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A Comprehensive Review of Pegfilgrastim Biosimilars Approved by The European Medicines Agency

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ABSTRACT

As of January 2021, eight Pegfilgrastim biosimilars to Amgen's Neulasta have been approved by European Medical Agency. The present study compared the quality, non-clinical and clinical aspects of the approved biosimilars to understand the regulatory expectations, requirements, and exceptions. The findings obtained from the European Public Assessment Reports (EPARs) of individual biosimilars are summarized in this report. The aspects compared were days taken for response approval, analytical methods for quality evaluation, non-clinical animal studies and utilization of their data to assess pharmacological parameters, number, and type of clinical studies, choice of endpoints for PK, PD, and efficacy, etc.

INTRODUCTION

Biosimilars constitute a large part of an ever-growing market of biological drugs. Biosimilars are defined by European Medical Agency as "a biological medicine highly similar to another biological medicine already approved in the EU in terms of structure, biological activity and efficacy, safety and immunogenicity profile". EMA became the first regulatory agency in October 2005 to issue a guideline for biosimilars [1]. The current version of "Guideline on similar biological medicinal products" [2] effective from April 2015 describes the general requirements for Marketing Authorization Application for a biological product claimed to be "similar" to a reference medicinal product, which has been granted marketing authorization in the European Economic Area. The similarity between a similar biological medicinal product and chosen reference medicinal product needs to be convincingly demonstrated in terms of quality, safety, and efficacy.

In addition, EMA has also issued a "Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance – quality [3] and non-clinical and clinical issues" [4], "Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins" [5] and Specific product-related guidelines issued by EMA.

The developer may seek scientific advice from EMA for timely and comprehensive development of safe efficacious medicine.

Chemotherapy-induced neutropenia is a major risk factor in patients undergoing chemotherapy. Chemotherapy results in a rapid reduction in the numbers of neutrophils leading to febrile neutropenia. This increases the risk of morbidity and mortality due to lower immunity resulting in a delay in chemotherapy or dose reduction.

Granulocyte Colony Stimulating Factor (G-CSF) is a glycoprotein that regulates the production and release of neutrophils from the bone marrow. G-CSF binds with granulocyte colony-stimulating factor receptor (G-CSFR) and stimulates the proliferation and differentiation of precursor cells in the bone marrow into mature granulocytes.

A recombinant methionylated Human Granulocyte Colony Stimulating Factor referred to as Filgrastim produced in *Escherichia coli* by Amgen under the brand name Neupogen has been approved by many regulatory agencies for the treatment of chemotherapy-induced neutropenia. Owing to the small size (175 amino acids, 18.8 KDa) it is rapidly cleared by the

Glomerulus filters in the kidney. The serum half-life of Filgrastim is estimated at 3.5 to 3.8 hours.

Pegfilgratim is the long-acting form of Filgrastim prepared by covalent linking of Polyethylene glycol (PEG) to the N-terminus of Filgrastiim. PEG is a neutral moiety that increases the molecular weight to 38.8 KDa and increases the serum half-time to 42 hours.

First Pegfilgratim to be approved by EMA was Neulasta by Amgen in 2002. The patents of Neulasta expired in the EU in 2017. This has led to the advent of biosimilars in the European market.

EMA "Guidance on Similar Medicinal Products Containing Recombinant Granulocyte-Colony Stimulating Factor" [6] describes the expectations from a biosimilar Filgrastim or Pegfilgrastim. The salient points of this guideline are as under:

- **Non-Clinical Studies**

  - "Before initiating clinical development, comparative non-clinical studies should be performed to detect differences in pharmaco-toxicological response."

  - *"In vivo* rodent models, neutropenic and non-neutropenic, should be used to compare the pharmacodynamic effects of the test and the reference medicinal product."

  - "Data from at least one repeat dose toxicity study in a relevant species should be provided."

  - "Data on local tolerance in at least one species should be provided."

  - "Safety pharmacology, reproduction toxicology, mutagenicity and carcinogenicity studies are not routine requirements for non-clinical testing."

