Welcome to Bioassays 2016: Scientific Approaches & Regulatory Strategies

On behalf of the Scientific Organizing Committee and CASSS, we are excited to welcome you to Bioassays 2016: Scientific Approaches & Regulatory Strategies and look forward to your participation and input April 4-5, 2016 at the DoubleTree by Hilton Hotel in Silver Spring, Maryland.

The CASSS Bioassays meeting has established itself as a premier conference and unique opportunity for participants and opinion leaders to discuss and debate current regulatory and industry topics regarding bioassays. Bioassays are a critical component of the analytical control strategies for biologics and other complex molecules. The ability of an assay to characterize and demonstrate biological activity is essential and developing such bioassays is becoming more difficult as biologic drugs are engineered to be more complex and/or have multiple modes of action. Companies are continuously challenged with developing assays that are biologically relevant for the analysis of multiple potential mechanisms. Bioassays are also used for lot release, stability, comparability and characterization studies, which requires that the assays be robust and, in most cases, suitable for a QC lab.

Bioassays 2016 is structured to encourage attendee interaction. Each session includes case study presentations followed by a panel discussion allowing for lively dialogue between attendees from academia, industry and regulatory agencies. As in previous years, we expect this format to result in additional focus on the technical and regulatory details of the topic. Regulatory participation from the US FDA, Health Canada and various European agencies has been strong each year. In addition, an exhibitor showcase at the end of day one and a dedicated poster session on day two will give attendees the opportunity to present additional topics and continue the day's discussion in an informal setting.

We would like to thank the speakers and the panel members who are giving generously of their time and resources and to you for your attendance. We would also like to acknowledge the generosity of our program partners for the continued support of the CASSS Bioassays meeting: AbbVie Inc.; Amgen Inc., Biogen, Eli Lilly and Company, MedImmune, A member of the AstraZeneca Group, Novo Nordisk A/S and Pfizer, Inc. We are grateful for the expert management from CASSSS and the audio-visual expertise of Michael Johnstone from MJ Audio-Visual Productions. Their experience and guidance in the preparation of this meeting has been invaluable.

We are sure you will find Bioassays 2016 to be informative and productive, and that it will provide you with current perspectives on bioassays.

Scientific Organizing Committee:

Thomas Arroll, Seattle Genetics, Inc., USA
Evangelos Bakopanos, Health Canada, Canada
Katrin Buss, Federal Institute for Drugs and Medical Devices, BfArM, Germany
Jill Crouse-Zeineddini, Amgen Inc., USA
Camille Dycke, Genentech, a Member of the Roche Group, USA
Chana Fuchs, CDER, FDA, USA
Denise Gavin, CBER, FDA, USA
Stephen Hartman, AbbVie Inc., USA
Xu-Rong Jiang, MedImmune, A member of the AstraZeneca Group, USA
Helena Madden, Biogen, USA
Bruce Meiklejohn, Eli Lilly and Company, USA
Thomas Anders Millward, Novartis Pharma AG, Switzerland
Michael Sadick, Catalent Pharma Solutions, USA
Sally Seaver, Seaver Associates LLC, USA
Max Tejada, Gilead Sciences, USA

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Bioassays 2016: Scientific Approaches & Regulatory Strategies
Scientific Program Summary

Monday, April 4, 2016

07:30 – 17:30  Registration in the Pinnacle Grand Foyer

07:30 – 08:30  Continental Breakfast in the Connection Ballroom

08:30 – 08:45  CASSS Welcome and Introductory Comments in the Pinnacle Grand Ballroom
Anthony Lubiniecki, Janssen R&D, LLC

Bioassays 2016 Welcome and Introductory Comments in the Pinnacle Grand Ballroom
Helena Madden, Biogen

New Bioassay Thinking / Bioassay Thinking for New Modality
Workshop Session One in the Pinnacle Grand Ballroom
Session Chairs: Michael Sadick, Catalent Pharma Solutions and Max Tejada, Gilead Sciences

