U.S. FOOD & DRUG ADMINISTRATION

Biosimilar User Fee Act (BsUFA) III Regulatory Science Pilot Program

ANNUAL REPORT

CONTENTS

Check if this report is Progress or Final Report:

☒**Progress report** ☐ **Final report**

1. REPORT OVERVIEW[1]

Project Title:	Critical Factors for Standardization and Accuracy of PK Assays of PEGylated Biosimilars
Investigator:	Kristina E. Howard, DVM, Ph.D.
Organization:	CDER/OTS/OCP/DARS
Grant No. (if applicable)	N/A
Project Objective:	To provide guidance/best practices to industry for evaluating pharmacokinetics (PK) associated with biosimilars that are conjugated to polyethylene glycol (PEG).

Specific Aim(s)	Progress	Outcomes	Communication Timeline
1. Validate existing assays used by biosimilar sponsors for pegfilgrastim PK	All work is completed. (The overall objective was a multi-year project and only manuscript publication remains to be completed.)	Using pegfilgrastim as a model drug, we showed that some commercially available G-CSF ELISA assays cannot accurately measure PK as required by the guidance.	Anticipate submitting for publication before the end of calendar year 2024
2. Develop an alternate pegfilgrastim PK assay as a proof of concept	All laboratory work proposed for the funded year has been completed. Preliminary manuscript draft completed. (The overall objective was part of a multi-year project, of which some lab work was completed in the past year.)	We used pegfilgrastim and its approved biosimilars to evaluate different PK assay methodologies that could potentially be used with other unrelated PEGylated biosimilars for PK assessment.	Expect to submit results for publication by the first quarter of 2025.

[1] This section will be used by program for broader research portfolio and regulatory impact analysis by the BsUFA III steering committee.

2. PROGRESS SUMMARY

Aim 1: Validate existing assays used by sponsors for pegfilgrastim PK

The objective of this aim was to determine if PEGylated biosimilars could fail PK biosimilarity assessments as a result of issues with the assay used to measure drug levels in the blood of clinical trial participants, rather than a result of actual differences between the biosimilar and reference product. We have completed a manuscript detailing our results using three unique assays, two of which have been used by biosimilar sponsors for 351K submissions. In this research, we show that the assay used can create questions about biosimilarity that stem from the assay rather than the product.

A paper has been drafted and is being prepared for submission. We expect submission prior to the end of calendar year 2024. Overall results from this project (prior years) were communicated directly to review staff through internal presentations.

Aim 2: Develop a ligand binding assay to assess PK of pegfilgrastim as a proof of concept

We wanted to determine if it was possible to build a ligand binding assay (to assess PK) that could be based on detection of the PEG portion of the pegylated drug. If sponsors did not have to develop monoclonal antibodies specific for the peptide portion of the therapeutic, and instead were able to use direct ligand binding to cells, followed by quantitation using anti-PEG antibodies, it would save time and money in the development of ADA assays. This type of methodology would make it easier to create these assays for biosimlars of PEGylated drugs used in rarer patient populations and could potentially accelerate development of biosimilars to less frequently used reference products.

As part of this study, we compared the standard PK assay used in sponsor applications for biosimilars to a cell-based assay that detects PEG, rather than the molecule it is conjugated to. If validated (as designated in the FDA bioanalytical method validation guidance), such an assay approach could be used by sponsors in their applications.

We have completed all laboratory research and are completing a final draft of the manuscript. Submission for publication is expected in early 2025.

No additional funds are needed for completion.

3. RESEARCH OUTCOMES

Aim 1 – Our results show that PK assay performance is critical to ensure that PEGylated biosimilars can be properly compared to their reference product. PEG can vary sufficiently to cause differences in a PK assay, even if it is the same weight, and apparent structure. This is because differences can exist in its manufacture, when procured from different sources, that ligand binding assays can detect.

Aim 2 – In this aim, we developed an assay to measure pharmacokinetics that binds to PEG, rather than the protein to which it is conjugated. This creates an approach to create a ligand binding assay that would apply to apply to the wide range of PEGylated products. Further, we compare this assay to a commercially created assay and compare drug concentrations of the reference and biosimilar products.

4. REGULATORY IMPACT

By developing a cell-based assay and detecting the PEG portion of the PEGylated drug, PK can be assessed without having to develop antibodies to the protein portion of the drug. This could lead to savings in the development of assays for biosimilars of PEGylated drugs.

5. COMMUNICATION AND DISSEMINATION

We presented some of this research (prior to BsUFA funding) in a poster presentation in the early stage of this research and collaborated with other investigators on a paper published in part from this study's funding. Some of this research has been shared internally through poster and seminar presentations to review staff.

We will be submitting the remaining work for publication this year and early next year.

- Shi D, Shah AB, Schrieber SJ, and Howard KE. PEGylation affects the pharmacokinetic assessment of pegfilgrastim using ligand binding assays. Poster. AAPS, PharmSci 360, 2019, San Antonio, TX.
- Xie T, Fang H, Ouyang W, Angart P, Chiang MJ, Bhirde AA, Sheikh F, Lynch P, Shah AB, Patil SM, Chen K, Shen M, Agarabi C, Donnelly RP, Brorson K, Schrieber SJ, Howard KE, Rogstad SM, Frucht DM. The ELISA Detectability and Potency of Pegfilgrastim Decrease in Physiological Conditions: Key Roles for Aggregation and Individual Variability. Sci Rep. 2020 Feb 12;10(1):2476. doi: 10.1038/s41598-020-59346-z. PMID: 32051479.

6. CHALLENGES

Over the course of the research project we experienced issues with the availability of certain reagents as vendors abruptly stopped offering an anti-PEG antibody that necessitated validation with a substitute. We also discovered that some commercially available reagents advertised as binding to the G-CSF receptor, did not actually bind to it. This makes it critical to ensure that all reagents are thoroughly validated regardless of how they are marketed.

7. NEXT STEPS

Our next steps are to publish the remaining manuscripts and provide a seminar to reviewers to inform them on the aspects of assay performance and development that affect the approval of biosimilar PEGylated products.

8. ABBREVIATIONS

This section includes all acronyms used in this document along with a corresponding definition.

ABBREVIATION	DEFINITION
PK	Pharmacokinetics
G-CSF	Granulocyte-colony stimulating factor
ELISA	Enzyme linked immunosorbent assay
PEG	Polyethylene glycol