- **Clinical Studies**

  - "The pharmacokinetic properties of the similar biological medicinal product and the reference medicinal product should be compared in single-dose crossover studies."

  - "The primary PK parameter is AUC and the secondary PK parameters are Cmax and T1/2."

- "The absolute neutrophil count (ANC) is the relevant pharmacodynamic marker for the activity. The CD34 + cell count should be reported as a secondary PD endpoint."

- "The pharmacodynamic effect of the test and the reference medicinal products should be compared in healthy volunteers."

- "The recommended clinical efficacy model is the prophylaxis of severe neutropenia after cytotoxic chemotherapy in a homogenous patient group."

- "Alternative models, including pharmacodynamic studies in healthy volunteers, may be pursued for the demonstration of comparability if justified."

- "Clinical safety data should be collected from a cohort of patients after repeated dosing preferably in a comparative clinical trial."

Following these guidelines, a total of eight biosimilars for Pegfilgrastim have been approved by EMA as of January 2021. Table 1 lists all the Pegfilgrastim biosimilars approved by EMA as of January 2021.

Table 1: Pegfilgrastim biosimilars approved by EMA as of January 2021

Product	Applicant	Manufacturer
Udenyca [7]	ERA consulting GmbH	Coherus
Pelgraz [8]	Accord Healthcare Limited	Intas Biopharma
Pelmeg [9]	Cinfa Biotech S L	3P Biopharmaceuticals
Ziextenzo [10]	Sandoz GmbH	Sandoz
Fulphila [11]	Mylan S.A.S	Biocon Limited
Grasustek [12]	Juta Pharma GmbH	USV Private Limited
Cegfila [13]	Mundipharma Biologics S.L.	3P Biopharmaceuticals
Nyvepria [14]	Pfizer Europe MA EEIG	Hospira Adelaide and Hospira Zagreb

METHODS

The key resources for this article were the European Public Assessment Reports published by EMA. The quality, non-clinical and clinical aspects of approved Pegfilgrastim were evaluated to understand the current and evolving expectations of EMA for approval of Pegfilgrastim biosimilars. The comparison was made on parameters listed in Table 2.

Table 2: Aspects evaluated for comparison

Aspects	Parameters
Quality	Analytical methods for the primary structure, higher-order structure, molecular size, purity & biological activity and stability
Non-clinical	Animal studies for PK, PD, Efficacy, Toxicity, Toxicokinetics and immunogenicity
Clinical	Healthy subjects or patients trials for sample size, PK, PD, efficacy and immunogenicity

REGULATORY TIMELINES FOR PEGFILGRASTIM BIOSIMILARS

The year 2016 saw the first application of Pegfilrastim to EMA, followed by five, zero and two applications in 2017, 2018 and 2019 respectively. Four Pegfilgrastim biosimilars were granted approvals in 2018, followed by three and two approvals in 2019 and 2020 respectively.

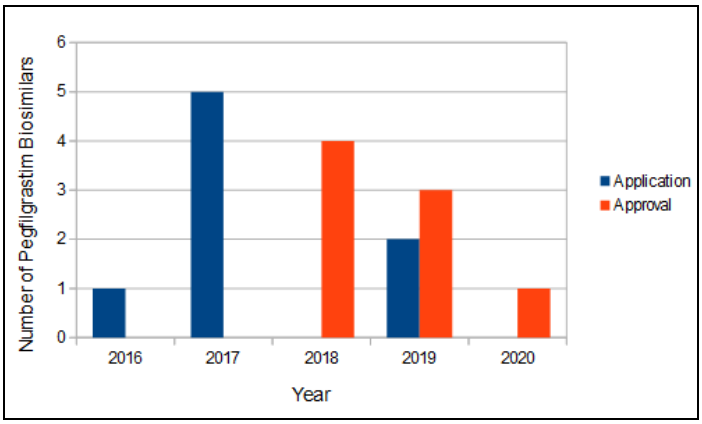
The maximum time taken from the application to approval was 629 days for Udenyca owing to two instances of outstanding issues. Whereas the minimum time taken was 79 days for Cegfila as the data for Pelmeg was used for MAA application after the acquisition of Cinfa Biotech by Mundipharma.