08:45 – 08:50  Introduction

08:50 – 09:15  Using Biosensor Analysis for Molecule Characterization
Joseph Papalia, Gilead Sciences, Oceanside, CA USA

09:15 – 09:40  Bioassay Development for Complex Molecules- Challenges with Bispecific Molecules
Bhavin Parekh, Eli Lilly and Company, Indianapolis, IN USA

09:40 – 10:05  Evaluation of High Content Imaging Technology and Associated Methods for the Acceleration of Cell-based Assay Development
Heather Anne Brauer, Seattle Genetics, Inc., Bothell, WA USA

10:05 – 10:30  Application of QbD Principles to Lifecycle Management of Bioassays
Wei Zhang, Biogen, Cambridge, MA USA

10:30 – 11:00  Networking Break – Visit the Exhibits and Posters in the Discovery Ballroom

11:00 – 12:15  PANEL DISCUSSION – Questions and Answers
Heather Anne Brauer, Seattle Genetics, Inc., USA
Tsai-lien Lin, CBER, FDA, USA
Joseph Papalia, Gilead Sciences, USA
Bhavin Parekh, Eli Lilly and Company, USA
Wei Zhang, Biogen, USA
Monday, April 4 continued…

12:15 – 13:30  **Hosted Lunch** in the Connection Ballroom

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<td><strong>Introduction</strong></td>
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<td>13:35 – 14:00</td>
<td><strong>Phase-appropriate Bioassay Strategy to Support a Novel Vaccine Product Development</strong></td>
<td>Jenny Wang, <em>Gilead Sciences, Oceanside, CA USA</em></td>
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<td>14:00 – 14:25</td>
<td><strong>Assessing and Controlling Potency of Vector and Drug Product for Chimeric Antigen Receptor T-cells</strong></td>
<td>David Hambly, <em>Kite Pharma, Inc., Santa Monica, CA USA</em></td>
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<td>14:25 - 14:50</td>
<td><strong>Potency Assay Development Strategies for Antibody Drug Conjugates with Multiple Potential Mechanisms of Action</strong></td>
<td>Jyoti Velayudhan, <em>Seattle Genetics, Inc., Bothell, WA USA</em></td>
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<td>14:50 - 15:15</td>
<td><strong>Design and Development of Cell-based Potency Assays for Therapeutic Biologics Targeting T-cell Co-stimulation and Co-inhibition Pathways</strong></td>
<td>Shihua Lin, <em>MedImmune, A member of the AstraZeneca Group, Gaithersburg, MD USA</em></td>
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<td>15:15 - 15:45</td>
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<td>Martin Némec, <em>Health Canada, Canada</em></td>
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<td>17:00 – 17:15</td>
<td><strong>Break</strong></td>
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Monday, April 4 continued…

Exhibitor Partner Scientific Showcase in the Pinnacle Grand Ballroom
Session Chairs: Helena Madden, Biogen and Bruce Meiklejohn, Eli Lilly and Company

17:15 – 17:20  Introduction

17:20 – 17:35  Novel Cell-based Immune Checkpoint and Combination Bioassays Improve Drug Development in Cancer Immunotherapy
Mei Cong, Promega Corporation, Madison, WI USA

17:35 – 17:50  The Future of Science is Sound
Johanna Mylet, Labcyte Inc., Sunnyvale, CA USA

17:50 – 18:05  Evaluation of PathHunter® Assays for Biocomparability Assessment of Exendin-4, Bevacizumab and Cetuximab Biosimilar Candidates
Abhishek Saharia, DiscoverX Corporation, Fremont, CA USA

18:05 – 18:20  Bioassay Method Transfer – A CRO Perspective
Maggie Bach, PPD, Inc., Middleton, WI USA

18:20 – 18:35  Development of Equivalence Margins with PLA 3.0
Ralf Stegmann, Stegmann Systems GmbH, Rodgau, Germany

