug reactions.	See safety.

	Secondary endpoint	The incidence, severity, and distribution of bone pain by brief pain inventory (BPI) form (Short Form) in Cycle 1 and Cycle 2 only.	See safety.
	Secondary endpoint	The incidence, severity, and distribution of infections.	See safety.
	Secondary endpoint	Injection site tolerance.	See safety.
	Secondary endpoint	Incidence, titer, and neutralizing capacity of antibodies against MYL-1401H and EU-Neulasta	See safety.
Results and Analysis			

Analysis description			
Analysis population and time point description	FAS		
Descriptive statistics and estimate variability		**MYL-1401H**	**Neulasta**
Descriptive statistics and estimate variability Effect estimate per comparison	Number of subject	N=126	N=67
	Primary endpoint Mean (SD)	1.2±0.93	1.2±1.10
	Median (Range)	1.0 (0.5)	1.0 (0-4)
	LS Mean for difference La-MYL-1401H – Neulasta (95% CI)	0.01 (0.148) (-0.285, 0.298)	

	Frequency of the worst Grade 3/4 Neutropenia (ITT); n (%)	120 (94.5%; 17 [13.4%] Grade 3 and 103 [81.1%] Grade 4	56 (83.6%; 7 [10.4%] Grade 3 and 49 [73.1%] Grade 4
	The depth of the ANC nadir in Cycle 1; Mean (std)	0.40 × 10^9/L (± 0.474)	0.78 × 10^9/L (± 1.426)
	Time to ANC recovery (days); Mean (std)	1.9 (± 0.85)	1.7 (± 0.91)
	ANC- time to nadir; days (std)	6.2 (± 0.98)	6.3 (± 1.57)
	Rate of febrile neutropenia; n(%)	7/127 (5.5%)	1/67 (1.5%)

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

The applicant did not submit analyses across trials.

Clinical studies in special populations

The applicant did not submit clinical studies in special populations (see clinical discussion).

Supportive study(ies)

The applicant did not submit supportive studies (see clinical discussion).

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The applicant has submitted the results of a parallel-group, active controlled, blinded trial to show equivalence in terms of DSN. The study design was in accordance with scientific recommendations as outlined in the respective EMA guideline currently in place.

There are minor criticisms on trial 3001 such as administering TAC to patients in neo-adjuvant intent, and not stratifying TAC for adjuvant/neo-adjuvant. These are, however, minor design and conduct issues which have, in essence, no effect on the biosimilar conclusion.

Efficacy data and additional analyses

The primary analysis, as well as the sensitivity analyses, of the primary endpoint are robust and allow the conclusion which read:

The primary objective of the study was met, where the median DSN was 1.0 day (range 0-5) and EU-Neulasta was 1.0 (0.4), the LS mean difference from Neulasta was 0.01 (95%CI -0.285, 0.298),

determined by the ANOVA analysis (with treatment group, country, and age group as factors for the difference in least square mean DSNs of MYL-1401H and EU-Neulasta). The results were found to be within the pre-specified equivalence range of [-1 day, +1 day]. In fact, the 95% CIs were very narrow allowing a firm conclusion of similar efficacy. These results show that there are no significant differences between the two products in terms of DSN, supporting the claim for biosimilarity.

There were small numerical differences for secondary efficacy endpoints (depths of nadir, frequency of severe neutropenia, frequency of febrile neutropenia) not precluding a conclusion of biosimilarity. However, these were not considered clinically relevant. Secondary endpoints and the result of the frequency of neutropenia for cycle 2 to 6 lend overall support to the therapeutic equivalence between Fulphila and Neulasta.

2.5.4. Conclusions on the clinical efficacy

The clinical data in the trial MYL-1401H-3001 in patients undergoing cytotoxic chemotherapy has shown comparable efficacy between Fulphila and Neulasta in reducing the duration of severe neutropenia. Hence, MYL-1401H and Neulasta EU-sourced offer therapeutic equivalence which supports the claim for biosimilarity.

2.6. Clinical safety

Mylan has conducted 3 clinical studies that have evaluated the comparability of safety between MYL-1401H and Neulasta: 2 studies in healthy subjects (MYL-1401H-1001 and MYL-1401H-1002) and 1 comparative safety and efficacy study in patients with Stage II/III invasive breast cancer (MYL-1401H-3001).

Due to differences in the study dose, study design, and populations, the safety data from the 3 studies (MYL-1401H-1001, MYL-1401H-1002, and MYL-1401H-3001) have not been integrated.

The clinical trial specifically dedicated to immunogenicity is trial MYL-1401H-1002. However a thorough assessment of immunogenicity was conducted across the 3 clinical studies (see below).

Patient exposure

A total of 232 healthy subjects and 127 patients diagnosed with breast cancer have received at least 1 dose of MYL-1401H.

In 3-way crossover Study MYL-1401H-1001, 216 healthy male and female subjects received at least one 2-mg SC injection of pegfilgrastim and 198 subjects received the planned 3 doses of pegfilgrastim: 207 subjects received at least 1 dose of MYL-1401H (test product), 208 subjects received at least 1 dose of EU-Neulasta and 207 subjects received atleast 1 dose of US-Neulasta.

In Study MYL-1401H-1002, 25 healthy male and female subjects received at least one 6-mg SC injection of MYL-1401H (test product) and 25 healthy male and female subjects received at least one 6-mg SC injection of US-Neulasta. Two 6-mg SC injections were received by 23 subjects in the MYL-1401H group and 21 subjects in the US-Neulasta group.

In Study MYL-1401H-3001, 127 patients received at least one 6-mg SC injection of MYL-1401H (test product) and 67 patients received at least one 6-mg SC injection of EU-Neulasta. One hundred twenty (94.5%) patients in the MYL-1401H group and 66 (98.5%) patients received all 6 doses of study drug. During each cycle, the majority of patients received their study drug on Day 2 of the cycle as scheduled.

Adverse events

Study MYL-1401H-1001

In Study MYL-1401H-1001, safety and tolerability were evaluated after the single 2-mg sc injection by evaluating all AEs, physical examinations, vital signs, ECGs, clinical laboratory, local tolerance, and immunogenicity (early development of ADA).

There were 1129 TEAEs reported by 200 (93%) subjects that were considered related to pegfilgrastim treatment with 177 (86%) subjects who received MYL-1401H, 182 (88%) subjects who received EU-Neulasta, and 181 (87%) subjects who received US-Neulasta (Table 24).

