U.S. FOOD & DRUG ADMINISTRATION

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

ANNUAL REPORT

CONTENTS

Check if this report is Progress or Final Report:

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

1. REPORT OVERVIEW[1]

Project Title:	**The bioassay initiative: enhanced biosimilar testing capabilities**
Investigator:	Carole Sourbier
Organization:	CDER/OPQ
Grant No. (if applicable)	
Project Objective:	Enhancing CDER bioassay capabilities to additional attributes to provide definition, standardization, and harmonization of expectations for assessing and reporting CQAs, while reducing inconsistencies and unnecessary testing.

Specific Aim(s)	Progress	Outcomes	Communication Timeline
1. Leverage these existing validated bioassays in studies aimed at assessing and harmonizing our efforts using international standards	A Material Transfer Agreement is in the process of being established between FDA and NISBC to transfer the international standards to OPQ laboratories.	We are expecting that the outcomes of these studies will generate a benchmark across laboratories and agencies for a comparison of biosimilar therapeutics.	Results will be communicated internally and externally by the end of Year 2 and during Year 3.
2. Develop and validate orthogonal assays to monitor and report related critical quality attributes that report the potency of biosimilar therapeutics.	Cell-based orthogonal assays are in the process of being developed and qualified for insulins and monoclonal antibodies products. Stability studies to assess whether our validated assays are stability indicating (heat stressed and photostability) are ongoing.	The development of orthogonal cell-based assays and the stability studies will provide information about the potential use of these assays and serve as resources to Stakeholders to develop bioassays to measure the potency of insulin products and monoclonal antibodies.	Results will be communicated internally and externally by the end of Year 2 and during Year 3.

2. PROGRESS SUMMARY

Project Objectives:

Biosimilars, drugs that are mimetic of previously licensed biological therapeutics, are rapidly becoming a major class of products being submitted to the Agency for review and approval. Our goal is to continue to establish and standardize cell-based biological activity assay capabilities in OPQ to support regulatory research, development, assessment, and harmonization of these biosimilar drug products. Cell-based biological activity assays or 'bioassays' are used to measure and control critical quality attributes central to the potency of regulated products. For this reason, bioassays play important roles in early drug development, comparative analytical assessments between the biosimilar and its innovator product, comparability to support licensure and

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

post-marketing manufacturing changes, and quality surveillance of marketed products. The OPQ Bioassay Initiative includes key collaborations with several OPQ laboratories that leverage their experience with cell-based bioassays and advanced analytics. Enhancing bioassay capabilities to additional attributes directly provides definition, standardization, and harmonization of expectations for assessing and reporting CQAs, while reducing inconsistencies and unnecessary testing.

Aim 1: Leverage existing validated bioassays in studies aimed at assessing and harmonizing our efforts using international standards.

The in-house standardization of bioassays amenable to a Quality Control laboratory environment will help address current gaps in control strategies for insulin and monoclonal antibody products by giving the Agency the ability to both review and conduct unbiased and rapid testing of insulin and monoclonal antibodies. Use of international standards will allow cross-validation of the bioassays that have been validated in CDER laboratories between laboratories and between international regulatory agencies. The outcome of this aim will be the generation of a benchmark across laboratories and agencies for a qualitative comparison of biosimilar therapeutics. The National Institute for Biological Standards and Control (NISBC) has World Health Organization international standards for both human insulin and monoclonal antibodies. An MTA agreement between FDA and NISBC is in the process of being established to allow for the transfer of material.

	Proposed timelines	Status
MTA establishment & transfer of material	Year 1	On going/On time
Use of international standards in validated bioassays (insulin and monoclonal antibodies)	Year 2: Month 1-12	N/A
Communication of results internally and externally	Year 2	N/A

Aim 2: Develop and validate orthogonal assays to monitor and report related critical quality attributes affecting the potency of biosimilar therapeutics.

Insulin Bioassay

A primary cell-based bioassay was validated, and its protocol has been published [Garige et al]. We are now developing an orthogonal assay (Year 1) that we are planning to qualify by the end of Year 2. We are also performing stability studies to assess whether our bioassay is stability indicating (Year 1-2), which would provide some insights on how to best detect changes in CQAs and whether that could lead to changes in biological activity of these products.

The proposed orthogonal assay is a luciferase-based reporter assay, using immortalized liver cells, and is dependent on the glucose-6-phosphatase transcription factor promoter. Glucose-6-phosphatase is involved in gluconeogenesis, which occurs mostly in the liver, and its activity is inhibited by insulin. This assay will provide a direct downstream readout of the biological activity of biosimilar insulin products.

The proposed timelines for the different parts of this aim, and their status are indicated in the table below.