18:35 – 18:50  Leverage Statistics to Reduce Bioassay Investment and Increase Assurance of Product Quality
Tara Scherder, Arlenda, Flemington, NJ USA

18:50 – 19:00  DISCUSSION – Questions and Answers

19:00 – 21:00  Exhibitor and Poster Reception in the Discovery Ballroom

21:00  Adjourn Day One
Tuesday, April 5, 2016

08:00 – 17:00  **Registration** in the Pinnacle Grand Foyer

07:30 – 08:30  **Continental Breakfast** in the Connection Ballroom

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<td>08:30 – 08:35</td>
<td>Introduction</td>
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| 08:35 – 09:00 | A Reviewer’s Perspective on the Use of Constrained Versus Unconstrained Models to Calculate Relative Potency  
Evangelos Bakopanos, *Health Canada, Ottawa, ON Canada*
| 09:00 – 09:25 | Statistical Surprises When Averaging Across the Rows  
Stan Deming, *Statistical Designs, Houston, TX USA*
| 09:25 – 09:50 | Qualification of a Liquid Handler in the Quality Control Environment and Application to a Commercial Bioassay Method  
Ayly Tucker, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*
| 09:50 – 10:15 | Keep the Tip! Acoustic Droplet Ejection Liquid Handling Improves Potency Assay Precision  
Jill Crouse-Zeineddini, *Amgen Inc., Thousand Oaks, CA USA*
| 10:15 - 10:45 | Networking Break – Visit the Exhibits and Posters in the Discovery Ballroom                  |
| 10:45 – 12:00 | PANEL DISCUSSION – Questions and Answers  
Evangelos Bakopanos, *Health Canada, Canada*
LeeAnn Benson Ingraham, *Bristol-Myers Squibb Company, USA*
Jill Crouse-Zeineddini, *Amgen Inc., USA*
Stan Deming, *Statistical Designs, USA*
Tsai-lien Lin, *CBER, FDA, USA*
Athar Masood, *CDER, FDA, USA*
Ayly Tucker, *Genentech, a Member of the Roche Group, USA*
| 12:00 – 13:15 | Hosted Lunch in the Connection Ballroom                                                      |
| 13:15 – 14:15 | Poster Session in the Discovery Ballroom                                                     |

**Data Analysis and Automation Challenges**  
**Workshop Session Three** in the Pinnacle Grand Ballroom
Session Chairs: Camille Dycke, *Genentech, A member of the Roche Group*, and Xu-Rong Jiang, *AstraZeneca*
Tuesday, April 5 continued…

Potency Assays: Cell-based versus Non Cell-based Formats

*Workshop Session Four* in the Pinnacle Grand Ballroom

Session Chairs: Thomas Anders Millward, *Novartis Pharma AG* and Sally Seaver, *Seaver Associates LLC*

14:15 – 14:20 **Introduction**

14:20 – 14:45 **Case Study: A Phase-driven Approach to the Development and Lifecycle Management of Potency Assays**
Kathleen Shields, *Pfizer, Inc.*, Andover, MA USA

14:45 – 15:10 **Potency Assays for Quality Control of Therapeutic Proteins – Comparison of Cell-based vs. SPR-based Formats**
Jan Hänisch, *Boehringer Ingelheim Pharma GmbH & Co. KG*, Biberach, Germany

15:10 – 15:35 **Strategies Used during Product Development for the Selection of Cell-based versus Binding Potency Assays**
Vineetha Jayasena, *Amgen Inc.*, Thousand Oaks, CA USA

15:35 – 16:00 **Phase-based Potency Assay Development and Implementation**
Marianne Hayes, *Janssen R&D, LLC*, Malvern, PA USA

