U.S. FOOD & DRUG ADMINISTRATION

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

ANNUAL REPORT

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Check if this report is Progress or Final Report:

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

Report Overview[1]

Project Title:	**Establishment of A Feasible Method to Quantify Major Glycoforms of Human IgG1 mAb Drugs and their Biosimilars in Culture Media as a Component of Process Analytic Technology**
Investigator:	Tongzhong Ju
Organization:	FDA/CDER/OPQ/ DPQRIII
Grant No. (if applicable)	N/A
Project Objective:	Develop mouse monoclonal antibodies against human non-glycosylated and glycosylated IgG1, and establish a Biolayer Interferometry (BLI) method to quantify the major glycoforms of human IgG1 mAb drugs

Specific Aim(s)	Progress	Outcomes	Communication Timeline
Aim 1: Further characterization of the purified mouse mAbs (mAb-a and mAb-c) using differently glycosylated human IgG1 mAb drugs to determine their affinity to their specific glycoforms of human IgG1 mAb using Biolayer Interferometry (BLI) or SPR	80% 1). Successfully characterized the specificities of mouse monoclonal antibodies (mAbs) generated against glycosylated and non-glycosylated human IgG1. 2). Successfully tested an ELISA format for measuring the affinity of the mAb-a and c with a human IgG1 drug (Humira) and a recombinant non-glycosylated human IgG1.	1). Mouse mAb-a and -c antibodies specifically recognized and bound non-glycosylated and glycosylated human IgG1, respectively. 2). Using an ELISA format, the affinity of mAb-c with Adalimumab (Humira) was determined at 20 nM range, and mAb-a with a non-glycosylated IgG1 was determined at 50 nM range.	Present an abstract and poster at a symposium within FDA. May 1st, 2023—Dec. 31st, 2023 (FY2 3)
Aim 2: Establishment of a BLI method to quantify the major glycoforms of human IgG1 mAb drugs and their *biosimilars in the production media*	Ongoing. 20% Developed an ELISA method to measure the major glycoforms of two human IgG1 drugs. Initial testing with BLI indicated that the orientation binding between mouse mAb-c and human IgG1 appears to be critical.	An ELISA format was able to quantify the major glycoforms of Adalimumab, Herceptin, and a control human IgG1 mAb, consistent with the results from the mass spectrometric (MS) analysis.	Publication in a scientific journal. Jan. 1st, 2024 - Dec. 31st, 2024
Aim 3: *Validation of the technology/method using the IgG1 mAb drugs and available biosimilars*	Not started yet.	N/A	Demonstration of the Technology to the stakeholders within FDA. Jan. 1st, 2025 – Sept. 30th, 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

IgG1 mAb drugs produced from CHO cells often contain three major glycoforms due to heterogenous N-glycosylation at Asn297 residue in their Fc domain: i, afucosylated; ii, fucosylated; and iii, High-mannose. N-glycosylation is a critical quality attribute (CQA) for many IgG1 mAb drugs as it can impact their effector function-related activities, such as ADCC, and PK/PD, and thus needs to be controlled and monitored. A small fraction of IgG1 mAb is not N-glycosylated (non-glycosylated) and consequently lacks effector activity. Mass spectrometry (MS) and (U)HPLC are the current tools for analysis of these glycoforms of the purified mAbs, which is not practical for an in-process analytical characterization such as PAT (process analytical technology) during biosimilar manufacturing process development, production cell line development and advanced manufacturing. To address the unmet analytical need, we developed mouse monoclonal antibodies against human non-glycosylated and glycosylated IgG1, and establish a Biolayer Interferometry (BLI) method to quantify the major glycoforms of human IgG1 mAb drugs. We successfully generated two sets of mouse mAbs (mAb-a and mAb-c) recognizing non-glycosylated and glycosylated human IgG1, respectively. We will use these mouse mAb-a and -c to quantify the major glycoforms of human IgG1 mAb drugs. We summarize the progress in each Aim for the project:

Aim 1: Further characterize the purified mouse mAbs (mAb-a and mAb-c) using differently glycosylated human IgG1 mAb drugs to determine their affinity to their specific glycoforms of human IgG1 mAb using Biolayer Interferometry (BLI) or SPR.