Table 24: Overview of treatment-emergent adverse events during the study (1001)

	MYL-1401H (N=207) n (%)	EU-Neulasta (N=208) n (%)	US-Neulasta (N=207) n (%)
Number of subjects with at least 1 TEAE	177 (86)	182 (88)	181 (87)
Number of subjects with at least 1 related TEAE	156 (75)	165 (79)	157 (76)
Number of subjects with at least 1 TEAE by severity:			
Grade 1 (mild) severity	158 (76)	172 (83)	166 (80)
Grade 2 (moderate) severity	86 (42)	92 (44)	84 (41)
Grade 3 (severe) severity	0 (0)	0 (0)	1 (>0)
Number of subjects withdrawn due to (S)AEs:	0 (0)	2 (1)	1 (>0)
SAE	0 (0)	0 (0)	1 (>0)
AE	0 (0)	2 (1)	0 (0)

Abbreviations: AE = adverse event; EU = European Union; SAE = serious adverse event; TEAE = treatment-emergent adverse event; US = United States

In MYL-1401H-1001, the most commonly reported TEAE by preferred term (PT) were back pain (81% of the subjects), headache (63% of the subjects), pain in extremity (36% of the subjects) and nasopharyngitis (22% of the subjects). There were no relevant differences in the frequencies of TEAEs or percentages of subjects reporting TEAEs among MYL-1401H and the reference treatments (EU-Neulasta and US-Neulasta).

Study MYL-1401H-1002

A summary of all Treatment-Emergent Adverse Events is provided in Table 25 below.

Table 25: Overview of Treatment-Emergent Adverse Events During the Study (1002)

	MYL-1401H (N=25) n (%)	US-Neulasta® (N=25) n (%)
Number of subjects with at least 1 TEAE	24 (96.0%)	25 (100.0%)
Number of subjects with at least 1 related TEAE	24 (96.0%)	25 (100.0%)
Number of subjects with at least 1 TEAE by severity:		
Grade 1 (mild)	23 (92.0%)	23 (92.0%)
Grade 2 (moderate)	19 (76.0%)	22 (88.0%)
Grade 3 (severe)	0 (0.0%)	1 (4.0%)
Number of subjects withdrawn due to (S)AEs:	2 (8.0%)	4 (16.0%)
SAE	0 (0.0%)	0 (0.0%)
AE	2 (8.0%)	4 (16.0%)

AE = adverse event; N = the # of subjects exposed to the treatment; n = the # of subjects that experienced the adverse event; SAE = serious adverse event; TEAE = treatment-emergent adverse event; % is calculated as (n/N)*100

There were 376 TEAEs reported by 49 (98%) subjects: 188 TEAEs by 24 (96.0%) subjects who received MYL-1401H and 188 TEAEs by 25 (100.0%) subjects who received the reference product US-Neulasta.

Generally, most TEAEs reported during the study were consistent with the clinical data of pegfilgrastim (Neulasta). No serious AEs (SAEs) or unexpected TEAEs were reported.

The number of TEAEs and percentage of subjects reporting TEAEs was comparable between MYL-1401H and the reference product US-Neulasta: 188 TEAEs reported by 24 (96.0%) subjects and 188 TEAEs reported by 25 (100.0%) subjects, respectively. The most frequently reported TEAEs by system organ class (SOC) (ie, reported by >50% of the subjects) were musculoskeletal and connective tissue disorders (by 90.0% of the subjects), nervous system disorders (72.0%), and general disorders and administration site conditions (60.0%). The most frequently reported preferred terms (PTs) (ie, reported by ≥20% of the subjects) were back pain (80.0%), headache (70.0%), injection site pain (30.0%), fatigue (26.0%), myalgia (24.0%), non-cardiac chest pain (24.0%), pain in extremity (20.0%), and abdominal pain (20.0%). There were no relevant differences in the frequency of TEAEs or percentage of subjects reporting TEAEs between MYL-1401H and US-Neulasta.

Study MYL-1401H-3001

In Study MYL-1401H-3001, 806 TEAEs were reported in 114 (89.8%) patients in the MYL-1401H group and 414 TEAEs were reported in 58 (86.6%) patients in the EU-Neulasta group. Among the patients with TEAEs, the majority had TEAEs that resolved during the study (104 [81.9%] patients in the MYL-1401H group and 47 [70.1%] patients in the EU-Neulasta group). An overview of TEAEs in Study MYL-1401H-3001 is provided in Table 26.

Table 26: Overview of treatment-emergent adverse events during the study (3001)

	MYL-1401H (N=127) n (%)	US-Neulasta (N=67) n (%)
Number of TEAEs	806	414
Number of patients with at least 1 TEAE	114 (89.8)	58 (86.6)
Number of patients with at least 1 pegfilgrastim-related TEAE	57 (44.9)	24 (35.8)
Number of patients with at least 1 TEAE by severity:		
Grade 1 (mild) severity	34 (26.8)	15 (22.4)
Grade 2 (moderate) severity	56 (44.1)	35 (52.2)
Grade 3 (severe) severity	24 (18.9)	8 (11.9)
Number of patients with SAE(s):		
Not related	8 (6.3)	1 (1.5)
Related	0	0
Number of patients withdrawn due to (S)AEs:		
Grade 1 (mild) severity	0	0
Grade 2 (moderate) severity	3 (2.4)	0
Grade 3 (severe) severity	1 (0.8)	0

Abbreviations: AE = adverse event; SAE = serious adverse event; TEAE = treatment-emergent adverse event; US = United States

The most commonly reported TEAE by preferred term was alopecia reported by 76 (59.8%) patients in the MYL-1401H group and 36 (53.7%) patients in the EU-Neulasta group. Of patients with this TEAE, most had CTCAE Grade 1 events (36 [28.3%] patients in the MYL-1401H group and 14 [20.9%] patients in the EU-Neulasta group) and Grade 2 events (36 [28.3%] patients in the MYL-1401H group and 22 [32.8%] patients in the EU-Neulasta group) (CSR MYL-1401H-3001). The events of alopecia were not considered related to the study drug by the investigator.

The TEAE of bone pain was reported for 51 (40.2%) patients in the MYL-1401H group and 24 (35.8%) patients in the EU-Neulasta group. Of the patients with this TEAE, most had CTCAE Grade 1 (21 [16.5%] patients in the MYL-1401H group and 10 [14.9%] patients in the EU-Neulasta group) and Grade 2 (26 [20.5%] patients in the MYL-1401H group and 13 [19.4%] patients in the EU-Neulasta group). Fifty (39.4%) patients in the MYL-1401H group and 23 (34.3%) patients in the EU-Neulasta group) had treatment-related TEAEs of bone pain. Bone pain was managed by simple analgetics, and no patients discontinued from the study as a result of their bone pain. The majority of the events of bone pain were reported in Cycle 1 (44 [34.6%] in the MYL-1401H group and 17 [25.4%] in the EU-Neulasta group). However, a higher rate of use of naproxen was reported during Cycle 1 in the EU-Neulasta group, 19 (28.4%) patients compared to 25 (19.8%) in the MYL-1401H group. Notably, the Brief Pain Inventory questionnaire, a sensitive and relevant measure of the intensity and interference of pain in the patient's life, was similar between the treatment groups.