Most of the candidates sought scientific advice from EMA. The maximum (four) was sought for Grasustek, whereas no scientific advice was sought for Fulphila.

Table 3 lists the timelines for approved biosimilars starting with the application, shared questions, outstanding issues and approval. Graph 1 represents the acceptance of Pegfilgrastim biosimilars by EMA over the years. Tables 4 and 5 list the number of scientific advices taken from EMA and a total time of approval respectively.

Table 3: EMA approvals timelines for Pegfilgrastim biosimilars

Product	Application received on	Procedure started on	Consolidated questions shared on	Response by applicant on	List of outstanding issues shared by EMA on	Response to outstanding issues by applicant on	Approval on
Udenyca	04/11/16	24/11/16	23/03/17	13/10/17	14/12/17 and 28/06/18	28/05/18 and 03/07/2018	26/07/18
Pelgraz	27/04/17	18/05/17	14/09/17	03/04/18	31/05/18	26/06/18	26/07/18
Pelmeg	08/09/17	28/07/17	25/01/18	26/04/18	28/06/18	14/08/18	20/09/18
Ziextenzo	06/10/17	26/10/17	22/02/18	24/05/18	26/07/18	20/08/18	20/09/18
Fulphila	03/11/17	23/11/17	22/03/18	22/08/18	NA	NA	20/09/18
Grasustek	06/11/17	23/11/17	22/03/18	12/10/10	13/12/18	20/03/19	26/04/19
Cegfila	30/07/19	19/08/19	NA	NA	NA	NA	17/10/19
Nyvepria	12/09/19	03/10/19	30/01/20	23/04/20	25/06/20	17/08/20	17/09/20



Graph 1: Acceptance of Pegfilgrastim Biosimilars by EMA

Table 4: Number of scientific advice sought from EMA

Product	Number of Scientific advices sought from EMA
Udenyca	2
Pelgraz	1
Pelmeg	1
Ziextenzo	1
Fulphila	0
Grasustek	4
Cegfila	1
Nyvepria	1

Table 5: Analysis of days taken for response and approval

Product	Days for response to consolidated questions	Days for response to outstanding issues	Total days for approval
Udenyca	204	201	629
Pelgraz	201	26	455
Pelmeg	91	47	377
Ziextenzo	91	25	349
Fulphila	153	NA	321
Grasustek	204	97	536
Cegfila	NA	NA	79
Nyvepria	84	53	371

ANALYTICAL DEVELOPMENT OF PEGFILGRASTIM BIOSIMILARS

Different analytical methods were used to analyze the primary structure, higher-order structure, molecular size and biological activity of Pegfilgrastim biosimilars.

In-vitro cell proliferation assay and Surface Plasmon resonance were used to assess the biological activity of all the Pegfilgrastim biosimilars; Nyvepria used a Competitive receptor binding assay in addition to these methods.

These methods were used to assess the biosimilarity of the candidate biosimilars with the reference drug Neulasta. Table 6 lists the analytical methods employed by Pegfilgrastim biosimilars.

Table 6: Comparison of methods used for Quality assessment

Product	Primary structure	Higher order structure	Molecular Size	Purity	Biological activity
Udenyca	"N-terminal sequencing, amino acid composition, peptide map, UV/MS, pegylation site and linkage, ESI-MS, western blot, SDS-PAGE (non-reduced silver and iodine stain)"	"CD, intrinsic fluorescence, 2D NMR, DSC"	"SEC-MALS, Intact mass by LC-MS, SEC MALS, Analytical ultracentrifugation"	"SE-HPLC, RP-HPLC, CEX-HPLC"	*"In-vitro* cell proliferation assay, Surface Plasmon resonance"
Pelgraz	"N-terminal sequencing, amino acid composition, peptide map, UV/MS, pegylation site and linkage, ESI-MS, western blot, SDS-PAGE (non-reduced silver and iodine stain)"	"CD, FTIR, intrinsic fluorescence, free cysteine estimation"	"ESI MS, UV/MS"	"SE-HPLC, SEC-MALS, Analytical ultracentrifugation, RP-HPLC, CEX-HPLC"	*"In-vitro* cell proliferation assay, Surface Plasmon resonance"
Pelmeg	"LC-MS, Edman"	"CD, near-UV CD, differential scanning calorimetry, fluorescence spectroscopy"	"MALDI TOF, Capillary gel electrophoresis, ESI-MS, SDS-PAGE"	"CEX, Capillary isoelectric focussing, RP-HPLC, Analytical ultracentrifugation, western blotting"	*"In-vitro* cell proliferation assay, Surface Plasmon resonance"
Ziextenzo	"RP-HPLC-UV Peptide mapping,	"CD-Near and Far UV,	"MALDI-TOF-MS"	"SEC, DLS, SEC-	*"In-vitro* cell