We have successfully characterized the specificities of mouse monoclonal antibodies (mAbs) generated against glycosylated and non-glycosylated human IgG1. 2). We have successfully tested an ELISA format for measuring the affinity of the mAb-a and -c with a human IgG1 drug (Humira) and a recombinant non-glycosylated human IgG1. The affinity measurement of mAb-c and mAb-a to their corresponding peptides and human IgG1 mAbs using BLI and SPR are ongoing.

Aim 2: Establish a BLI method to quantify the major glycoforms of human IgG1 mAb drugs and their biosimilars in the production media.

We developed an ELISA method to measure the major glycoforms of two human IgG1 drugs. Initial testing with BLI indicated that the orientation of binding between mouse mAb-c and human IgG1 appears to be critical for the measurement using BLI format. We are trying other strategies in BLI.

Aim 3: Validate the technology/method using the IgG1 mAb drugs and available biosimilars.

The proposed experiments have not been conducted yet. As mentioned in Aim 2, we will test any unprocessed bulk of human IgG1 drugs and their biosimilars to quantify % glycoforms once the BLI method is established.

Project Objectives:

1. Comprehensively characterize the purified mouse mAbs (mAb-a and mAb-c) in their specificities and determine their affinity to the specific glycoforms of human IgG1 mAb.
2. Establish a BLI method to quantify the major glycoforms of human IgG1 mAb drugs and their biosimilars in the production media.
3. Validate the technology/method using the human IgG1 mAb drugs and available biosimilars.

Research Outcomes

Aim 1

The current research outcomes are: 1). Mouse mAb-a and -c antibodies were shown to specifically recognize and bind non-glycosylated and glycosylated human IgG1 mAb drugs, respectively; 2). Using an ELISA format, the affinity of mAb-c with Adalimumab was determined to be in 20 nM range, and mAb-a with non-glycosylated IgG1 was determined at about 50 nM.

In the previous relevant project, we have successfully generated two different murine monoclonal antibodies by traditional immunization method: mAb-a recognizes non-glycosylated human IgG1 peptide, and mAb-c recognizes both aFucosylated and Fucosylated IgG1 peptides with high binding affinity on ELISA. We have obtained 50mg of purified mAb for each. Next, we investigated the respective binding of mAb-c and mAb-a to human IgG1 drugs (Adalimumab, and Herceptin) and human IgG1 mAb controls which include the non-glycosylated human IgG1, and the aFucosylated human IgG1 mAb, anti-βGal (InvivoGen). By mass spectrometry (MS), we confirmed N-glycans from the human IgG1 drugs. In Western/Lectin blots (WB/LB), and ELISA analyses, mAb-a specifically bound to NG-IgG1 with no cross reactivity to glycosylated IgG1s; and in contrast, mAb-c only bound to glycosylated human IgG1s, but not NG-IgG1. In an ELISA format, we calculated the affinity constant (KD) of mAb-c to Adalimumab to be about 20 nM; and mAb-a with non-glycosylated IgG1 was determined at about 50 nM. We are currently working on the Biolayer Interferometry (BLI) and SPR methods to accurately measure the affinity constant (KD) of mAb-a and mAb-c with their appropriate targets.

Aim 2

The current research outcome is that an ELISA format was established, and it can quantify the major glycoforms of Adalimumab, Herceptin, and a control human IgG1 mAb, the quantity (%) of IgG1 glycoforms was consistent with the results from the mass spectrometric (MS) analysis.

The experiments for this Aim are ongoing. In an ELISA format, we quantified % glycoforms of IgG1 drugs with mAb-c, showing that the results were consistent with that from MS analysis. To establish a BLI method, we firstly biotinylated mAb-c for the immobilization on the BLI streptavidin-biosensor. Biotinylation did not interfere the mAb-c binding to the target glycopeptides and human IgG1 mAbs. We preliminarily tested the binding of biotinylated mAb-c on the BLI sensor with glycopeptides and human IgG1 as analytes in solution, however, a precise resonance unit (RU) was not detected, suggesting that binding orientation may be critical as the BLI has an opposite orientation for mAb-c binding to human IgG1 mAb compared to an ELISA format. We are currently testing the binding kinetics on BLI by switching the orientation (biotinylated peptides and glycopeptides on the BLI biosensor, and mAb-a and mAb-c as analytes in solution, respectively). In the meantime, we are collecting the biosimilars of Herceptin, and Herceptin in cell culture media to test the optimal condition for quantification of their major glycoforms to establish the BLI method.