There were 8 patients with thrombocytosis in the MYL-1401H group that were Grade 1 or 2 in severity and resolved without any intervention. The actual laboratory values of platelets were similar between the treatment groups (approximately 65 [51.2%] patients in the MYL-1401H group had at least 1 episode of elevated platelet count >450 compared with 35 [52.2%] in the EU-Neulasta group and about half of these were single isolated episodes in both the groups). At the end-of-study visit, the mean and median platelets and the change from baseline in platelet counts were similar between the treatment groups. Additionally, the AE reporting of thrombocytosis appeared to be subjective, with only 3 of 24 sites reporting all the 8 events of thrombocytosis in the MYL-1401H group.

Serious adverse event / deaths / other significant events

Study MYL-1401H-1001

In Study MYL-1401H-1001, 1 serious AE (SAE) of appendicitis in the US-Neulasta group occurred and resulted in subject withdrawal. The SAE was not considered to be related to pegfilgrastim.

Study MYL-1401H-1002

No SAEs were reported in Study MYL-1401H-1002.

Study MYL-1401H-3001

In Study MYL-1401H-3001, SAEs were infrequent. A total of 9 (4.6%) patients in the safety population had at least 1 SAE (8 [6.3%] patients in the MYL-1401H group and 1 [1.5%] patients in the EU-Neulasta group. There were no SAEs considered by the investigator to be related to study drug.

Six of 127 (4.7%) patients had FN in the MYL-1401H group and 1 of 67 (1.5%) patients had FN in the EU-Neulasta group, which were considered to be SAEs. All the events of FN lasted less than 5 days, no documented infections nor sepsis events were observed during the events of FN, and all the FN events resolved without the use of rescue therapy. Of 7 patients with FN considered SAEs, only 3 patients met the ESMO definition for FN while 4 other patients had insufficient data. However, these patients were conservatively included under the category of FN.

There was 1 patient with an SAE of erysipelas and 1 patient with SAEs of hypokalemia and anemia in the MYL-1401H group, all of which were deemed resolved at the time of data analysis. All SAEs were deemed unrelated to the study drug by the investigator.

All SAEs of FN were deemed related to the chemotherapy and unrelated to treatment with MYL-1401H or EU-Neulasta by the investigator. There was no significant difference in the rate of FN between the treatment groups ($p=0.35$) based on a chi-square test comparing the proportion of patients with FN between the treatment groups. Given the 2:1 randomization, small sample size, and frequency of ANC assessments based on safety considerations, it is believed that these minor differences are incidental findings. All events of FN lasted less than 5 days, no documented infections or sepsis events were observed during the events of FN, and all FN events resolved without the use of rescue therapy.

No deaths occurred during any of the MYL-1401H clinical studies.

Laboratory findings

In study MYL-1401H-1002 all clinical laboratory parameters were measured at screening and follow-up, and each period at baseline on Day -1 and on Day 2. Absolute neutrophil count was also measured on Days 3, 8, 15, and 22 as part of the PD assessments. For both treatments, mean ALP and LDH levels on Day 2 of both periods were elevated compared with baseline but remained below the ULN. Also individual ALP and LDH levels during the study remained below ULN. In summary, all observed hematological and clinical chemistry changes were expected and were primarily related to the PD effects of pegfilgrastim.

Summary of Hematology

Across all 3 studies (MYL-1401H-1001, MYL-1401H-1002, MYL-1401H-3001), there were no notable differences observed in the hematology measurements between the MYL-1401H groups and Neulasta groups. Across treatments, similar transient shifts in neutrophils and leukocytes occurred, and these parameters had returned to baseline levels by Day 13 and Day 15, respectively. White blood cell (counts of 100 $\times$ 10^9/L or greater) have been observed in less than 1% of patients receiving Neulasta and are consistent with the PD effects of pegfilgrastim.

Summary of Liver and Kidney Function Tests

Overall, there were no notable new differences observed in the liver or kidney function tests between MYL-1401H and Neulasta treatment groups. Liver function abnormalities are consistent with the PD effects of pegfilgrastim.

Vital signs, ECG, and physical findings in study 1002 can be summarized that they were insignificant for a population of healthy volunteers. There were no findings of splenomegaly or symptoms of splenic rupture during the physical examinations of the abdomen throughout the study. One subject (US-Neulasta) had 'left side tenderness', which was considered to be of no clinical relevance.

Local tolerability (including ISR and VAS) in study 1002 was assessed each period at pre-dose and at 1, 4, 24 (Day 2), and 48 hours (Day 3) post-dose. Mostly, the ISR scores were 'none' (0). For 9 subjects that received MYL-1401H, at 1 or more time points following drug administration, a mild reaction was observed (ISR score of 1). This was mainly at 1 hour post-dose, but in some instances also at 4, 24, or 48 hours post-dose. For 5 subjects that received US-Neulasta, at 1 or more time points following drug administration, a mild reaction was observed (ISR score of 1). This was mainly at 1 hour post-dose, but in some instances also at 4, 24, or 48 hours post-dose. Most subjects had a score of 0 mm on a 0-100 mm VAS scale, indicating no pain at the injection site. There were 2 scores of 7 mm, all other scores were 4 mm or lower. The difference in the frequency of injection site reactions (ISR) 9/25 (36%; MYL-1401H) vs. 5/25 (20%; US-Neulasta) could reach statistical significance (not statistically analysed by the applicant). In trial 1001 identical but in 1002 different syringes were used. A higher frequency of injection site reactions (9/25 MYL-1401H vs 5/25 US-Neulasta) has been observed and is noticeable in trial 1002 (all grade 1) but absent in pivotal 3001.

Safety in special populations

N/A

Immunological events

A thorough assessment of immunogenicity was conducted across the 3 clinical studies. The clinical program included Study MYL-1401H-1001 and Study MYL-1401H-1002, which were conducted in normal healthy volunteers, and Study MYL-1401H-3001, which was conducted in patients with breast cancer who were receiving chemotherapy. Serum samples were analyzed for the presence of ADA against MYL-1401H or Neulasta (either EU-Neulasta and/or US-Neulasta). Samples that were positive in the screening assay were further evaluated in a confirmatory assay. The samples confirmed as ADA-positive were titrated to quantify the ADA response and were further evaluated for moiety characterization to determine if the antibodies were specifically directed against the PEG and/or the filgrastim moiety of the molecule.

The immunogenicity assessment in the pivotal PK/PD **Study MYL-1401H-1001** was limited. It evaluated a 2-mg dose, which is sub-therapeutic, and had a 3-way crossover design. Since subjects crossed over to other treatments, immunogenicity data from baseline through Day 29 in Period 1 (i.e., Period 2 pre-dose) are the most relevant for discussion, while data from Period 2 and Period 3 are potentially confounded.