	RP-HPLC-MS peptide mapping, MALDI-TOF-MS"	NMR Spectroscopy"		MALS,SDS-PAGE,MFI, CE, RP-HPLC"	proliferation assay, Surface Plasmon resonance"
Fulphila	"Peptide mass fingerprinting (Glu-C digest), Intact MALSI TOF MS, MALDI TOF, M-terminal pegylation by GluC / CNBr-Trypsin / Trypsin digestion"	"Non-reduced peptide mass fingerprinting Glu-C digest (disulphide), Far UV CD spectroscopy, FTIR, Ellman's reagent (free cysteine), Extrinsic fluorescence, Near UV CD spectroscopy, DSC, Intrinsic fluorescence, 1D NMR"	"cIEF (Isoelectric point)"	"SEC-UV, Analytical ultracentrifugation, SEC-MALS, CIEX, RP-HPLC"	*"In-vitro* cell proliferation assay, Surface Plasmon resonance"
Grasustek	"Non-reducing / reducing peptide mapping (MS)"	"CD Spectroscopy, FTIR, DSC, fluorescence spectroscopy"	"ESI MS"	"SEC, Analytical ultracentrifugation, DLS, RP-HPLC, IEC, RP-HPLC-ELSD"	*"In-vitro* cell proliferation assay, Surface Plasmon resonance"
Cegfila	"LC-MS, Edman"	"CD, DSC, intrinsic	"Capillary SDS-PAGE,	"RP-HPLC, CEX-HPLC,	*"In-vitro* cell

		fluorescence spectroscopy”	LC-MS”	IEF, UPLC-UV-MS, Western blot, SEC-HPLC, AUC, RP-HPLC-ELSD”	proliferation assay, Surface Plasmon resonance”
Nyvepria	“Glu-C Peptide Mapping (RP-UPLC-MS), Ellman's assay, Capillary isoelectric focussing”	“CD Spectroscopy, Non-reduced Peptide Mapping, Hydrogen-Deuterium exchange, Sedimentation velocity AUC, NMR, DSC”	“RP-UPLC intact mass method”	“RP-HPLC, Ion chromatography, SEC, SDS-PAGE, RP-HPLC-ELSD, RP-UPLC-MS, Glu-C Peptide Mapping”	*“In-vitro* cell proliferation assay, Surface Plasmon resonance, Competitive receptor binding assay”

NON-CLINICAL DEVELOPMENT OF PEGFILGRASTIM BIOSIMILARS:

All the candidate biosimilars conducted studies on animal models to evaluate PK, PD, Efficacy, Toxicity, Toxicokinetics, local tolerance, and immunogenicity. A maximum number of non-clinical animal studies were conducted by Pelgraz and Ziextenzo. Both conducted five animal studies. Minimum number of non-clinical animal studies was conducted by Pelmeg and Cegfila. Both conducted only one animal study.

Table 7 lists all the animal studies conducted by Pegfilgrastim biosimilars along with the dosages and parameters evaluated in each study.