16:00 – 16:15 **Networking Break** in the Discovery Ballroom

16:15 – 17:30 **PANEL DISCUSSION – Questions and Answers**
Katrin Buss, *Federal Institute for Drugs and Medical Devices (BfArM)*, Germany
Kathleen Clouse, *CDER, FDA*, USA
Jan Hänisch, *Boehringer Ingelheim Pharma GmbH & Co. KG*, Germany
Marianne Hayes, *Janssen R&D, LLC*, USA
Vineetha Jayasena, *Amgen Inc.*, USA
Martin Nemec, *Health Canada*, Canada
Kathleen Shields, *Pfizer, Inc.*, USA

17:30 – 17:45 **Bioassays Workshop Recap**
**Closing Remarks and Invitation to Bioassays 2017**
Bruce Meiklejohn, *Eli Lilly and Company*

17:45 **Adjournment**
Bioassays are critical components of drug discovery, development and analytical control strategies for biologics. The ability of bioassays to reflect the relevant biologic activities is essential; however, developing such assays is increasingly challenging as biologic drugs are engineered with more inherent complexity and/or multiple mechanisms of action. Companies are continuously challenged with developing assays that are both functionally relevant, and which reflect these complex mechanisms of action. Bioassays that support lot release, stability, comparability and extended characterization, have the additional requirements to be robust, precise and, in the case of potency assays, suitable for use in a GMP-compliant environment.

In this session we will discuss bioassay strategies for molecules with novel formats or complex modalities including, but not limited to anti-viral molecules, bispecific molecules including T-cell engagers e.g. BiTE, antibody-drug conjugates, immune-modulators including T-cell immune-therapies e.g. CAR.

NOTES:
**Heather Ann Brauer**  
*Seattle Genetics, Inc.*

Heather Ann Brauer received her PhD from the University of Washington in the molecular and cellular biology program. Her dissertation utilized MALDI-TOF mass spectrometry methods for the detection of blood-based biomarkers associated with diet and the early detection of breast cancer. She did her post-doc at the University of North Carolina Chapel Hill with a focus on the metabolomics and genomics of the breast tumor microenvironment. Here she ran cell-based assays to understand the stroma-driven metabolic phenotypes of breast cancer and correlated these findings with the intrinsic subtypes of these tumors as identified with genomic microarrays. Heather Ann has been a scientist at Seattle Genetics for the past two years where she has implemented new technologies for accelerated bioassay development, as well as developed a number of assays for pipeline programs. She has also facilitated the development of best practices for the potency assay group and served as a representative on CMC teams.

**Tsai-lien Lin**  
*CBER, FDA*

Bio was not available at the time of printing.

**Joseph Papalia**  
*Gilead Sciences*

Dr. Papalia (Joe) received his undergraduate degree in biology from the University of Ottawa in Canada. He pursued biophysical studies for both his masters and PhD degrees at the University of Toronto. Both theses included work using various biophysical techniques including NMR and CD spectroscopy as well as X-ray crystallography. For his post-doctoral work, Joe moved to Salt Lake City, Utah to study with Dr. David Myszka, a pioneer in biosensor studies to follow-up on his main interest, the thermodynamics and kinetics of molecular interactions. During his time with Dr. Myszka, Joe worked on various projects including GPCRs as well as helping to coordinate several benchmark studies with participants from around the world. These studies helped establish various standards in the biosensor field. In time, he was promoted to staff scientist at the University of Utah. Joe was subsequently recruited to the Bay Area by Dr. Scott Klakamp to work at Takeda San Francisco where he added to existing biosensors capabilities at Takeda and additionally, complemented his strength in biosensors with studies using KinExA technology. In time, he was recruited by Gilead Sciences as a senior scientist to fulfill various roles including leading biosensor work in the department of biochemistry and serving on various core teams where he provides both biophysical insight and guidance.