A 7% (16 of 216 subjects overall) baseline frequency of ADA+ subjects is notable, as well as a small imbalance (9 (4%) subjects prior to administration of MYL-1401H, 4 (2%) subjects prior to EU-Neulasta and 3 (1%) subjects prior to US-Neulasta). Most of these baseline ADAs were directed against PEG, or PEG and filgrastim, but not filgrastim alone. A volunteer having ADAs directed against neither the PEG nor the filgrastim portion of the molecule seems to be a false positive ADA result.

Prior to dosing on Day 1 of Period 2 (Table 27), which was Day 29 of Period 1 and the most relevant for immunogenicity assessment, 27 of the 208 (13%) subjects had positive ADA results at this time point with median ADA titre of 4 for each of the 3 treatments. Of the 27 subjects with confirmed positive results at pre dose in Period 2, 10 subjects had pre-existing ADAs at baseline and the other 17 (8%) subjects developed ADAs after the first dose of study drug (5 subjects after MYL-1401H, 5 subjects after EU-Neulasta, and 7 subjects after US-Neulasta). For these 17 subjects, the increased ADAs were considered to be treatment-induced positive ADA results. Thus, the incidence of treatment induced ADA positivity was similar across all the 3 dosing groups (7.2-9.7%) in Period 1.

Table 27: Summary of subjects with treatment-induced anti-drug antibodies at pre-dose in period 2 (1001)

	ADA Results at Predose (Day 1) in Period 2, by Administration of the First Dose			
First dose in Period 1	Total confirmed positive for ADAs n	Treatment-induced ADAs[b] n (%)	Mean titer of treatment-induced positives[a]	Predose positives with >3-fold ADA titer increase n (%)
MYL-1401H (N=69)[a]	11	5 (7.2)	4	1 (1.4)
EU-Neulasta® (N=67)[a]	7	5 (7.5)	2	1 (1.5)
US-Neulasta® (N=72)[a]	9	7 (9.7)	9	1 (1.4)
Total (N=208)	27	17 (8.2)		3 (1.4)

Abbreviations: ADA = anti-drug antibody; EU = European Union; N = number of patients; n = number of patients in the sample; US = United States

[a] The number of subjects shown and used for the calculation of percentages in this table are the number of subjects with data available at Period 2 predose (Study Day 29).

[b] Subjects who were confirmed positive ADA prior to administration of the first dose in Period 1 were excluded

Prior to dosing in Period 3, 13 of the 198 (6%) subjects continued to have positive ADA results. The median ADA titers were comparable across treatments (median titer: 2, 4, and 4 for MYL-1401H, EU-Neulasta and US-Neulasta, respectively).

At follow up, a total of 14 of the 213 (6%) subjects were found positive for ADA that included 6 subjects with ADAs present prior to the first dose of study drug and 8 subjects that were considered to have treatment-induced positive ADA results (including 4, 1, and 3 subject[s] who received MYL-1401H, EU-Neulasta, or US-Neulasta as first dose of study drug, respectively). All ADA titers were <30 at follow up.

The follow-up result make clear why a cross-over design is all but optimal for testing immunogenicity: The treatments actually consisted of 6 different sequences of 3 different products.

Samples that were ADA positive were further assessed for NAb. A total of 72 subjects with ADA positive samples were analyzed for NAbs (Table 28).

The term “72 subjects with ADA positive samples” gives approximately the same proportion of “immunogenicity” as in trial 1002 the wording “ADA was positive at 1 or more time points for 8 of 25 (32.0%) subjects who received MYL-1401H and for 8 of 25 (32.0%) subjects who received US-Neulasta”. 72/216 (see above) is 33.3%. Thus, this “phenomenon” is not dose related.

Table 28: Summary of neutralizing antibodies by visit (1001)

Visit	NAb Assay	MYL-1401H (N=72) n (%)	EU-Neulasta (N=72) n (%)	US-Neulasta (N=72) n (%)	Total (N=216) n (%)
Period 1 Predose (Day 1)	Total # of samples	72	72	72	216
	Negative	5 (6.9)	3 (4.2)	3 (4.2)	11 (5.1)
	Positive	4 (5.6)	1 (1.4)	0 (0.0)	5 (2.3)
	Not Reportable	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	No Assay	63 (87.5)	68 (94.4)	69 (95.8)	200 (92.6)
Period 1 Day 8	Total # of samples	72	72	72	216
	Negative	18 (25.0)	16 (22.2)	21 (29.2)	55 (25.5)
	Positive	3 (4.2)	3 (4.2)	3 (4.2)	9 (4.2)
	Not Reportable	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	No Assay	51 (70.8)	53 (73.6)	48 (66.7)	152 (70.4)
Period 2 Predose (Day 1)	Total # of samples	69	67	72	208
	Negative	7 (9.7)	4 (5.6)	9 (12.5)	20 (9.3)
	Positive	4 (5.6)	2 (2.8)	0 (0.0)	6 (2.8)
	Not Reportable	0 (0.0)	1 (1.4)	0 (0.0)	1 (0.5)
	No Assay	58 (80.6)	60 (83.3)	63 (87.5)	181 (83.8)

Abbreviations: ADA = anti-drug antibody; EU = European Union; N = number of patients; n = number of patients in the sample; NAb = neutralizing antibodies; US = United States

Note: Day 8 of Period 1 immunogenicity samples consist of pooled pharmacokinetic samples that were collected on Day 8 and Day 9 of Period 1. There are 2 subjects with samples collected on Day 7 and Day 8 due to missing visit on Day 9.

Study MYL-1401H-1002 was specifically designed to assess immunogenicity and evaluated a 6-mg repeated dose in normal healthy volunteers. It also evaluated both an early (IgM) and late (IgG) immunogenic response in a controlled setting.

Samples for determination of ADA were taken each period on Day -1, on Days 8, 15, and 22, and at follow-up.

Based on the SAF set, the confirmatory assay for ADA was positive at 1 or more time points for 8 of 25 (32.0%) subjects who received MYL-1401H and for 8 of 25 (32.0%) subjects who received US-Neulasta. There was no time-dependent increase in ADA titre following dosing of either MYL-1401H or US-Neulasta. Two Subjects (MYL-1401H) and one Subject (US-Neulasta) had a positive ADA result before first dosing on Day -1 of the first period.

Two subjects (MYL-1401H) continued to have positive ADA results at all time points measured, including follow-up, whereas one Subject (US-Neulasta) had no positive ADA results after dosing. Positive ADA results at follow-up were seen for 4 subjects who received MYL-1401H and 2 subjects who received US-Neulasta. A maximum titer of 30 was measured once for one Subject (on Day 15, MYL-1401H) and once for other Subject (at follow-up, US-Neulasta).

