U.S. FOOD & DRUG ADMINISTRATION

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

ANNUAL REPORT

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Contents

REPORT OVERVIEW 2
PROGRESS SUMMARY 3
Project Objective: 3
Aim 1. Test biological drug products with known clinical immunogenicity, either alone or in combination, and evaluate humanized mice for adaptive immune responses to the products. 3
Aim 2. Compare the responses of Neo/thy (neonatal thymus) humanized mice, that have a human thymus, with CD34 humanized mice, that only have a murine thymus to determine which, if either, can produce adaptive immune responses to biological drug products. 3
RESEARCH OUTCOMES 3
REGULATORY IMPACT 4
COMMUNICATION AND DISSEMINATION 4
SCIENTIFIC AND TECHNICAL CHALLENGES 5
NEXT STEPS 5
REFERENCES 5

Report Overview[1]

Project Title:	**Validation of a non-clinical immunogenicity model**
Investigator:	Kristina E. Howard, DVM, Ph.D.
Organization:	CDER/OTS/OCP/DARS
Grant No. (if applicable)	N/A
Project Objective:	This project evaluates the ability of humanized mice to serve as a non-clinical immunogenicity model by evaluating several biological drug products (with clinically moderate to high immunogenicity) alone or in combination in the two most commonly used humanized mouse models.

Specific Aim(s)	Progress	Outcomes	Communication Timeline
1. Test biological drug products with known clinical immunogenicity, either alone or in combination, and evaluate humanized mice for adaptive immune responses to the products.	All laboratory research is complete. Draft manuscript is being reviewed internally prior to submission	We show that immune humanized mice can IgG class switch and can produce ADAs to biologics known to have high rates of immunogenicity in patients.	Paper will be submitted following internal review. We anticipate publication before the end of the calendar year.
2. Compare the responses of Neo/thy (neonatal thymus) humanized mice, that have a human thymus, with CD34 humanized mice, that only have a murine thymus to determine which, if either, can produce adaptive immune responses to biological drug products.	All laboratory assays have been completed; with the final neutralizing ADA assay completed in 1st Quarter FY 2026.	Thus far data shows that while CD34-humanized mice may have the same immune populations as Neo-thy mice (using the same donor), they do not have equivalent immune function as Neo-thy-humanized mice.	Some data was presented in a poster at SOT in March 2024. Additional data was presented at ACT and the Immunogenicity Summit in 2024. The manuscript is expected to be completed and submitted in 2nd quarter FY 2026.

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

Progress Summary

Project Objective:

This project evaluates the ability of humanized mice to serve as a non-clinical immunogenicity model by evaluating several biological drug products (with clinically moderate to high immunogenicity) alone or in combination, in the two commonly used humanized mouse models.

Aim 1. Test biological drug products with known clinical immunogenicity, either alone or in combination, and evaluate humanized mice for adaptive immune responses to the products.

All animal studies were completed during the funding year. We successfully developed anti-drug antibody (ADA) assays to test humanized mice for antibodies to infliximab and interferon-β. All testing for immunoglobulin isotype switching, and ADAs were completed. All analysis of flow cytometry data was completed. We currently have a draft manuscript completed that is undergoing internal review and edits prior to planned submission.

Aim 2. Compare the responses of Neo/thy (neonatal thymus) humanized mice, that have a human thymus, with CD34 humanized mice, that only have a murine thymus to determine which, if either, can produce adaptive immune responses to biological drug products.

All animal studies were completed during the funding year. We successfully developed anti-drug antibody (ADA) assays to test immune-humanized mice for antibodies to infliximab. All testing for immunoglobulin isotype switching and binding and neutralizing ADA assays have been completed. We are currently completing assay development for detection of neutralizing antibodies to salmon calcitonin. We are currently preparing a manuscript draft and plan submission by the end of the calendar year.

Research Outcomes

The data that have been analyzed thus far show that immune humanized mice have cellular distribution of immune cells that are similar in diversity and representation to that observed in humans. They show the ability to make antibody responses to biological/biosimilar drugs, including class switching and anti-drug antibodies. Through isolation and optimization of lymph node cells, they show the ability to produce cytokines and upregulate activation markers. These responses were found to be most significant in mice that were made with a human thymus and CD34+ hematopoietic stem cells, as compared to mice made using the same donor cells with no human thymus present.

Our results show that this advanced rodent model has a sufficiently human immune system to be used for biological/biosimilar drug product risk assessment, drug development and non-clinical studies and can serve as a replacement for non-human primate models.

Regulatory Impact

These studies demonstrate the utility of these mice as an advanced animal model for biological drug product testing. Through the comparison studies, we show that for the assessment of immune function and immunogenicity, the humanized mouse model with human thymus present had more clear and interpretable results as compared to the CD34 humanized mouse model, which may inform the selection of models for different types of studies in the future.

While animal studies are not required for biosimilar development nor have animal studies traditionally been conducted, having an available animal model may help with de-risking products prior to transitioning to *in vivo* clinical studies or may help address questions regarding immunogenicity in situations where such *in vivo* clinical studies cannot be performed.

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)
Assessing Immunogenicity Using Immune Humanized Mice	Presentation	CHI Immunogenicity Summit; October 2024; Washington DC	N/A
Can the Humanized Mouse Model Inform Immunogenicity Risk Assessment?	Presentation	American College of Toxicology, November 2024; Austin, TX	N/A
Neo-Thy Immune-Humanized Mice can produce anti-drug antibodies to support immunogenicity assessment for biological drug products	Manuscript	Drafted; under internal review	N/A
Neo-Thy, but not CD34 immune-humanized mice are capable of producing anti-drug antibodies to biological drug products	Manuscript	In preparation	N/A

Aim 1: We expect to submit the manuscript by the end of calendar 2025.

Aim 2: We expect to submit the manuscript before the end of calendar 2025.

Scientific and Technical Challenges

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

Next Steps

Aim 1: Submit manuscript for publication.

Aim 2:

- Complete analysis of recently completed in vitro assays (neutralizing antibodies)
- Draft manuscript for publication.
- Submit manuscript by end of 2025.

References

N/A