	Proposed timelines	Status
Secondary Assay development	Year 1: Month 1-12	On going/On time
Secondary assay qualification/validation	Year 2: Month 1-12	N/A
Stability studies: stressed samples	Year 1: Month 1-12	On going/On time
Stability studies: photostability	Year 1/Year 2	On going/On time
Communication of results internally and externally	Year 2	N/A

Fc Effector Function Assay:

Having successfully developed and validated a Surface Plasmon Resonance (SPR) assay for the purpose of monitoring Fc-dependent activity of IgG1 mAbs, we are in the process of establishing and qualifying a commercial orthogonal cell-based assay to measure antibody-dependent cellular cytotoxicity (ADCC) in Jurkat cells (Year 1-2). We are also employing our SPR validated protocol to qualify and measure the effects of product quality attributes on Fc binding activity following stresses, as part of stability studies (heat- stress and photostability). These data will inform us whether our SPR assay is stability indicating.

The proposed timelines for the different parts of this aim, and their status are indicated in the table below.

	Proposed timelines	Status
Secondary assay development	Year 1: Month 1-12	On going/On time
Secondary assay qualification/validation	Year 2: Month 1-12	N/A
Stability studies: stressed samples	Year 1: Month 1-12	On going/On time
Stability studies: photostability	Year 1/Year 2	On going/On time
Communication of results internally and externally	Year 2	N/A

3. RESEARCH OUTCOMES

Insulin Bioassay

1. Develop and qualify an orthogonal bioassay

We are in the process of generating a HepG2 knock-in stable cell line expressing a luciferase reporter gene under the transcriptional control of the Glucose-6-phosphatase (G6P) promoter. After transfection, cells were selected and maintained in 1.0 ug/ml puromycin containing growth medium. Selected cells were mono-cloned and identified by genotyping polymerase chain reaction and Sanger sequencing.

2. Analysis of degraded samples to assess the stability-indicating nature of the bioassay.

Two stability studies were designed: a heat-stressed stability study and a photostability study.

For the heat-stressed study, three batches of three different insulin drug products (insulin lispro, aspart and glargine) were stressed and evaluated over 3 months. Control samples were stored at 5°C, while stressed samples were stored at 40 °C for 1-month and 3-month prior to analysis for bioidentity (i.e. bioassay for insulin), potency and impurities (reversed phase-high-performance liquid chromatography, RP-HPLC), and aggregates (size exclusion chromatography, SEC). Using our validated bioassay, all the control and stressed samples presented a stable biological activity with only a trend of decreased activity with the lispro samples at 3 months.

Using RP-HPLC, control samples met US Pharmacopeia specification (95-105%), while the stressed samples were degraded by 3-month, with insulin aspart showing degradation at 1-month. Impurities were found in stressed samples as well (while controls met the USP specifications). SEC analysis showed that all stressed samples had an increased high molecular weight species at 3-month, with marginal increased at 1-month.

For the photostability study, two batches of three insulin drug products (insulin lispro, aspart and glargine) were stressed according to current ICHQ1B Guidelines (UV exposure levels of 200-watt hours/square meter, Visible light exposure levels of 1.2 million lux hours, Temperature 25 ± 2 °C and 65% relative humidity). Similar to the samples generated for the stressed stability study previously described, samples will be analyzed for bioidentity (bioassay), potency and impurities (RP-HPLC), and aggregates (SEC). Samples have been generated and analysis is ongoing.

Fc effector function assay

1. Develop and qualify a cell-based assay

Commercial human CD16-positive Jurkat reporter cells are being used as a surrogate assay for a biological ADCC assay using human Natural Killer cells as effector cells. In this system, expression of Lucia luciferase is controlled by Nuclear factor of activated T-cells (NFAT) promoter. NFAT pathway is activated in Jurkat cells when rituximab is engaged with its target antigen, CD20, and simultaneously binds to Jurkat cells via its Fc domain leading to the increased expression of Luciferase. Thus, the activity of Lucia luciferase reporter can indirectly reflect ADCC activity. This commercial assay is being established and optimized in the laboratory and several lab personnel are currently being trained to perform it.

2. Analysis of degraded samples to assess the stability-indicating nature of the validated SPR assay.

To assess whether our validated SPR assay is stability-indicating, we are in the process of performing stability studies with the following stressor conditions: ICHQ1B light, oxidative stress, heat, and agitation. In this study, rituximab was used as a reference standard and was subjected to the different stressor conditions and later analyzed for by MFI (purity), SEC-HPLC (particle formation, aggregates) and SPR (binding activity). A pilot was conducted to determine optimal running conditions for the endpoint assays. The light stress was set at 25^{o}C, 65% humidity, 1.2 million lux hours fluorescent light and 200-Watt hours/square meter ultraviolet light; using a light control of aluminum foil-wrapped tube exposed to the same conditions. The initial heat condition was done at 40^{o}C for 72 hours, but later expanded to 50^{o}C for one and two weeks. While the initial agitation conditions of rotation at 500rpm for 7 days at 4^{o}C was expanded to include inversion for one week and two weeks. Preliminary data show distinct patterns of change throughout different stressors. Now that experimental conditions have been established, further analyses will be performed and will include stressed and unstressed biosimilars and their reference products.

