U.S. FOOD & DRUG ADMINISTRATION

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

ANNUAL REPORT

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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. Manuscript is undergoing review prior to submission.	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.	Manuscript will be submitted for publication after receiving internal clearance; anticipated submission in 1st quarter FY2026.
2. Develop an alternate pegfilgrastim PK assay as a proof of concept	All work is completed. Manuscript is undergoing review prior to submission.	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.	Manuscript will be submitted for publication after receiving internal clearance anticipated submission in 1st quarter FY2026.

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

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.

Manuscript is under review prior to submission. 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 develop 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 biosimilars of PEGylated drugs 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 pegfilgrastim biosimilars to a cell-based assay that detects PEG, rather than the molecule to which it is conjugated. Results show that the cell-based assay was similar in result to the standard ELISA assay for reference product and biosimilars. In addition, in vivo PK testing using immune humanized mice showed no significant differences between methods. There data indicate this approach could be appropriate for some biosimilar product development programs.

Manuscript is drafted and under review prior to submission.

Research Outcomes

Aim 1: Summary of results (first manuscript)

Polyethylene glycol (PEG) is added to therapeutic proteins to prolong half-life; however, the presence of PEG can cause variability in ligand binding assays (LBA) used to assess serum concentrations of the biologic/biosimilar. This variability may result in pegylated biosimilars failing to meet requirements for FDA approval. We evaluated LBAs using pegfilgrastim and the non-pegylated form, filgrastim, for potential variability. We assessed two commercially available LBAs using selected parameters from FDA's Bioanalytical Method Validation Guidance. Pegfilgrastim

samples showed increased variability overall, with effects of matrix and storage showing higher variability for pegfilgrastim as compared to filgrastim samples. Factors such as donor sex, short-term storage at 4°C, and long-term storage at -20°C did not affect filgrastim or pegfilgrastim detection. Variability observed in the calibrator, matrix, and storage conditions contributed primarily to inconsistent pegfilgrastim measurement. Therefore, commercial LBAs designed to detect filgrastim may require protocol modifications to reliably report pegylated peptide concentrations of pegfilgrastim.

Aim 2: Summary of results (second manuscript)

Pegylation can pose challenges for biosimilar drug development, as replicating the reference product's PEG characteristics to achieve similarity to the reference product pharmacokinetics (PK) can be difficult. A few biosimilar product submissions have highlighted issues with PK assays, potentially contributing to failures in PK similarity assessment. With many approved pegylated protein therapeutics becoming eligible for biosimilar development, developing alternative methods for PK assessment that can be applied across multiple product categories may expedite pegylated biosimilar development and approval.

We previously validated an ELISA-based method and observed significant variability even in samples prepared with known drug concentrations. We determined that a cell-based assay (CBA) might offer greater translatability to other pegylated products because the antibodies detecting drug concentration bind to the PEG moiety rather than the peptide itself. This allows a cell line to be used to bind the drug product being tested, using anti-PEG antibodies for detection. In this study, we utilized pegfilgrastim, a pegylated granulocyte-colony stimulating factor (G-CSF) product, for PK determination using the CBA method.

Our results demonstrate this methodology offers a wider detection range as compared to conventional ELISA assays for pegfilgrastim. It also has good reproducibility and higher throughput, presenting a viable option for the rapid development of PK assays for other pegylated drug products.

Regulatory Impact

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. Further, 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.

Communication and Dissemination

Two manuscripts are under review prior to submission to journals.

Scientific and Technical Challenges

No scientific or technical challenges were reported for this past year.

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.

References

N/A