Table 7: Comparison of Non-Clinical studies

Product	Animal Model	Dose	Non-clinical study data used for						
			Efficacy	PK	PD	Toxicity	TK	LT	Immunogenicity
Udenyca	"Sprague-Dawley Rats"	30, 100, 300 or 1000 µg/kg	No	Yes	Yes	No	No	No	No
	"Cynomolgus monkeys"	0.075, 0.25 or 0.75 mg/kg	No	No	Yes	Yes	Yes	No	Yes
Pelgraz	"Neutropenic Swiss albino mice"	250, 500 and 1000 mg/kg	Yes	No	No	No	No	No	No
	"Neutropenic balb/C mice"	Not given	Yes	No	No	No	No	No	No
	"Wistar Rats"	30, 180, 1100 µg/kg, 10 doses	No	No	No	Yes	Yes		No
	"Female New Zeeland rabbits"	Not given	No	No	No	No	No	Yes	No
	"Guinea pigs"	Not given	No	No	No	No	No	Yes	No
Pelmeg	"Normal and Neutropenic male rats CD/Crl: CD (SD)"	15 or 100 µg/kg	No	Yes	Yes	No	No	No	No

Ziextenzo	"Sprague-Dawley Rats"	50,100,200 or 1000 µg/kg	No	Yes	Yes	No	Yes	Yes	Yes, 28/128 and 42/128 ADA animanls in Ziextenzo and Neulasta respectively
	"Sprague-Dawley Rats"	12.5, 25, 50, 75, 100, 1000µg/kg	No	No	No	Yes	Yes	No	No
	Rabbits	99µg/kg	No	Yes	Yes	No	No	No	No
	Dogs	Not given	No	Yes	Yes	No	No	No	No
	"Rabbit Himalayan White"	0, 2, 5, 50, 100 µg/kg	No	No	No	Yes (Embryo Fetal developmental Toxicity)		No	No
	"Wistar Rats"	0,100,500,1000 µg/kg	No	No	No	Yes	Yes	No	Yes
Fulphila	"Male CD / Crl:CD (SD) rats"	100, 300 ,1000 or 3000 mg/kg, 12 doses	No	No	Yes	No	No	Yes	No
	"Sprague-Dawley Rats"	0.15 mg/kg, 0.65mg/kg and 1.5mg/kg (low, mid and high) for 28 days	No	No	No	Yes	Yes	Yes	No

Grasustek	"Rat model with or without pre-treatment with 50mg/kg of cyclophosphamide"	0, 50, 150 or 450 µg/kg	No	Yes	Yes	No		No	No
	"CD strain rats"	100, 300 or 1000 µg/kg, 5 doses	No	No	No	Yes	Yes	No	Yes, higher incidence of ADA in Grasustek arm
	"NZW Rabbit"	6mg	No	No	No	No	No	Yes	No
Cegfila	"Normal and Neutropenic male rats CD/Crl: CD (SD)"	15 or 100 µg/kg	No	Yes	Yes	No	No	No	No
Nyvepria	"CD/Crl: CD (SD) rats"	200 or 1800 µg/kg	No	Yes	Yes	Yes	Yes	No	Yes
	"Sprague-Dawley Rats"	200, 600 or 1800 µg/kg	No	Yes	Yes	Yes	No	No	No

CLINICAL DEVELOPMENT OF PEGFILGRASTIM BIOSIMILARS

All the Pegfilgrastim biosimilar candidates conducted clinical studies on healthy volunteers to assess PK, PD, and immunogenicity. Only four out of eight Pegfilgrastim biosimilars conducted an efficacy study on cancer patients. The remaining four got the approval without any efficacy data as per "EMA Guidance on Similar Medicinal Products Containing Recombinant Granulocyte-Colony Stimulating Factor."

A maximum number of patients (589) in the efficacy study was enrolled for Pelgraz whereas Phulfila enrolled a minimum number of patients (194). Primary endpoint for efficacy for all biosimilars was DSN C1.

The most commonly monitored PK parameters were Cmax, AUC 0-t, and AUC 0-inf. Pelgraz additionally monitored Tmax, K el, and T1/2 as PK parameters. Most commonly monitored PD parameters were ANC AUC0-t and ANC Cmax. Five out of eight Pegfilgrastm biosimilars also monitored CD34+ cells Cmax and CD34+ cells Tmax as PD parameters.