**Bhavin Parekh**  
*Eli Lilly and Company*

Dr. Bhavin Parekh received his PhD in microbiology and molecular genetics from the University of California at Irvine followed by a postdoctoral fellowship in molecular and cell biology from Harvard University. Dr. Parekh has been in the pharmaceutical and biotechnology industry for over 14 years and is currently the bioassay group leader at Eli Lilly and Company. He leads a group of scientists in the development of cell-based bioassays as well as non cell-based assays to support potency testing of Lilly’s bioproduct portfolio.
Wei Zhang

Biogen

Dr. Zhang is currently working at the bioassay group in the analytical development department of Biogen and is responsible for development and qualification of binding and cell-based potency assays as well as impurity assays for process development, product release and stability testing of Biogen’s therapeutic products. His group also manages inventory and production of critical reagents for bioassays. Before he joined Biogen, he had worked at Genzyme and Phylos on assay development for various projects, including gene and cell therapies and protein binder screening. He received his BS from Peking University and PhD from Cornell University. He did his post-doctoral work at the Brigham and Women’s Hospital of Harvard Medical School.
Using Biosensor Analysis for Molecule Characterization

Joseph Papalia

Gilead Sciences, Oceanside, CA USA

Biosensors are now a well-established technology in the study of molecular interactions. As with any other biophysical tool, understanding the limitations to this technology is key to the successful interpretation of biosensor data. The talk will focus on A) An Introduction to SPR: The origin of the SPR response, the fundamentals of global fitting as well as the most commonly used model in fitting, the simple kinetic model, will be discussed. B) Problems Associated with Describing Avidity: Bivalent interactions such as antibody-antigen interactions comprise a large portion of the biosensor literature. However, the immobilization of the wrong binding partner can unnecessarily introduce complications to data; experimental strategies to simplify this will be discussed. C) Describing Mass Transport: Mass transport is a common phenomenon in biosensor data that can affect the uniqueness of solutions derived from fits. The origins of mass transport will be described as well as how mass transport has been modeled quantitatively to help explain the lack of uniqueness in solutions. How mass transport manifests itself with respect to the shape of sensorgrams will also be described. D) Reproducibility of Biosensor Data: Biosensors are commonly used to derive rate and equilibrium constants for molecular interactions. Many of these values appear in publications and technical reports. A commonly asked question about this technology relates to the magnitude of experimental errors that can typically be expected. Some case examples will be discussed to help orient the audience as to the reliability of values of cited.

NOTES:
Bioassay Development for Complex Molecules- Challenges with Bispecific Molecules

Bhavin Parekh

*Eli Lilly and Company, Indianapolis, IN USA*

Biologics are one of the fast growing parts of the pharmaceutical portfolio with many companies focusing on the development of biologics as part of its key innovation strategy. Biologics continue to grow in molecular diversity and complexity and pose unique challenges to the development of potency assays. My talk will focus on challenges encountered with developing bioassay for bispecifics, from understanding the MOA to changing the fundamental rules for the analysis of potency.

NOTES:
Evaluation of High Content Imaging Technologies and Associated Methods for the Acceleration of Cell-based Bioassay Development

Heather Ann Brauer

Seattle Genetics, Inc., Bothell, WA USA

There is growing diversity in therapeutic targets that require robust bioassays for quantification of biological activity. The complexity of characterizing the interplay between cells and these biological compounds can be addressed through new technologies that may accelerate bioassay development while maintaining high standards of assay accuracy and precision. High-content imaging instruments have the ability to elucidate cytotoxic effects and phenotypic changes across cell lines with diverse biologically active reagents in real-time. Cell proliferation assays can monitor proliferation by analyzing confluence with high definition phase-contrast imaging or evaluating cells that express fluorescent constructs. Stable cell lines with promoter driven recombinant GFP/RFP reporter gene expression features can allow for cell proliferation quantification of fluorescence over time. Additional fluorescent labeling reagents can be used to quantify apoptosis and cytotoxicity over time. For cytotoxicity, a probe that enters the cell upon the loss of membrane integrity fluorescently labels the nuclei for cell counting in real-time. For apoptosis, reagents that parallel targets used in qualified assays (e.g. caspase-3/7) can be used to quantify apoptotic cells. High-definition phase contrast images can be used in tandem with all of these methods for further validation through morphological assessment of cell viability. Data analyses can be run over time and at specific points to determine key parameters for continued assay development independent of these instruments. The combination of live imaging technologies and fluorescent constructs will support accelerated development of robust potency assays with sophisticated monitoring of complex molecules for the ever changing landscape of cell-targeted therapies.

