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:	**Production & optimization of humanized mice**
Investigator:	Kristina E. Howard, DVM, Ph.D.
Organization:	CDER/OTS/OCP/DARS
Grant No. (if applicable)	N/A
Project Objective:	Use an established protocol for producing immune humanized mice to generate a cohort for answering regulatory questions related to pharmacokinetics, pharmacodynamics, immunogenicity, and adverse events that have not been successfully addressed with other nonclinical models.

Specific Aim(s)	Progress	Outcomes	Communication Timeline
1. Using a previously established protocol, produce a cohort of immune humanized mice for use in a separate project for evaluating *in vivo* immunogenicity	This was the final year of a multi-year project to make immune humanized mice to determine if they could be used to test human biological drug products and their biosimilars.	In previous years, a protocol for producing immune humanized mice was established. In the award year, this protocol was used to generate a cohort of immune humanized mice for a nonclinical study for validation of the mouse model under a range of conditions (details described in the annual report for the nonclinical study). Presentations with results of studies using humanized mice for immunogenicity assessment were given at the Immunogenicity Summit (October 2024), and the American College of Toxicology (Nov 2024)	A protocol manuscript outlining methods for producing immune humanized mice has been written and will be submitted for publication in 2025.

[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:

Produce immune-humanized mice to answer important questions related to pharmacokinetics, pharmacodynamics, immunogenicity and adverse events that have not been successfully addressed with other models.

Aim 1: Using a previously established protocol, produce a cohort of immune humanized mice for use in a separate project for evaluating in vivo immunogenicity

Animal models have generally not been useful for the testing of biologics/biosimilars as many of the therapeutic protein products have human specific receptors which do not cross-react with traditional models such as mice, rats and dogs. Non-human primates (NHP) may have cross-reactive receptors, however, their immune biology can be quite different from humans, as receptors may be expressed on different cell types or have alternate functions. To determine if an immune-humanized mouse model could be useful for the evaluation of biologics/biosimilars, this multi-year project evaluated various experimental steps for producing an immune humanized mouse model. Best practices derived from these experiments, including tissue source and mouse strains, will be published as a protocol in 2025. Concurrently, to validate the immune humanized mouse model, a series of studies were outlined as part of a separate regulatory project. Under this project, the developed protocol was followed to produce a cohort of immune humanized mice for the studies. This is the final year of a multi-year project that supported the production of the mice needed for these studies, as the most useful model had not been commercially available until very recently (2024). The commercial development of this advanced mouse model was facilitated by this project, as we had a research collaboration agreement with the commercial vendor to assist them with development and validation of their model. Other deliverables from prior years are noted in research outcomes. In this final year of the project, funding was used to produce mice for a final set of large *in vivo* studies evaluating the ability of different humanized mouse models to demonstrate immunogenicity, as described in the project titled “Validation of a non-clinical immunogenicity model”. At this time all production of immune-humanized mice is complete, and the project is closed.

Research Outcomes

In previous years, this multi-year project established a protocol for producing immune humanized mice for evaluating pharmacokinetic, pharmacodynamic, and immunogenicity regulatory questions for biologics and biosimilars. In prior years, we used mice from this project to compare reference biologics to biosimilar products; determine if these mice could effectively demonstrate cytokine release syndrome and evaluate their ability to model adverse effects of checkpoint inhibitors (see references for selected papers). For the most recent award year, this protocol was used to produce a cohort of immune humanized mice for the study “Validation of a non-clinical immunogenicity model”. All work is completed on this project, apart from submitting a manuscript

protocol outlining methods for producing immune humanize mice according to procedures developed by our lab. No additional funds are required to complete this outcome.

Regulatory Impact

Mice produced through this project were used for (1) demonstration that immune-humanized mice could be used to test reference versus biosimilar drug products, and (2) that these mice could demonstrate immunogenicity (neutralizing antibodies) to biological/biosimilar drug products. These two outcomes provide an advanced animal model that sponsors can use to address issues related to biosimilar drug products without having to conduct a human clinical trial or utilize non-human primates for biological drug studies.

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)
Can the Humanized Mouse Model Inform Immunogenicity Risk Assessment?	Presentation	American College of Toxicology; San Antonio, Tx; November 2024	N/A
Assessing Immunogenicity Using Immune-Humanized Mice	Presentation	CHI Immunogenicity Summit, Washington DC, October 2024	N/A
Alternate methods for immunogenicity assessment of biosimilar drug products	Presentation	BsUFA III Regulatory Science Pilot Program:Progress Update; Virtual; January 2025	N/A
Neo-Thy immune-humanized mice can produce anti-drug antibodies to support immunogenicity assessment for biological drug products	Poster	BsUFA III Regulatory Science Pilot Program Interim Public Meeting, September 2025	N/A

Two manuscripts are expected to be published, that include (1) best practices for non-clinical studies with advanced humanized mouse models (submission expected in 2nd Quarter FY 2026), and (2) a separate protocol manuscript for the production of these mice (submission expected in calendar 2025). Information from this project may be utilized as part of a future public workshop, currently planned for calendar 2026, that will help delineate best practices for use of the immune-humanized mouse model.

Scientific and Technical Challenges

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

Next Steps

Next steps for the project include publishing a protocol manuscript detailing methods evaluated and validated by our laboratory for producing immune-humanized mice that drug developers can utilize. Information from this project may be utilized as part of a future best practices workshop on immune humanized mice with drug developers, academics, and regulatory agencies.

References

N/A