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:	Addressing fundamental issues for in vitro immunogenicity testing
Investigator:	Kristina E. Howard, DVM, Ph.D.
Organization:	CDER/OTS/OCP/DARS
Grant No. (if applicable)	N/A
Project Objective:	To validate methods for in vitro immunogenicity testing that could be used by industry to reduce/eliminate the need for clinical trials assessing immunogenicity for biosimilar drug products

Specific Aim(s)	Progress	Outcomes	Communication Timeline
1. Survey therapeutic protein submissions to FDA (regardless of regulatory pathway), select those with in vitro methods, and examine in detail to determine methods submitted by industry.	Survey of approved 351k applications has been completed.	Identified (6) unique assays used for in vitro assessment of immunogenicity. Not every assay was used by every sponsor, but most sponsors did include at least one of them.	Data is being summarized for inclusion in a publication. Expect completion by Q1 2025.
2. Review the published literature to identify in vitro methods used by industry and compare with those submitted to FDA. Summarize all methods identified and determine where gaps, inconsistencies, and issues with methodology exist.	Survey of published literature is approximately 50% complete.	Thus far it appears that more methods are in the literature than were identified in our survey of 351k approved biosimilars.	This data will be included in the manuscript that has the data mining results.
3. Compile clinical immunogenicity data for potential control and test therapeutic proteins to be the basis of comparison to estimate the predictive power of the in vitro assays. Compare previously identified in vitro data with clinical data when possible.	This survey has not started. We expect completion by the end of the calendar year.	N/A	This data will be included in the manuscript that has the data mining results.

[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

Project Objective:

The primary objective of this project is to determine the different types of assays used by sponsors to assess immunogenicity *in vitro*. It is anticipated that the use of *in vitro* immunogenicity testing could reduce or eliminate the need for clinical studies for biosimilar products. By comparing assays in drug applications to those in the literature and clinical study results, we can develop a standardized and consistent approach to in vitro testing and identify the roadblocks to more efficient use of them in the application review process.

Aim 1: Survey therapeutic protein submissions to FDA, select those with in vitro methods, and examine in detail to determine methods submitted by industry.

To date, we have reviewed 64 biologics license applications (BLAs) that included 12 reference products with approved biosimilars. As expected, the most common types of assays were to identify binding and neutralizing anti-drug antibodies (ADA). A range of ADA assay approaches were used including enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence (ECL) assay for higher sensitivity. Additional *in vitro* immunogenicity-related assays included cytokine release, mixed lymphocyte reaction (MLR), proliferation, apoptosis, dendritic cell/T cell proliferation (DC: T cell assay) and Enzyme-Linked Immunosorbent Spot (ELISpot). Each of these assay types were selected by sponsors based on the drug target(s) and its biological effects. A summary of the results is provided in Table 1 of section 9, Appendix A. **(Appendix A was redacted at the request of the awardee.)**

In addition, we identified 58 additional applications that contained DC: T, ELISpot, and/or MLR assays. However, these were excluded from the results because they were either still undergoing review (351k) or solely 351(a) applications. Nonetheless, they further highlighted the fact sponsors are using and submitting immunogenicity assays, but they are not being used effectively in the review process due to the wide variability in assay conduct making them difficult to interpret. We also concluded that sponsors use a range of cell types and sources for their assays, and that cell type and sourcing varies widely by sponsor. The wide variability in the conduct of these assays, especially for an individual reference product, makes it more difficult to evaluate the output.

Aim 2: Review the published literature to identify in vitro methods used by industry and compare with those submitted to FDA. Summarize all methods identified and determine where gaps, inconsistencies, and issues with methodology exist.

As part of this project, in Aim 2, we are reviewing the published literature to determine what assay types have been published by sponsors, and if those assays were submitted in 351K applications. We anticipated finding a greater diversity of approaches to *in vitro* immunogenicity assessment than is observed in the applications to FDA. While this aim is not yet completed, we have generally found more assay diversity in the literature.

Aim 3: Compile clinical immunogenicity data for potential control and test therapeutic proteins to be the basis of comparison to estimate the predictive power of the in vitro assays. Compare previously identified in vitro data with clinical data when possible.

This survey has not started yet. We expect it will occur during September-October 2024.

Note: No additional funding is needed to complete this project.

3. RESEARCH OUTCOMES

We identified 122 biologics applications that contained in vitro immunogenicity assays. Of these, 52 were approved 351K applications. The remaining 70 reviewed were either 351a (reference products) or biosimilars that were under review or otherwise not approved. We identified a range of in vitro assays that aimed to address immunogenicity of a product and found there was great diversity in the cell types used, assay parameters and protocols, as well as the source of the cells. This will enable comparison to the literature and clinical trial results for immunogenicity to determine the predictive potential of these assays as currently conducted.

4. REGULATORY IMPACT

This research has already identified that sponsors are conducting *in vitro* studies to address immunogenicity assessment as part of biosimilar applications. Through the identification of assays being conducted for submissions and comparison with assays reported in the literature and in results from clinical trials, we can identify a set of assays that may be able to consistently provide meaningful insight into immunogenicity of biosimilars.

The eventual goal is to identify methodologies that might permit a clinical assessment of immunogenicity for biosimilars to shift to *in vitro* assessment. This could enable biosimilar products to complete development and testing more quickly. More biosimilar products represent cost savings for patients who need these therapies.

5. COMMUNICATION AND DISSEMINATION

We plan on disseminating our results using the timeline below:

1. Complete Aims 2 & 3 - October 2024
2. Poster presentation (conference to be determined) – Fall/Winter 2024
3. Manuscript drafted and submitted for publication – First quarter 2025

6. CHALLENGES

Major challenges included obtaining access to all necessary internal information systems. Further challenges occurred because the FDA currently has a range of old legacy systems and is migrating to a new system, which makes finding specific information through queries difficult.

A recurrent issue has been the terminologies that sponsors use to describe their assays, which varied greatly, even from how they are described in the literature. This issue is significant as it highlights the need for standardized *in vitro* immunogenicity assay submission to make workflow more efficiently. For example, some

sponsors use different assay names in their applications such as the mixed lymphocyte reaction (MLR) assay being called a “macrophage induction” assay by some sponsors; also, the enzyme linked immunosorbent spot (ELISpot) is referred to as a “T-SPOT” by some sponsors. Some other assay terminologies used in the applications are also unclear. Similar issues were found when sponsors use terms such as “cytokine binding” vs “cytokine release”, which seem to be used interchangeably but have different biological effects.

7. NEXT STEPS

Our future directions include completion of the literature review, clinical immunogenicity study result comparisons and de-identification of assay results gathered from the data mining to protect the privacy of sponsors. Once complete, we will submit a scientific manuscript in early 2025 to share our results. In the interim, we will present our findings at a conference in poster format.