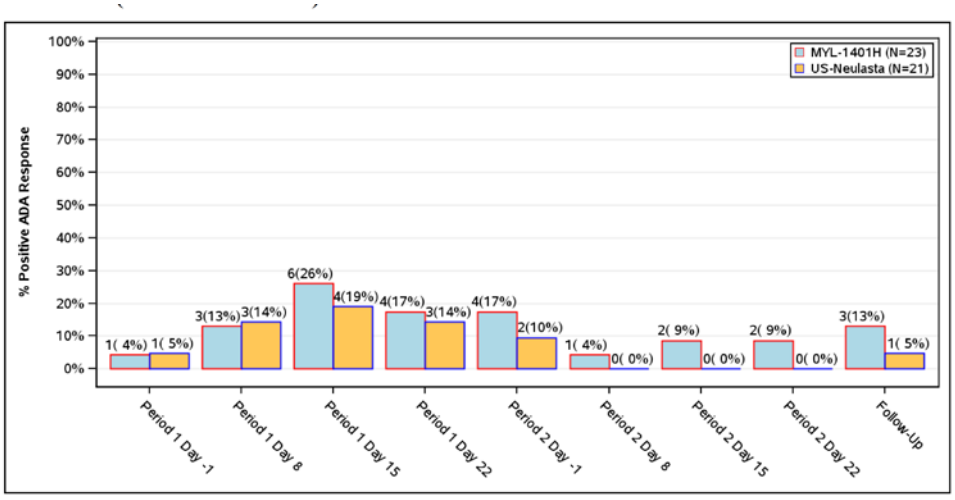
Based on the per-protocol (PP) set (subjects who received both doses of study drug), at the majority of the 9 time points measured, subjects who received MYL-1401H had slightly more positive ADA results than subjects who received US-Neulasta.

All samples confirmed as positive for ADA (mainly against PEG) were further analyzed for NAb using a validated cell-based assay.

Based on the PP set (subjects who received both doses of study drug), no positive NAb results were seen for any of the subjects. Based on the SAF set however, positive NAb results were seen for one Subjects (MYL-1401H) and one subject (US-Neulasta).

One Subject (in the MYL-1401H arm) had positive ADA results at 4 time points in Period 1 (pre-dose [Day -1], Day 8 and Day 15, and at follow up after Period 1). At the first 3 of these time points (with ADA titers of 7 [pre-dose], 4, and 30), the NAb results were also positive. This subject was withdrawn after the first period due to a treatment-emergent adverse event (TEAE) of headache and was not included in the PP set; therefore, the subject had a follow-up visit after Period 1. At this follow-up visit, the subject was not positive for NAb.

One subject had a treatment-emergent, positive ADA result at 1 time point (with an ADA titer of 2 at Period 1, Day 8) at which time the NAb results were also positive. The subject did not have positive ADA prior to study start and therefore, her ADA and NAb positivity was treatment-emergent. As with one Subject, this subject was also withdrawn from the study after Period 1 due to a TEAE of headache and therefore was not included in the PP set.



ADA = anti-drug antibody

Figure 7: Percentages of Subjects with Positive ADA Versus Time by Treatment (PP set)

- Relationship Between Immunogenicity and Pharmacodynamic Results

For the 2 subjects that had a maximum ADA titre of 30, one Subject (Day 15, MYL-1401H) and other Subject (follow-up, US-Neulasta), the effect of pegfilgrastim treatment on ANC levels appeared not to be different from the subjects that had no positive ADA counts or positive ADA counts with lower titers. Based on this it appeared that the formation of ADA had no effect on the PD effects of pegfilgrastim.

Finally, immunogenicity was also evaluated in the relevant patient population within Study MYL-1401H-3001, in which patients with breast cancer received multiple doses of MYL-1401H or Neulasta in addition to their chemotherapeutic dosing regimen. Thus, the overall immunogenicity assessment includes evaluation of early and late immune response, response after multiple dosing in healthy volunteers as well as in patients, and response after low and therapeutic doses of MYL-1401H and Neulasta.

Table 29 and Table 30 summarize the immunogenicity data at the sample and subject levels integrated across the 3 studies.

The proportions of ADA-positive samples were similar in MYL-1401H and EU-Neulasta groups (8.3-8.7%) and slightly higher (12.7%) in US-Neulasta group. At a subject level, 22.3% and 23.7% of subjects were positive at least once in MYL-1401H and EU-Neulasta arm respectively, while the proportion was slightly higher at 33% in the US-Neulasta arm. Data from US-Neulasta is only from healthy subjects and it could have contributed to higher proportion of ADA positive response in that arm. Both at subject and sample level, most of the ADA positivity was against the PEG moiety of the molecule across the 3 groups.

Table 29: Integrated summary of all immunogenicity results by sample (1001, 1002, 3001, ITT population)

Parameter	MYL-1401H	EU-Neulasta	US-Neulasta
Total # of Samples	1050	545	417
Positive ADA samples at least once	91 (8.7)	45 (8.3)	53 (12.7)
PEG+ only at least once	46 (4.4)	31 (5.7)	33 (7.9)
GCSF+ only at least once	13 (1.2)	1 (0.2)	0 (0.0)
PEG+ & GCSF+ at least once	23 (2.2)	10 (1.8)	16 (3.8)
PEG- & GCSF- at least once	9 (0.9)	3 (0.6)	4 (1.0)
NAb+ at least once	12 (1.1)	1 (0.2)	1 (0.2)

Abbreviations: ADA=antidrug antibody; NAb=neutralizing antibody

Table 30: Integrated summary of all immunogenicity results by subject (1001, 1002, 3001, ITT population)

Parameter	MYL-1401H	EU-Neulasta	US-Neulasta
Total # of Subjects	224	139	97
Positive ADA samples at least once	50 (22.3)	33 (23.7)	32 (33.0)
PEG+ only at least once	29 (12.9)	27 (19.4)	23 (23.7)
GCSF+ only at least once	3 (1.3)	1 (0.7)	0 (0.0)
PEG+ & GCSF+ at least once	17 (7.6)	7 (5.0)	11 (11.3)
PEG- & GCSF- at least once	8 (3.6)	3 (2.2)	4 (4.1)
NAb+ at least once	6 (2.7)	1 (0.7)	1 (1.0)

Abbreviations: ADA=antidrug antibody; NAb=neutralizing antibody

It is known that healthy subjects and patients are exposed to PEG-containing chemicals in the environment, and that anyone has a potential to develop antibodies against this moiety. This was apparent based on the pre-dose positive samples across each of the 3 studies. Table 30 summarises the pre-dose ADA-positive samples across the studies.

The proportions of samples that were ADA-positive were similar in the MYL-1401H and EU-Neulasta groups (13.5% and 11.5%, respectively) but was quite low in the US-Neulasta group (4.1%), which appears to be a chance finding. Many of the subjects who were ADA-positive prior to dosing continued to remain positive throughout the study. The majority of these subjects had antibodies against the PEG moiety of the molecule.

