U.S. FOOD & DRUG ADMINISTRATION

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

ANNUAL REPORT

CONTENTS

1. REPORT OVERVIEW 2
2. PROGRESS SUMMARY 2
3. RESEARCH OUTCOMES 3
4. REGULATORY IMPACT 3
5. COMMUNICATION AND DISSEMINATION 3
6. CHALLENGES 4
7. NEXT STEPS 4
8. REFERENCES 4

Check if this report is Progress or Final Report:

☐ **Progress report** ☒ **Final report**

1. REPORT OVERVIEW[1]

Project Title:	Production & optimization of humanized mice
Investigator:	Kristina Howard, DVM, Ph.D.
Organization:	CDER/OTS/OCP/DARS
Grant No. (if applicable)	
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 current 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).	A protocol manuscript outlining methods for producing immune humanized mice has been written and will be published in 2025.

2. 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.

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

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

biologics/biosimilars, this multi-year project evaluated various experimental steps for producing an immune humanized mouse model. An overview of deliverables from prior years is provided in research outcomes to provide additional context for the current fund year's research outcomes.

In this final year of the project, BsUFA III Regulatory Science Pilot Program 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 to biological/biosimilar drug products, as described in the project annual progress report titled "Validation of a non-clinical immunogenicity model". At this time all production of immune-humanized mice is complete, and the project is closed.

3. RESEARCH OUTCOMES

In previous years, using PDUFA and BsUFA funding, 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 presentations and papers).

For the current year, using BsUFA III Regulatory Science Pilot Program funds, this protocol was used to produce a cohort of immune humanized mice for the study "Validation of a non-clinical immunogenicity model". Please see that annual report for additional details. All work is completed on this project, except for 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.

4. REGULATORY IMPACT

This project's high level impact from prior years of funding includes (1) the ability to develop the humanized mouse model to the point that we were able to collaborate with a commercial vendor to establish a rigorous and reproducible protocol for producing humanized mice, (2) provide sufficient training and education on the model to non-clinical reviewers, so that sponsors could confidently use the model as part of their development program, (3) validate the use of neonatal, rather than fetal tissue, for the production of these mice, so that a viable, non-fetal source of tissue was available, (4) identified the best humanized mouse model to use to address different study questions, e.g. cytokine release and checkpoint inhibitor adverse events, and (5) immune-humanized mice could demonstrate adaptive immune responses to biological drug product and, as such, could be used to compare reference versus biosimilar drug products.

Specific studies that mice produced through this year's project were used for, include (1) testing biological drug products with known clinical immunogenicity, either alone or in combination, and evaluate humanized mice for adaptive immune responses to the products., and (2) compare the responses of BLT (bone marrow-liver-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.

5. COMMUNICATION AND DISSEMINATION

From this year's funding, two manuscripts are expected to be published, that include (1) a direct comparison of reference vs biosimilar products and (2) a separate protocol manuscript for the production of these mice. Information from this project may be utilized as part of a future public workshop that will help delineate best practices for use of the immune humanized mouse model. Please see the annual report for 'Validation of a non-clinical immunogenicity model' for information on this year's communication and dissemination plan.

6. CHALLENGES

For the current year, there were no new challenges in the production and use of this animal model. It is recognized the *in vivo* animal models are not currently utilized as part of biosimilar development. However, as discussions continue regarding truncation or elimination of *in vivo* clinical elements of biosimilar development programs, the use of *in vivo* animal studies may serve a role for reducing residual uncertainties and de-risking products. The use of such models would be at the discretion of the sponsor and would likely only be applicable for a subset of complex biologics.

7. 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.

8. REFERENCES

- Yan H, Semple KM, Gonzaléz CM, Howard KE. Bone marrow-liver-thymus (BLT) immune humanized mice as a model to predict cytokine release syndrome. Transl Res. 2019 Apr 22. pii: S1931-5244(19)30081-7.
- Yan H, Bhagwat B, Sanden D, Willingham A, Tan A, Knapton AD, Weaver JL, Howard KE. Evaluation of a TGN1412 analogue using in vitro assays and two immune humanized mouse models. Toxicol Appl Pharmacol. 2019 Jun 1;372:57-69.
- Weaver JL, Zadrozny LM, Gabrielson K, Semple KM, Shea KI, Howard KE. BLT-Immune Humanized Mice as a Model for Nivolumab-Induced Immune-Mediated Adverse Events: Comparison of the NOG and NOG-EXL Strains. Toxicol Sci. 2019 May 1;169(1):194-208.
- Semple KM, Gonzaléz CM, Zarr M, Austin JR, Patel V, Howard KE. Evaluation of the Ability of Immune Humanized Mice to Demonstrate CD20-Specific Cytotoxicity Induced by Ofatumumab. Clin Transl Sci. 2019 May;12(3):283-290.
- Yan H, Knapton AK, Weaver JL and Howard KE. Modeling Cytokine Release Syndrome Using BLT Immune Humanize Mice Treated with anti-CD28. American College of Toxicology Annual Meeting. Palm Springs, CA. November 2017.
- Howard KE, Zadrozny L, Semple KM, Shea K, and Weaver JL. Autoimmunity Induced by Ipilimumab in Immune-Humanized Mice. Society of Toxicology Annual Meeting. Baltimore, MD. March 2017.
- Yan H, Semple KM, Gonzalez CM and Howard KE. Modeling Cytokine Release Syndrome Using Immune Humanized Mice. Society of Toxicology Annual Meeting. Baltimore, MD. March 2017.
- Howard KE, Zadrozny L, Weaver JL. Immune Humanized Mouse Model: Autoimmunity Induced by Nivolumab. American College of Toxicology Annual Meeting, Baltimore, MD. November 2016.
- Gonzaléz CM, Li XH, Austin J, Patel, V and Howard KE. Comparison of anti-CD20 Innovator versus Follow-on Biologics. Fifth International Workshop on Humanized Mice. Zurich, Switzerland, January 2016.