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Comparison of the physicochemical properties of a biosimilar filgrastim with those of reference filgrastim

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ABSTRACT

Recombinant human granulocyte-colony stimulating factor (filgrastim) is a therapeutic protein used primarily to reduce incidence and duration of severe neutropenia and its associated, and serious, complications. We have developed a biosimilar filgrastim (Hospira filgrastim; Nivestim™) designed to be comparable to Amgen filgrastim (Neupogen®).

An extensive characterization study assessed the physiochemical similarity of Hospira filgrastim to Amgen filgrastim. Both drugs were supplied in 1 ml glass, single-use, prefilled syringes (five batches of each product at 480 μg/0.5 ml and one batch of each product at 300 μg/0.5 ml). Samples were evaluated using state-of-the-art analytical methods (validated in accordance with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use or Pharmeuropa guidelines) to determine physicochemical properties, molecular characteristics, purity and biological activity. Samples were compared after long-term storage at 2–8 °C and storage at 40 °C (stress conditions) to evaluate their degradation impurity profiles.

Hospira filgrastim and Amgen filgrastim were shown to have similar physicochemical properties, molecular characteristics, purity and biological activity. No significant differences in product-related impurities were recorded between Hospira filgrastim and Amgen filgrastim following storage for 12 weeks under stress conditions. These data show that the physicochemical profile of Hospira filgrastim is similar to that of Amgen filgrastim.

1. Introduction

In the majority of cases, a single bioequivalence study is sufficient to demonstrate that a generic version of a small-molecule pharmaceutical is as safe and effective as its reference product [1], due, in part, to the relatively basic chemical structures of these molecules and the highly reproducible manufacturing processes. In contrast, biopharmaceuticals are extremely complex products, consisting of mixtures of highly similar molecules, with manufacturing processes that cannot be exactly replicated. As a result, 'generic' biopharmaceuticals do not exist, but legislation has enabled the development of similar biological medicinal products [2–4]. Biosimilars are considered to be mixtures of product variants that, although highly similar, may have an acceptable level of minor differences from the reference product. Furthermore, there may be qualitative differences between the process-related impurities present in biosimilars and their reference products.

Over the next few years, the field of biosimilars is expected to rapidly expand with the imminent patent expiry of a number of important biopharmaceuticals. Filgrastim, a recombinant human granulocyte colony-stimulating factor (rhG-CSF), was first approved under the trade name Neupogen® (Amgen Inc., Thousand Oaks, CA, USA) in 1991, in both Europe and the US, and has already undergone patent expiry. Human G-CSF is a growth factor, produced by endothelial cells, macrophages and other immune cells, which influences the survival, proliferation and differentiation of cells of the neutrophil lineage [5]. In light of the haematopoietic activity of human G-CSF, filgrastim is primarily used to reduce the incidence and duration of neutropenia and associated complications.

Amgen filgrastim is a 175-amino acid recombinant protein with a molecular weight of 18 800 Da. While human G-CSF is a glycosylated protein, Amgen filgrastim is a non-glycosylated protein, produced in genetically modified *Escherichia coli* (*E. coli*). Its amino

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Table 1
Analytical methodologies employed for assessment of Hospira filgrastim and acceptance criteria for similarity with reference product (Amgen filgrastim).

Method	Acceptance criteria
Physical and chemical parameters	
Appearance	
Visually	A clear, colorless solution
pH	
Ph. Eur. method	consistent with the reference product
IEF	
In-house method	One principal band between pI 5.85 and 6.55. Other bands: no more than trace
Ph. Eur. method	Identity: The principal band in electropherogram obtained with the test solution is similar in position to the principal band in the electropherogram obtained with reference solution Impurities: No band is more intense than the principal band in the electropherogram obtained with reference solution (10%)
SDS-PAGE	
In-house method	Coomassie staining: one principal band with molecular weight between 21 kDa and 14 kDa compared to molecular weight standards. Other bands: no more than trace amount Silver staining: diffused principal band with molecular weight between 21 kDa and 14 kDa compared to molecular weight standards, additional bands of lower molecular weight that correspond to additional bands in reference product. Other bands: no more than trace amount
Ph. Eur. method	Identity: The principal band in the electropherogram obtained with test solution is similar in the position to the principal band obtained with the reference solution Impurities: Impurities with molecular masses lower or higher than that of filgrastim – no band is more intense than the principal band in the electropherogram obtained with test solution (2.0%)
Protein concentration (UV/VIS)	0.86–1.06 mg/ml
RP-HPLC assay	0.86–1.06 mg/ml
Intact molecule mass determination	18 800 ± 1 Da
Biological assay identify	Cell number proliferation (induced by filgrastim) relative to control cell group
Purity	
SEC-HPLC	
In-house method	Filgrastim dimer and higher molecular weight related impurities should be within the range demonstrated by the reference product
Ph. Eur. method	Impurities with molecular masses higher than that of filgrastim should be within the range demonstrated by the reference product
RP-HPLC	
In-house method	Filgrastim oxidised forms and Filgrastim-related impurities should be within the range demonstrated by the reference product
Ph. Eur. method	Any individual impurity should and total impurities should be within the range demonstrated by the reference product
IC	
In-house method	f-met filgrastim and more acidic-related impurities should within the range demonstrated by the reference product
Potency	
Biological assay activity	
In-house method	Consistent with the reference product
Additional	
Fluorescence spectroscopy	
In-house method	Complies with reference product Amgen filgrastim spectra
Circular dichroism	
In-house method	Complies with reference product Amgen filgrastim spectra
Peptide mapping with C- and N-terminus AA sequencing	
In-house method	Complies with reference product Amgen filgrastim primary AA sequence
Disulphide bridges and AA sequence determination by peptide mapping	
In-house method	Complies with literature data (confirmed disulphide bridges position and amino acid sequence)

Ph. Eur. = European Pharmeuropa; IEF = isoelectric focusing; pI = protein isoelectric point; SDS-PAGE = sodium dodecyl sufate polyacrylamide gel electrophoresis; UV/VIS = ultraviolet-visible spectroscopy; RP-HPLC = reverse phase high-performance liquid chromatography; SEC-HPLC = size-exclusion high-performance liquid chromatography; IC = ion chromatography.

acid sequence is identical to that of human G-CSF, except for an additional N-terminal methionine.

A number of filgrastim biosimilars are currently approved for medicinal use or are in development. If comparable quality, safety and efficacy can be demonstrated to Amgen filgrastim, these agents could provide cost-effective alternatives. Here we report the data from a comparability study that was conducted to determine physicochemical and biological similarity between a biosimilar filgrastim (Hospira filgrastim; Nivestim™) manufactured and co-developed by PLIVA and Hospira, and reference filgrastim (Amgen filgrastim).

2. Materials and methods

Test product samples were: Hospira filgrastim 480 μg/0.5 ml, lot numbers: 6241116, 6242116, 6243116 (24 months old at the time of testing), 4621067, 4626067 (17 months old at the time of testing); Hospira filgrastim 300 μg/0.5 ml, lot: 4623107 (13 months old at the