Table 31: Integrated summary of pre-dose immunogenicity results by sample (1001, 1002, 3001, ITT population)

Parameter	MYL-1401H	EU-Neulasta	US-Neulasta
Total # of Samples	223	139	97
Positive ADA samples pre-dose	30 (13.5)	16 (11.5)	4 (4.1)
PEG+ only	14 (6.3)	13 (9.4)	3 (3.1)
GCSF+ only	2 (0.9)	0 (0.0)	0 (0.0)
PEG+ & GCSF+	9 (4.0)	3 (2.2)	1 (1.0)
PEG- & GCSF-	5 (2.2)	0 (0.0)	0 (0.0)

Abbreviations: ADA=antidrug antibody; NAb=neutralizing antibody

To assess the treatment-induced impact on immunogenicity, an analysis was conducted to evaluate the post-dose ADA-positive results excluding the subjects who were ADA-positive at baseline. The data at the sample and subject level is presented in Table 32 and Table 33.

Table 32: Integrated summary of post-dose immunogenicity results from subjects who were ADA-negative at baseline by sample (1001, 1002, 3001, ITT population)

Parameter	MYL-1401H	EU-Neulasta	US-Neulasta
Total # of Samples	723	350	306
Positive ADA samples post-dose	36 (5.0)	22 (6.3)	44 (14.4)
PEG+ only	23 (3.2)	15 (4.3)	29 (9.5)
GCSF+ only	1 (0.1)	1 (0.3)	0 (0.0)
PEG+ & GCSF+	9 (1.2)	4 (1.1)	11 (3.6)
PEG- & GCSF-	3 (0.4)	2 (0.6)	4 (1.3)
NAb+	2 (0.3)	0 (0.0)	0 (0.0)

Abbreviations: ADA=antidrug antibody; NAb=neutralizing antibody

Table 33: Integrated summary of post-dose immunogenicity results from subjects who were ADA-negative at baseline by subject (1001, 1002, 3001, ITT population)

Parameter	MYL-1401H	EU-Neulasta	US-Neulasta
Total # of Subjects	192	123	93
Positive ADA samples post-dose	20 (10.4)	17 (13.8)	28 (30.1)
PEG+ only	13 (6.8)	13 (10.6)	20 (21.5)
GCSF+ only	1 (0.5)	1 (0.8)	0 (0.0)
PEG+ & GCSF+	7 (3.6)	4 (3.3)	8 (8.6)
PEG- & GCSF-	3 (1.6)	2 (1.6)	4 (4.3)
NAb+	2 (1.0)	0 (0.0)	0 (0.0)

Abbreviations: ADA=antidrug antibody; NAb=neutralizing antibody

The data indicate that at the sample level, 5.0% and 6.3% of post-dose samples were treatment-emergent ADA-positive in the MYL-1401H and EU-Neulasta groups, respectively.

The proportion was higher (14.4%) in the US-Neulasta group. At a subject level, the proportions of subjects with post-dose treatment-emergent ADA-positive data were also similar for MYL-1401H and EU-Neulasta groups (10.4% and 13.8%, respectively), while it was 30.1% in the US-Neulasta group. Although the proportion of subjects who were treatment-emergent ADA-positive was higher in the US-Neulasta group, the ADA in most cases were against only the PEG moiety, the titers were very low, and the antibodies were non-neutralizing. Only 2 subjects (1 each in the MYL-1401H and EU-Neulasta groups) had antibodies against the GCSF moiety only.

Two subjects were NAb-positive in the MYL-1401H group while none were NAb-positive in either Neulasta group. Table 34 presents the post-dose ADA-positive results excluding the subjects who were NAb-positive at baseline. This analysis is slightly different from analysis in Table 45 as it includes subjects who might have been ADA-positive but NAb-negative prior to dosing. The data indicate that there were 2 subjects who were treatment-emergent NAb-positive in the MYL-1401H group, 1 subject who was NAb-positive in the EU-Neulasta group, and 1 subject who was NAb-positive in the US-Neulasta group.

Table 34: Integrated summary of post-dose immunogenicity results from subject Nab-negative at baseline by subject (1001, 1002, 3001, ITT population)

Parameter	MYL-1401H	EU-Neulasta	US-Neulasta
Total # of Subjects	218	139	97
Positive ADA Sample at least once	26 (11.9)	21 (15.1)	31 (32.0)
PEG+ only at least once	15 (6.9)	16 (11.5)	21 (21.6)
GCSF+ only at least once	3 (1.4)	1 (0.7)	0 (0.0)
PEG+ & GCSF+ at least once	8 (3.7)	6 (4.3)	11 (11.3)
PEG- & GCSF- at least once	4 (1.8)	3 (2.2)	4 (4.1)
NAb+ at least once	2 (0.9)	1 (0.7)	1 (1.0)

Abbreviations: ADA=antidrug antibody; NAb=neutralizing antibody

Safety in special populations

The applicant did not submit safety in special populations (see clinical safety).

Safety related to drug-drug interactions and other interactions

The applicant did not submit safety related drug-drug interactions (see clinical safety).

Discontinuation due to adverse events

Discontinuation from MYL-1401H due to TEAEs was infrequent. In *Study MYL-1401H-1001*, 3 TEAEs led to subject withdrawal (rash and abnormal liver function test in 2 subjects after EU-Neulasta, and a SAE of appendicitis in 1 subject after US-Neulasta).

In *Study MYL-1401H-1002*, 6 subjects were withdrawn from the study due to TEAEs after dosing in Period 1 (vomiting in 1 subject and headache in 3 subjects in the US-Neulasta group and pain in extremity and headache in 1 subject each in the MYL-1401H group).

In *Study MYL-1401H-3001*, a Grade 2 TEAE of influenza led to discontinuation of MYL-1401H. The event was considered resolved at the time of data analysis. A Grade 3 TEAE of erysipelas led to discontinuation of the patient from the study, although it was considered resolved at the time of data analysis. A Grade 2 TEAE of pneumonitis led to discontinuation of MYL-1401H and from the study. The event was considered resolved at the time of data analysis. Among the patients who received EU-Neulasta, a Grade 3 TEAE of increased ALT led to the discontinuation from the study drug. The event was considered resolved at the time of data analysis.

None of the described events were deemed related to the study drug by the investigator.

Post marketing experience

There is no post marketing experience with Fulphila.

2.6.1. Discussion on clinical safety

To assess clinical safety of MYL-1401H intended to be bio-similar to Neulasta (EU) based on the dossier submitted has several challenges.

Trial MYL-1401H-1001, has a cross-over design and therefore mainly period 1 can contribute to immunogenicity and safety assessment. For immunogenicity, results at the end of period 1 suggest that MYL-1401H is comparable to the reference product.

