U.S. FOOD & DRUG ADMINISTRATION

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

ANNUAL REPORT

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Contents

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 review 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 (351a and 351k) to FDA, select those with in vitro assay methods, and examine in detail to determine methods submitted by industry.	All research is complete.	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.	Manuscript is under review and will be submitted after internal clearance.
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.	All research is complete.	There are more methods in the literature than were identified in our survey of 351k approved biosimilar applications. Wide range of assays, protocols and cell types used can make interpretation and comparability difficult.	These data will be included in the manuscript noted in Aim 1.

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

Specific Aim(s)	Progress	Outcomes	Communication Timeline
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.	All research is complete.	Many published clinical studies had binding and neutralizing ADA data. In general, *in vitro* ELISpot assays were most in line with published clinical trial outcomes.	These data will be included in the manuscript noted in Aim 1.

Progress Summary

Project Objective:

The primary objective of this project is to better understand the different types, and conduct, of *in vitro* assays used by sponsors to predict immunogenicity. It is anticipated that the use of *in vitro* immunogenicity testing could more prominently factor in de-risking immunogenicity concerns from biosimilar products. By comparing assays in drug applications to those in the literature and clinical study results, we can begin to identify best practices so that a standardized approach to *in vitro* testing can be formulated. This will identify roadblocks to more efficient use in the application review process.

Aim 1: Survey therapeutic protein submissions (351a and 351k) to FDA, select those with in vitro assay methods, and examine in detail to determine methods submitted by industry.

We reviewed 55 biologics license applications (BLAs) that included 12 reference products with 43 approved biosimilars. A broad range of search terms were used including “immunogenicity”, “assay”, and “in vitro”. While we were searching specifically for predictive in vitro assays, these terms also identified assays used to assess the development of anti-drug antibodies in clinical trial participant. As a results, the most common types of assays found were those identifying binding and neutralizing anti-drug antibodies (ADAs). A range of ADA assay approaches were used including enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence (ECL) assay for higher sensitivity. With respect to al *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.

In addition, we identified 59 additional applications that contained DC: T, ELISpot, and/or MLR assays. However, these were excluded from the results because they were either undergoing review (351k) or otherwise not approved. Nonetheless, they further highlighted the fact sponsors are conducting and submitting immunogenicity assays. Based on our review, we believe they could be more effectively used in the review process if: 1) assays are consistently located in the same folder of the application, 2) consistent naming practices are used for assays, and 3) best practices and standardization of parameters (such as relevant positive and negative controls) were employed to enable more efficient review.

Aim 2: Review the published literature to identify potentially predictive 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.

In review of the published literature, we identified 73 studies, of which, 36 included at least one of the 12 products surveyed in our data mining of applications. These studies employed predictive immunogenicity assays including T cell proliferation, cytokine release, ELISpot, major histocompatibility complex-associated peptide proteomics (MAPPs), CD134/CD137 activation assay, transwell DC migration assay, and PBMC *in vitro* comparative immunogenicity assessment. We also identified assays that assessed immunogenicity (ADA assays), as well as assays such as competitive binding ELISA for HLA-DR molecule activities, L929 (murine fibrosarcoma) cell assay for NAbs and direct ELISA binding, that may have been included in the same paper. Again, as with internal database mining, it was difficult to identify only predictive in vitro immunogenicity assays in the studies we identified.

Even when the overall assay was the same, e.g. ELISpot or proliferation, the methods for conduct, positive and negative controls, number of donors, reporting of HLA-typing, varied widely. This is not to say that assays were not conducted properly, but instead, the wide variability can make correlation to clinical results challenging.

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.

ADA results from clinical trials in the literature were explored, with the initial search identifying 190 studies. After cross-referencing results and removing duplicates across databases, 111 scientific reports were reviewed. Of those reviewed, 77 published clinical trials reports included testing for ADAs for 59 biosimilars, including three with undisclosed names.

Assays from the *in vitro* and clinical literature reviews were then compared to each other to determine whether *in vitro* results reflected clinical ADAs experience. With a threshold of two studies or more reports available in each category, only results from adalimumab, bevacizumab, etanercept, infliximab, rituximab, trastuzumab and ustekinumab were evaluated with each study representing a data point. BAbs and NAbs for RP from USA and European Union (EU), and biosimilars, were more closely correlated to ELISpot than T cell proliferation results.

Research Outcomes

We identified 12 reference products with 52 biosimilars in data mining of FDA submissions. We found that sponsors were submitting predictive *in vitro* immunogenicity assays with their applications, but as compared to the literature, fewer assay types were submitted. As noted previously, due to the broad range of search terms used, we identified both predictive assays as well as those used to assess ADA responses in clinical trial participants. For the data mining, the assays found included 42% binding ADAs, 32% NAbs, and 26% predictive immunogenicity assays such as ELISpot, DC:T-cell and proliferation among others. The ELISpot assay results were most correlated with clinical ADAs found in published clinical trial data. Overall, we found that *in vitro* assays do have predictive capacity but should be carefully selected based on the product's mechanism of action/target, and patient population characteristics (e.g. oncology patients are generally immune suppressed).

However, variability in the assays, protocols, and cell types used can make results difficult to interpret and highlights the need for best practices to facilitate data interpretation and usability in de-risking biosimilar products.

Regulatory Impact

This research has 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 serve a more prominent role in de-risking immunogenicity concerns from biosimilar products. In turn, this may also enable biosimilar products to complete development and testing more quickly as well as potentially reducing biosimilar development costs by leveraging in vitro studies to address regulatory questions regarding immunogenicity.

Communication and Dissemination

Table 2: Summary of communications and dissemination of information, results, outcomes, etc. related to this study.

Title	Type of Communication (e.g., poster, manuscript, presentation)	Source	Link (if available)
Data mining study: In vitro immunogenicity assay submissions in biosimilar drug applications	Poster	BsUFA III Regulatory Science Pilot Program Interim Public Meeting, September 2025	N/A
Alternate methods for immunogenicity assessment of biosimilar drug products	Presentation	BsUFA III Regulatory Science Pilot Program: Progress Update; January 2025	N/A

We have completed a manuscript with our findings and expect to publish it this year. The research will also be presented in poster form at the BsUFA Regulatory Science Forum in September 2025.

Scientific and Technical Challenges

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

Next Steps

We have a completed manuscript draft, and it is currently under review prior to submission for publication. We anticipate submission for publication during calendar year 2025.

References

N/A