4. REGULATORY IMPACT

The validation and standardization of specific bioassays to assess biosimilar mAbs and insulins have recently been accomplished by OPQ laboratories and are now available for deployment based on regulatory needs for biotechnology-derived biosimilars. The current proposed studies to harmonize and standardize our bioassays using international reference standards will support consistent regulatory decision-making and evidence-based risk assessment of biosimilar monoclonal antibodies, Fc fusion proteins, and insulin products. In addition, the development and qualification/validation of orthogonal cell-based bioassays will provide added resources to CDER and to our Stakeholders for testing the potency of biosimilar monoclonal antibodies, Fc fusion proteins, and insulin products. Ongoing stability studies will provide information about the stability-indicating nature of these assays and allow an assessment of comparability between the stability profiles with biosimilars.

5. COMMUNICATION AND DISSEMINATION

Data generated will be communicated internally and externally according to the communication schedule shown in the table below:

Insulin Bioassay	Communication timelines	Status
Orthogonal Assay	Technical report (development): Year 2 Technical report (validation): Year 2 Publication (development): submission during Year 2	n/a n/a n/a
Stability studies: stressed samples	Technical reports: Year 1	60% complete
Stability studies: photostability	Technical reports: Year 2	n/a
Overall report stability	Technical report: Year 2	n/a
Overall communication of the insulin bioassay initiative to Stakeholders	Presentation at scientific conferences Year 2 (internal) and Year 3 (external)	n/a

Fc Effector Function Assay	Communication timelines	Status
Orthogonal Assay	Technical report (development): Year 2 Technical report (validation): Year 2 Publication (development): submission during Year 2	n/a n/a n/a
Stability studies (stressed samples and photostability)	Technical report: Year 2	n/a
Overall communication of the Fc effector function initiative to Stakeholders	Presentation at scientific conferences Year 3 (internal/external)	n/a

6. CHALLENGES

Development of a G6P reporter assay:

Challenges have been faced during the development of a monoclonal cell line expressing the G6P luciferase reporter. After a few iterations with no/weak vector expression, a HepG2 knock-in stable cell line has been generated and monoclonal cells are being selected. These challenges are not expected to affect the overall proposed timeline, and even if the development of the assay may be slightly delayed, its qualification should be performed within Year 2 (pending OPQ funds in FY25).

Qualification of the ADCC bioassay:

Major challenges were met using a commercially available ADCC cell-based assay. They include, but are not limited to, variation control between different scientists, different time points, using different passages of Jurkat reporter cell lines and target cells, etc. We are currently working on controlling these variants to ensure repeatability and reproducibility in the assay by (1) training the scientists to gain more experience; (2) testing

different luciferase assay kits from different vendors; (3) purchasing large amounts of same lots of reagents and cell lines. To be able to stay within the proposed timeline (qualification of the secondary assay by the end of Year 2), in case we are unable to qualify a reliable assay using commercial ADCC kits, we are also developing a flow cytometry based ADCC assay using human NK cells. This assay may potentially be used as an orthogonal assay for cell based ADCC activity.

7. NEXT STEPS

The following next steps are expected to be taken by year 2 (pending availability of OPQ funds):

- Internal standards will be used in our validated assays to generate a benchmark across laboratories and agencies for a qualitative comparison of biosimilar therapeutics.
- Once the orthogonal assays are established, they will be qualified/validated and compared to our in-house validated assays.
- Ongoing stability studies will be finalized by the end of year 2.
- Data will be communicated internally (technical reports, internal seminars) and externally (scientific conferences and potential scientific publications - *pending CDER clearance*).

8. APPENDIX: ABBREVIATIONS

ABBREVIATION	DEFINITION
ADCC	Antibody-Dependent Cellular Cytotoxicity
CDER	Center for Drug Evaluation and Research
G6P	Glucose-6-Phosphatase
NFAT	Nuclear Factor of Activated T-Cells
NISBC	National Institute for Biological Standards And Control
OPQ	Office of Pharmaceutical Quality
RP-HPLC	Reversed Phase-High-Performance Liquid Chromatography
SEC	Size Exclusion Chromatography
SPR	Surface Plasmon Resonance