Trial MYL-1401H-1002, in healthy volunteers was specifically dedicated to investigate immunogenicity. Healthy subjects are in fact considered a more sensitive model to compare immunogenicity of two pegfilgrastims than immunosuppressed patients, although differences in the frequency of AEs have to be large to be detected in a small trial such as 1002. The confirmatory assay for ADA was positive at 1 or more time points for 8 of 25 (32.0%) subjects who received MYL-1401H and for 8 of 25 (32.0%) subjects who received Neulasta suggesting comparable immunogenicity of both products. Of note, this study used US-sourced Neulasta. The data are however relevant for the present application since an analytical bridge has been established between EU- and US-reference product.

Trial MYL-1401H-3001, a phase III trial with parallel group design comparing Fulphila with Neulasta EU sourced during 6 cycles of TAC showed similar ADRs that occurred at similar frequencies for Fulphila and Neulasta.

Overall, the AE profile of test and reference appeared similar. There is a high frequency of injection site reactions for MYL-1401H in study 1002. The relative high frequency of injection site reactions (grade 1) in the MYL-1401H arm of trial 1002 was is an isolated finding in the smallest clinical trial and hence is not clinically relevant.

Immunogenicity data derived from the 3 studies suggest similar immunogenicity profiles of test and EU reference. In the integrated analysis, immunogenicity appeared to be higher with US-reference which may be due to the fact that US-Neulasta was only administered to healthy subjects that are more likely to mount an immune response to an antigen than immunocompromised patients on chemotherapy as treated in study 3001. Most of the ADA positivity, including that at predose, was directed against the PEG moiety of the molecule across the 3 groups, which is unsurprising as it is known that exposure to PEG-containing chemicals in the environment may lead to development of antibodies against this moiety.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

Overall, the results from the 3 clinical studies did not show any relevant difference in ADRs or immunogenicity following Fulphila administration compared to Neulasta. The safety of Fulphila supports the claim for similarity with Neulasta.

2.7. Risk Management Plan

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 acute respiratory distress syndrome) • Sickle cell crisis in patients with sickle cell disease • Musculoskeletal pain-related symptoms • Leukocytosis • Thrombocytopenia • Glomerulonephritis
Important potential risks	• Acute myelogenous leukaemia/ myelodysplastic syndrome • Cytokine release syndrome • Medication errors including overdose • Drug interaction with lithium • Off-label use • Immunogenicity (incidence and clinical implications of anti-G-CSF antibodies) • Extramedullary haematopoiesis
Missing information	• Use in paediatric patients • Use during pregnancy and breastfeeding

Pharmacovigilance plan

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

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

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important identified risks		
Severe splenomegaly / splenic rupture	Routine risk minimization measures: SmPC sections: 4.2, 4.4, 4.8 and 5.3. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Cutaneous vasculitis	Routine risk minimization measures: SmPC sections: 4.2 and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Sweet's syndrome	Routine risk minimization measures: SmPC sections: 4.2 and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Anaphylactic reaction	Routine risk minimization measures: SmPC sections: 4.2, 4.3, 4.4 and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Capillary leak syndrome	Routine risk minimization measures: SmPC sections: 4.2, 4.4 and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities, including follow up questionnaire
Serious pulmonary adverse events (including interstitial pneumonia and acute respiratory distress syndrome)	Routine risk minimization measures: SmPC sections: 4.2, 4.4. and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Sickle cell crisis in patients with sickle cell disease	Routine risk minimization measures: SmPC sections: 4.2, 4.4. and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Musculoskeletal pain-related symptoms	Routine risk minimization measures: SmPC sections: 4.2 and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Leukocytosis	Routine risk minimization measures:	Routine pharmacovigilance

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	SmPC sections: 4.2, 4.4, 4.8 and 5.3. Additional risk minimisation measures: None.	activities.
Thrombocytopenia	Routine risk minimization measures: SmPC sections: 4.2, 4.4 and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Glomerulonephritis	Routine risk minimization measures: SmPC sections: 4.4 and 4.8. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Important identified risks		
Acute myelogenous leukaemia/ myelodysplastic syndrome	Routine risk minimization measures: SmPC sections: 4.2 and 4.4. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Cytokine release syndrome	Routine risk minimization measures: SmPC sections: 4.2. Additional risk minimisation measures: None.	Routine pharmacovigilance activities, including follow up questionnaire
Medication errors including overdose	Routine risk minimization measures: SmPC sections: 4.2. Additional risk minimisation measures: None.	Routine pharmacovigilance activities, including follow up questionnaire
Drug interaction with lithium	Routine risk minimization measures: SmPC sections: 4.2. and 4.5. Additional risk minimisation measures: None.	Routine pharmacovigilance activities, including follow up questionnaire
Off-label use	Routine risk minimization measures: SmPC sections: 4.1, 4.2, 4.4. Additional risk minimisation measures: None.	Routine pharmacovigilance activities, including follow up questionnaire
Immunogenicity (incidence and clinical implications of anti-G-CSF antibodies)	Routine risk minimization measures: SmPC sections: 4.2 and 4.4. Additional risk minimisation measures: None.	Routine pharmacovigilance activities, including follow up questionnaire
Extramedullary haematopoiesis	Routine risk minimization measures: SmPC sections: 4.2 and 5.3. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Missing information		
Use in paediatric patients	Routine risk minimization measures: SmPC sections: 4.1, 4.2, 4.8 and 5.2. Additional risk minimisation measures: None.	Routine pharmacovigilance activities.
Use during pregnancy and breastfeeding	Routine risk minimization measures: SmPC sections: 4.2 and 4.6. Additional risk minimisation measures: None.	Routine pharmacovigilance activities including follow up questionnaire

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

Conclusion

The CHMP and PRAC considered that the risk management plan version 2 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

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

Periodic Safety Update Reports submission requirements

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

2.9. Product information

2.9.1. User consultation

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

2.9.2. Additional monitoring

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

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

3. Biosimilarity assessment

3.1. Comparability exercise and indications claimed

The claimed indication is identical to the reference product Neulasta: “Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes)”. Clinical studies supporting the application were carried out in healthy volunteers as part of the biosimilarity exercise as well as a phase III clinical trial in breast cancer patients.

The claim of biosimilarity is based on comparative analytical, nonclinical and clinical data.

Quality:

To establish biosimilarity of Fulphila to EU Neulasta on the quality level, a comprehensive analytical comparability exercise was performed comparing Fulphila to EU Neulasta. Up to 12 batches of Fulphila and up to 34 batches of EU Neulasta were included in the analytical similarity studies.

Non-clinical:

Comparative *in vitro* and *in vivo* non-clinical studies were performed in order to demonstrate biosimilarity between Fulphila and the reference product Neulasta. In addition, one comparative 28 day repeat dose toxicity study in neutropenic rats was performed.