Exceptions

The biosimilars got approval despite showing the following exceptions as they were not considered significant to prove that the candidate biosimilar is different than Neulasta.

- PK/PD, immunogenicity, and tolerability study for Udenyca failed to meet acceptance criteria for Cmax, AUC 0-last, and AUC 0-inf.
- PK/PD, immunogenicity, and tolerability study for Udenyca failed to meet acceptance criteria for AUC 0-inf and AUC 0-last.
- Biosimilarity could not be demonstrated for AUC 0-last, Cmax, and AUC 0-inf for the pivotal PK/PD study of Ziextenzo.
- Supportive PK/PD study for Ziextenzo 90% CI of GMR for AUC last, Cmax, and AUC 0-inf: 80-125%. The 90% CIs did not cover 100% of all endpoints.
- A low dose PK/PD study for Grasustek was not powered to establish PK equivalence, and provided supporting evidence of similarity at 2mg although PK equivalence failed.
- 9 out of a total of 11 FN instances were in Grasustek.

Table 8 lists all the clinical studies conducted by Pegfilgrastim biosimilars.

Table 8: Comparison of Clinical studies

Product		Study design	Population	No of subjects	Dose	PK parameters assessed	PD parameters assessed	Efficacy endpoints	Immunogenicity
Udenyca	"PK/PD, immunogenicity, tolerability study"	"Randomized, Single dose, 2-period, single site, crossover"	Healthy subjects	78	6 mg	• Cmax • AUC 0-t • AUC 0-inf	• ANC • ANC AUC 0-last	NA	TE ADA were 6/37 (16.2%) in Udenyca and 7/39 (7.7%) in Neulasta.
	"PK/PD, immunogenicity, tolerability study"	"Randomized, double-blind, 2-period, Single dose, crossover"	Healthy subjects	116	6 mg	• Cmax • AUC 0-t • AUC 0-inf	• ANC AUC 0-last • ANC Cmax	NA	TE ADA were 15/50 (30%) in Udenyca and 18/52 (34.6%) in Neulasta. No Nabs.
	"Immunogenicity study for impact of ADA on PK/PD and tolerability"	"Two dose, parallel arm"	Healthy subjects	303	6 mg	NA	NA	NA	12/122 (9.8%) in Udenyca group and 6/120 (5.0%) in Neulasta group were ADA positive. No TE ADA were neutralizing. ADA had no impact on PK/PD and tolerability

	"PK/PD, immunogenicity, local tolerance including impact of ADA on; tolerability"	"Randomized, single blind, Crossover, 3 sequence, 3 period"	Healthy subjects	122	6 mg	• Cmax • AUC 0-inf	• ANC AUC • ANC Cmax	NA	Treatment emergent ADA was 28.6% in Udenyca and 33.3% in Neulasta.
Pelgraz	"PK/PD study"	"Randomized, Single dose, 2-way crossover, assessor blinded, active controlled"	Healthy subjects	66	6 mg	• Cmax • AUC 0-t • AUC 0-inf • Tmax • K el • T1/2	• ANC AUCt • ANC Cmax • ANC Tmax • CD34+ cells-AUCt • CD34+ cells Cmax	NA	NA
	"Comparative PK/PD study"	"Randomized, Assessor blinded, Single dose, cross over"	Healthy subjects	Not given	Two dose levels	• Cmax • AUC 0-t • AUC 0-inf	• ANC AUC0-t • ANC Cmax	NA	NA
	"Safety and efficacy study"	"Multicenter, randomized, assesor-blinded, active controlled"	Cancer patients suffering from stage IIA, IIB or IIA breast cancer	589	6 mg			Primary Endpoint: DSN C1	TE ADA: 3/294 for Pelgraz, 1/148 for US Neulasta and 1/147 EU Neulasta. No Nabs