Aim 3

The proposed experiments have not been conducted yet. As mentioned in Aim 2, we will test any unprocessed bulk of human IgG1 drugs and their biosimilars to quantify % glycoforms once the BLI method is established.

Regulatory Impact

The successful project has both regulatory and scientific impacts.

To industry, the technology will: 1) assist in development of production cell lines for biosimilars by quickly assessing the glycosylation patterns/glycoforms of mAb drugs in the cultivation media; 2) provide the ability to reliably quantify the levels of four major glycoforms of mAbs in unprocessed bulk media or in drug products; 3) function as a component of process analytical technology (PAT) to improve control strategies by optimizing upstream process through identifying critical material attributes (CMA) to produce better mAb drugs with consistent amount of aFucosylated mAb species; and 4) facilitate the development of advance manufacturing for production of mAb drugs with higher quality and better safety.

To Quality Assessors, the ultimate regulatory impact includes: 1) the development of a reliable method will enable the regulatory agency and reviewers from OPQ to make correct quality assessments of mAb drugs, and thus make adequate and effective risk-based decisions; 2) success of the project will facilitate the development of OPQ guidance in advanced manufacturing and quality assessment of the mAb drugs and their biosimilars with regard to glycosylation and glycoforms.

Communication and Dissemination

After validation, the method for profiling the major glycoforms of IgG1 mAb drugs and their biosimilars for in-process testing and characterization will be published in a scientific journal. A workshop to demonstrate the method and data interpretation will be provided to Assessors and other stakeholders.

In FY23, A poster titled "Establishment of A Feasible Method to Quantify Glycoforms of Human IgG1 mAb Drugs in the Production Media as a Component of Process Analytical Technology" was presented at "The 2023 FDA Science Forum: Advancing Regulatory Science Through Innovation" (https://www.fda.gov/science-research/fda-science-forum/establishment-feasible-method-quantify-major-glycoforms-human-igg1-mab-drugs-production-media) on June 13-14, 2023.

Challenges

The major challenges include:

1. The orientation of binding between mouse mAbs and human IgG1 mAb drugs appears to be critical for the interaction of mouse mAbs to human IgG1 Fc. In the ELISA format, the human IgG1 mAbs that were coated or immobilized can be recognized and bound by the mouse mAbs. In the BLI format, we tested the opposite binding orientation, and no specific binding was detected. This causes the delay of the project progress and publication.
2. We also tested several endo-glycosidases for specific cleavage of afucosylated complex N-glycans on human IgG1, thus far, none of them tested was specific.

Next Steps

1. Identify and optimize the condition in which mouse mAb-c can recognize and bind to human IgG1 in the BLI format. The experiments next include: a) denature the human IgG1 first, then measure the binding and affinity constant; b) digest human IgG1 mAbs with IdeZ to generate the Fc regions for testing; c) identify the secondary antibody that recognizes the CH3 or C-terminus of human IgG1 Fc for immobilizing and generating the biosensor.
2. Continue searching for the endo-glycosidases with the specificity for afucosylated complex N-glycans on human IgG1 by collaborating with the experts in the field. Even if the specific enzyme cannot be identified, we can use the amounts of total glycosylated (100%), fucosylated (x %), and high mannose-glycan (y%) to estimate the afucosylated complex N-glycoform (z %) of IgG1 in an IgG1 mAb sample.

Appendix: Abbreviations

Abbreviation	Definition
mAb	Monoclonal antibody
ELISA	Enzyme-linked immunosorbent assay
BLI	Biolayer Interferometry
afucosylated glycans	N-glycans without core fucose or fucosylation
WB/LB blots	Western/Lectin blots
IdeZ	Highly specific protease for IgG cleavage into $F(ab')_2$ and Fc fragments
SPR	Surface plasmon resonance
ADCC	Antibody-dependent cellular cytotoxicity
PK	Pharmacokinetics
PD	Pharmacodynamics