Clinical:

With the present application (EMEA/H/C4915) the applicant provides study results from 3 clinical trials, of which MYL-1401H-1001 is the pivotal PK/PD study.

MYL-1401H-1001 was a single centre, randomized, double-blind, 3-period, 3 treatments, 3-way crossover trial to evaluate the PD, PK, safety and tolerability of pegfilgrastim from test product (MYL-1401H) compared to reference products EU- and US-Neulasta in healthy subjects. Primary objectives were comparison of PK and PD profiles after a single injection of a 2 mg dose of MYL-1401H and a single injection (2 mg) of EU- and US-Neulasta.

Trial **MYL-1401H-1002** was a single-centre, randomized, open-label, repeated dose, parallel group trial intended to evaluate immunogenicity, PD, safety, and tolerability of the test product, MYL-1401H, compared with the reference product, US-licensed Neulasta. Healthy subjects received 2 single SC injections of 6 mg of either the test product, MYL-1401H, or the reference product, US-Neulasta, in 2 separate periods with a washout period of 4 weeks between study drug administrations.

The phase III trial **MYL-1401H-3001** was a multicentre, randomized, double-blind, therapeutic equivalence study in breast cancer patients receiving 6 cycles TAC for adjuvant or neo-adjuvant treatment. The primary objective of the study was to compare the efficacy of MYL-1401H versus Neulasta during chemotherapy cycle 1 using duration of severe neutropenia (DSN), defined as days with ANC $< 0.5 \times 10^9$/L, as endpoint.

3.2. Results supporting biosimilarity

From a quality perspective:

With respect to primary, secondary and higher order structures comparability of Fulphila with the reference product EU Neulasta has been confirmed. Fulphila has been demonstrated to have an overall similar purity and impurity profile compared to Neulasta which refers in particular to oxidized and reduced, deamidated and charged variants, dimers, di-PEGylated variants and aggregates of pegfilgrastim as well as free filgrastim.

In addition, analytical similarity of Neulasta sourced from US and EU was established.

From a non-clinical perspective:

The results of *in vitro* and *in vivo* studies underline comparability between the two products. In addition, no relevant differences were observed in the 28 day repeat dose toxicity study in neutropenic rats.

From a clinical perspective:

- Pharmacokinetics and Pharmacodynamics
    - Study MYL-1401H-1001 demonstrated similar PK profiles of Neulasta-EU sourced, Neulasta-US sourced, and MYL-1401H (in all comparison-pairs).
    - For the comparison test vs. EU reference, the 90% CIs of the primary PK endpoints Cmax and $AUC_{0\text{-}inf}$ ([0.984; 1.16] and [0.979; 1.12], respectively] lay well within the predefined acceptance range of 0.8 to 1.25.
    - The PD profiles were also similar between the 3 treatments.
    - For the comparison test vs. EU reference, the 95% CIs of the primary PD parameters ANC C_{max} and ANC $AUC_{0\text{-}t}$ ([0.960; 1.028] and [0.959; 1.045], respectively) were well contained within the predefined equivalence range of 0.8500 - 1.1765. Also the 95% CIs of the secondary PD parameters CD34+ C_{max} and CD34+ $AUC_{0\text{-}t}$ met these margins, further supporting biosimilarity.
    - The PD parameters of all three products tested demonstrate that they are equivalent in terms of PD.
    - Although study MYL-1401H-1001 was not powered to evaluate equivalence of the primary PD parameters for ANC in a smaller subgroup of ADA negative subjects, these results indicate that the primary PD parameters continued to be similar between MYL-1401H and the reference treatments EU-Neulasta and US-Neulasta in a subgroup of subjects without any ADA positive response at any time point. Also the secondary PD parameters appeared to be similar between MYL-1401H and the reference treatments in this subgroup.
    - There were no clinically relevant differences in immunogenicity as shown in the trial MYL-1401H-1002 where there were no detectable neutralizing antibodies detected.
    - A secondary PD endpoint, however, was ANC which was descriptively analysed and supported the primary endpoints (C_{max} and AUC of ANC) as of trial MYL-1401H-1001. The study 1002 is considered supportive of the overall biosimilarity of Fulphila
- Efficacy
    - Trial MYL-1401H-3001 met its primary objective. The mean (± SD) DSN in the MYL-1401H group was 1.2 (± 0.93), the median DSN was 1.0, and the DSN ranged from 0 to 5 days.

In the EU-Neulasta group, the mean (± SD) DSN was 1.2 (± 1.10), the median DSN was 1.0, and the DSN ranged from 0 to 4 days. The 95% CI (-0.285, 0.298) for the difference in least square mean DSN of MYL-1401H and EU-Neulasta was found to be within the pre-specified equivalence range of [-1 day, +1 day].

- Safety
    - The safety and immunogenicity profiles of MYL-1401H and EU-sourced Neulasta appeared generally similar in all 3 studies. The applicant presented within this application an integrated immunogenicity analysis which provided supportive evidence on the similarity of the immunogenicity profile.

3.3. Uncertainties and limitations about biosimilarity

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

3.4. Discussion on biosimilarity

Analytical similarity of MYL-1401H to the reference product Neulasta (EU) has been shown in a satisfactory manner. Likewise, analytical similarity of Neulasta sourced from EU and US was also demonstrated. Therefore, results obtained in comparison to US-reference product can be bridged and are relevant in supporting the overall biosimilarity exercise in this application.

Non-clinical

In vitro assays are considered more sensitive than *in vivo* studies to detect potential differences between test and reference product and hence, the results have shown equivalent similarity between the two products. Results from the *in vitro* study support a conclusion of functional similarity. The *in vivo* studies can be considered supportive of the biosimilarity.

Clinical

The clinical pharmacology studies have shown that the PK and PD data were within the acceptance range for the criteria for biosimilarity and immunogenicity was comparable between Fulphila and Neulasta. In addition, the clinical efficacy and safety data support the claim for biosimilarity as demonstrated by showing equivalent DSN and rates of febrile neutropenia as well as comparable safety profiles between the two products.

Therefore, considering the totality of the evidence on the quality, non-clinical and clinical data, biosimilarity of Fulphila with the reference product EU Neulasta can be concluded.

3.5. Extrapolation of safety and efficacy

The claimed indication is the only indication currently approved for EU-Neulasta (“Reduction in the duration of neutropenia and the incidence of febrile neutropenia in adult patients treated with cytotoxic chemotherapy for malignancy [with the exception of chronic myeloid leukaemia and myelodysplastic syndromes”]).

3.6. Additional considerations

Not applicable.

3.7. Conclusions on biosimilarity and benefit risk balance

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

4. Recommendations

Outcome

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

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

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

Conditions or restrictions regarding supply and use

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

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

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

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

Risk Management Plan (RMP)

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

An updated RMP should be submitted:

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

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.