Pelmeg	"PK/PD study"	"Single dose, Randomized, double blind, 2-stage, 2-way, cross over study"	Healthy subjects	172	6 mg	• Cmax • AUC 0-t • AUC 0-inf	• ANC AUC 0-t • CD34+ cells Cmax • CD34+ cells Tmax	NA	34/171 (19.9%) positive, mainly due to PEG. 9/34 for Pelmeg, 7/34 for Neulasta. No antibodies against Filgrastim or Nab
	"PD and immunogenicity / safetty study"	"Multiple dose, randomized, double-blind, 3-periods, 2-sequences, crossover study"	Healthy subjects	96	3 mg	NA	• ANC AUEC0-t • ANC Cmax • ANC Tmax • CD34+ cell count	NA	No significant ADA, No Nabs
Ziextenzo	"Pivotal PK/PD study"	"Single dose, randomized, double blind, two period cross-over PK/PD study"	Healthy subjects	184	6 mg	• Cmax • AUC 0-t • AUC 0-inf	• ANC AUEC0-t	NA	1 and 4 subjects were ADA positive in Ziextenzo and Neulasta respectively. None positive for Nab.
	"Supportive PK/PD study"	"Single dose, randomized, double blind,three arm, parallel group, PK/PD study"	Healthy subjects	279	6 mg	• Cmax • AUC 0-t • AUC 0-inf	• ANC AUEC0-t	NA	5 and 1 subjects were ADA positive in Ziextenzo and Neulasta respectively.

	"Pivotal confirmatory efficacy and safety study, supportive PK sub-study"	"Randomized, double-blind, parallel group, active-controlled, multi-center study in US, ROW countries and Asia"	Female patients with breast cancer undergoing myelosuppressive chemotherapy	308 (60 for PK/PD sub-study)	6 mg upto 6 cycles	• Cmax • AUC 0-t • AUC 0-inf	• ANC AUC0-t	Primary Endpoint: DSN C1 Secondary Endpoint: ANC nadir C1 and FN C1	23 and 29 patients tested positive for ADA in Ziextenzo and Neulasta group respectively. None positive for Nab.
Fulphila	"PK/PD, safety study"	"Single dose, Randomized, double blind, 3-treatment, 3-period,3-way cross over study"	Healthy subjects	216	2 mg	• Cmax • AUC 0-inf	• ANC AUC0-t • ANC Cmax • CD34+ cells-AUC0-t • CD34+ cells Cmax	NA	ADA positive and negative ratios: Fulphila- 62:142 EU Neulasta- 62:141 US Neulasta- 64:143
	"Immunogenicity, safety study"	"Single center, randomized, open-label, 2-dose, parallel study"	Healthy subjects	50	6 mg		ANC vs Time profile	NA	32% ADA positive for Flphila and US Neulata at 1 or more time points.

	"Efficacy, Safety, immunogenicity study"	"Multicentre, randomized, double-blind, therapeutic equivalence study"	Female patients with stage II/III invasive breast cancer in the adjuvant / neo-adjuvant setting who were receiving TAC	194	6 mg	NA	NA	Primary Endpoint: DSN C1	19 out of 126 (15%) and 13 out of 67 (19.4%) were tested positive for ADA for Fulphila and EU Neulasta respectively. Only 1 patient in both group was positive for GCSF antibody. 2 subjects were Nab positive for Fulphila and 1 subject was Nab positive for both Neulasta group across 3 studies.
Grasustek	"PK/PD study"	"Single dose, Randomized, double blind, 2-treatment, 2-period, 2-sequence, cross over study"	Healthy subjects	156	6 mg	• Cmax • AUC 0-t • AUC 0-inf	• ANC AUC • ANC Cmax • ANC Tmax • CD34+ cells-AUC • CD34+ cells Cmax • CD34+ cells Tmax	NA	None out 454 samples were ADA positive

	"PK/PD study"	"Single dose, Randomized, double blind, 2-treatment, 2-period, 2-sequence, cross over study"	Healthy males	64	2 mg	Study not powered to establish PK equivalence, provided supporting evidence of similarity at 2mg although PK equivalence failed	• ANC AUC • ANC Cmax • ANC Tmax • CD34+ cells-AUC • CD34+ cells Cmax • CD34+ cells Tmax	NA	5 out of 188 samples (from 3 subjects) were ADA positive. (2.7%) All negative for Nab
	"Efficacy Safety study"	"2:1 Randomized, multi-centre, double-blind, parallel group"	Female patients with breast cancer undergoing myelosuppressive chemotherapy	254	6mg uppto 6 cycles	NA	NA	Primary Endpoint: DSN C1 Secondary Outcome: ANC nadir C1 and FN C1	2 out of 949 samples were ADA positive, 1 was Nab positive. Additionally antibodies against impurities (His-Filgrastim and EK) were detected in Pre-dose samples

