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]

Table 1: High-level overview of the project objective, aim(s) progress, outcomes, and timelines for communication and regulatory impact.

Project Title:	**The bioassay initiative: enhanced biosimilar testing capabilities**
Investigator:	Carole Sourbier
Organization:	CDER/OPQ
Grant No. (if applicable)	N/A
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 existing validated bioassays in studies aimed at assessing and harmonizing our efforts using reference standards	Procurement of reference standards.	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 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 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 during Year 3.

[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:

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 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 human insulin and monoclonal antibodies reference standards will allow cross-validation of the bioassays that have been validated in CDER laboratories across laboratories. The outcome of this aim will be the generation of a benchmark across laboratories for a qualitative comparison of biosimilar therapeutics.

Table 2. Proposed timelines and status of activities.

Activities	Proposed timelines	Status
Procurement of reference standards	Originally Year 1, now Year 2 or 3	On going
Use of reference standards in validated bioassays (insulin and monoclonal antibodies)	Year 3	N/A
Communication of results internally and externally	Year 3	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.

Our 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 provides a direct downstream readout of the biological activity of biosimilar insulin products.

The proposed timelines for the different parts of this aim have been updated in Table 3 below to reflect the status of this project.

Table 3. Proposed timelines and status of insulin bioassay activities.

Activities	Proposed timelines	Status
Secondary Assay development	Year 1	Completed
Secondary assay qualification/validation	Year 2 and Year 3	On going
Stability studies: stressed samples	Year 1	Completed
Stability studies: photostability	Year 1/Year 2/Year 3	On going
Communication of results internally and externally	Year 2 and Year 3	On going

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 1-3). 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, agitation, metal catalyzed oxidation and photostability stress conditions). These data will inform us whether our SPR assay is stability indicating.

The proposed timelines for the different parts of this aim have been updated in Table 4 below to reflect the status of this project.

Table 4. Proposed timeline and status of Fc E effector function assay activities.

Activities	Proposed timelines	Status
Secondary assay development	Year 1 – Year 3	On going
Secondary assay qualification/validation	Year 2 and Year 3	On going
Stability studies: stressed samples	Year 1 – Year 3	On going
Stability studies: photostability	Year 1 – Year 3	On going
Communication of results internally and externally	Year 2 and Year 3	On going

Research Outcomes

Insulin Bioassay:

1. Develop and qualify an orthogonal bioassay

We have generated a HepG2 knock-in stable cell line expressing a luciferase reporter gene under the transcriptional control of the Glucose-6-phosphatase (G6P) promoter. The development and qualification of this assay is on-going.

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 for potency and impurities (RO-HPLC) as well as bioidentity (ICW) was performed. Analysis of aggregates by SEC is currently paused due to lack of reagents.

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 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 SEC (purity), MFI (particle formation), ELISA (Carbonyl content), LC-MS (PTMs) and SPR (binding activity). Light exposure, heat, and metal-catalyzed oxidation (MCO) resulted in a loss of protein purity, as evidenced by decreased main peak area percentage in size-exclusion chromatography (SEC) analysis. These conditions led to the formation of high molecular weight aggregates and/or low molecular weight fragmented species, depending on the specific stress condition. Micro-fluidic Imaging (MFI) analysis revealed that heat exposure caused the largest increase in subvisible particle generation, particularly for particles above 10 and 25 µm in size. Light exposure significantly increased carbonyl content, observed by ELISA results. Additionally, LC-MS/MS analysis demonstrated that light exposure was the main factor causing methionine oxidation at multiple sites. Metal-catalyzed oxidation (MCO) was primarily responsible for non-methionine oxidation. No changes were observed in glycan species and only MCO stress showed a detectable change outside the pre-established-phase 1 parameters for the SPR binding affinity. Further confirmatory testing is underway with multiple biosimilars of rituximab.

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.

Communication and Dissemination

Completed and on-going communication materials that have been created in support of this project are listed in Table 5 below.

Table 5: 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)
Development and validation of an SPR-based bioassay for Fc Receptor binding	Poster	SSC meeting (internal FDA)	N/A
The insulin bioassay initiative: bioassay validation and stability study	Poster	SSC meeting (internal FDA)	N/A
The insulin bioassay initiative: bioassay validation and stability study	Poster	OPQR Poster Day (internal FDA)	N/A
Impact of structure and formulation changes on the function of insulin products	Manuscript (review)	Under review	N/A
Assessment of the potency of therapeutic antibodies: A surface plasmon resonance approach	Manuscript	Manuscript in preparation	N/A

All data generated will be communicated internally and externally according to the proposed communication schedule shown in Table 6 below:

Table 6. Proposed schedule for internal and external communications.

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

Fc Effector Function Assay	Communication timelines	Status
Orthogonal Assay	Technical report (development): Year 3 Technical report (validation): Year 3	N/A N/A
Stability studies on reference standard (stressed samples and photostability)	Technical report: Year 2	90% complete
Confirmatory Stability studies on biosimilars (stressed samples and photostability)	Technical report: Year 3	50% complete
Overall communication of the Fc effector function initiative to Stakeholders	Presentation at scientific conferences Year 3	N/A

Scientific and Technical Challenges

Qualification of the ADCC bioassay: Significant challenges have arisen in the implementation of a commercially available ADCC cell-based assay. These issues include, but are not limited to, controlling variations between different scientists, managing inconsistencies across different time points, and addressing variability when using different passages of Jurkat reporter cell lines and target cells. These technical challenges have complicated the assay's reliability and reproducibility.

Next Steps

We will continue our efforts to develop and qualify orthogonal assays.

References

N/A