Cegfila	"PK/PD study"	"Single dose, Randomized, double blind, 2-stage, 2-way, cross over study"	Healthy subjects	172	6 mg	• Cmax • AUC 0-t • AUC 0-inf	• ANC • AUC 0-t • CD34+ cells Cmax • CD34+ cells Tmax	NA	34/171 (19.9%) positive, mainly due to PEG. 9/34 for Cegfila, 7/34 for Neulasta. No antibodies against Filgrastim or Nab
	"PD and immunogenicity / safety study"	"Multiple dose, randomized, double-blind, 3-periods, 2-sequences, crossover study"	Healthy subjects	96	3 mg	NA	• ANC AUC0-t • ANC Cmax • ANC Tmax • CD34+ cell count	NA	No significant ADA, No Nabs
Nyvepria	"Comparative PK/PD study"	"Open label, randomized, single dose, comparator controlled, 3-treatment,3-period, 6-sequence, crossover study"	Healthy subjects	153	6 mg	• Cmax • AUC 0-t • AUC 0-inf	• ANC AUC • ANC Cmax • ANC 0-last	NA	ADA positives: 6/153 for Nyveria, 2/153 each for EU and US Neulasta. 2 Nabs positive for Nyvepria, None for Neulasta. Design not considered optimal for impact of ADA on PD.

	"Comparative immunogenicity study"	"Randomized, open label, multiple dose, parallel design non-inferiority study"	Healthy subjects	422	6 mg	NA	NA	NA	ADA positive: 5.9% for Nyvepria, 7.5% for US Neulasta. 1 Nab positive for Nyvepria. Design not considered optimal for impact of ADA on PD.
	"Non-comparative PK/PD study"	"Open label, non-comparative study"	Metatatic breast cancer patients	25 (in two phases)	3mg and 6 mg	Supportive	Supportive	NA	NA

CONCLUSION

High variability is observed between the quality, non-clinical and clinical aspects. Half of the approved Pegfilgratim biosimilars did not conduct any efficacy study in cancer patients. There are also some instances of failure to meet the acceptance criteria in clinical studies. EMA has taken a balanced approach in approving the Pegfilgrastim biosimilars on case-to-case basis based on available data and provided justification in absence of that. It can be concluded that there is a fair degree of flexibility shown by EMA for granting MAA to Pegfilgrastim biosimilars as there have been multiple approved products from the same class of drug with proven safety and efficacy record. Therefore, establishing bio similarity with innovator products is generally considered adequate. This approach by EMA is likely to be extended to other biosimilars which have adequately proven record of safety and efficacy. Exemption from conducting redundant clinical efficacy studies will result in rapid and low cost development of biosimilars, ultimately benefitting the patients.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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APPENDIX

DEFINITIONS

- AUC 0-t = "Area under the concentration-time curve (time 0 to time of last quantifiable concentration."
- AUC0-inf = "Area under the serum concentration-time curve (from time 0 to infinity) calculated as as AUC0-last + Clast/λz, where Clast is the last measureable concentration and λz the apparent first-order terminal elimination rate constant"
- Cmax = "Observed maximium concentration of Pegfilgrastim in plasma over the sampling interval."
- Tmax = "Time to attain maximum serum concentration."
- kel = "Terminal elimination rate constant"
- T half = "Apparent terminal elimination half-life."
- ANC AUC0-t = "Area under the ANC curve above baseline values versus time curve (time 0 to time of last data collection point)."
- ANC Cmax = "Maximum absolute neutrophil count."
- ANC Tmax = "Time of maximum change from baseline for ANC."
- 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."
- ANC nadir = "Lowest ANC in Cycle 1."

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