Slides were not available at the time of printing.

NOTES:
Application of QbD Principle to Lifecycle Management of Bioassays

Wei Zhang

Biogen, Cambridge, MA USA

The quality-by-design (QbD) principle has been used in pharmaceutical product development. A similar approach can be applied to development and lifecycle management of bioassays. With the QbD approach, an analytical target profile (ATP), which includes requirements for performance of an analytical test, is defined based on objective of the test and quality requirements, instead of based on performance of any specific method. This approach offers several benefits for lifecycle management of analytical methods, including increased method robustness and ruggedness, more flexibility in development, use, transfer and change of analytical methods, and a more efficient regulatory filing strategy. Two case studies, a potency assay and a process-related impurity assay, will be presented to demonstrate the application of the QbD approach to bioassays.

Slides were not available at the time of printing.

NOTES:
PANEL DISCUSSION – Questions and Answers
Heather Anne Brauer, Seattle Genetics, Inc., USA
Tsai-lien Lin, CBER, FDA, USA
Joseph Papalia, Gilead Sciences, USA
Bhavin Parekh, Eli Lilly and Company, USA
Wei Zhang, Biogen, USA

The following questions will guide the panel discussion:

- What are current bioassay development strategies for new or novel antibodies and/or antibody formats? Are binding assays being used successfully as surrogates for very complex assays? While there are examples or bridging assays being used to reflect ADCC or ADCP activities are there examples for other complex bioactivities? This has been a successful strategy for early phase, but what about and later phases? Are there any current examples of bridging, or other surrogate formats, being used commercially?

- What are some bioassay development strategies molecules with complex modalities? Are there newer technologies/formats (non plate-based assay formats, SPR e.g. Biacore, Forte Octet, Essen Incucyte, high content imaging, digital scanning calorimetry) that have been/are being implemented? What are approaches that are being used to facilitate implementation of these technologies/formats in bioassays?

- What are current lifecycle approaches to bioassay strategy?
  - Examples of creative or ‘outside-the-box’ thinking for bioassay development, including the implementation of design-of-experiments as part of bioassay development and/or validation.
  - Drivers to replace existing assays during clinical phases or post-commercialization?
  - Strategies for implementing new technologies or assay formats to replace existing assays during clinical phases, post-commercialization?

NOTES:
Bioassays for Complex Therapeutics
Session Abstract


Many novel therapies are being designed with a diversity of mechanism(s) of action. These typically represent complex modalities, such as multi-target/multi-functional antibodies, and gene or cell-based therapies. These complex modalities pose a variety of new challenges for bioassay development and implementation.

This session will include presentations on an array of different therapeutic modalities: a chimeric antigen receptor therapy, an antibody drug conjugate, a yeast-based vaccine, and a cancer immunotherapeutic antibody. The approaches used to characterize the biological activities of these therapeutics will be discussed, as well as the unique challenges posed during the development of bioassays for atypical mechanism(s) of action. Method qualification/validation, transfer, implementation, and maintenance will also be highlighted. The case studies presented in this session will highlight the practical aspects of developing bioassays for lot release and characterization of these types of therapeutics.

NOTES:
Chana Fuchs
CDER, FDA

Bio was not available at the time of printing.

Denise Gavin
CBER, FDA

Bio was not available at the time of printing.

David Hambly
Kite Pharma, Inc.

David completed his PhD in biochemistry developing mass spectrometric methods to characterize protein structure. He joined Amgen and travelled the US and organization moving from formulation and analytical sciences in R&D to operations attribute sciences over a decade. He was the attribute sciences team leader for the first FDA approved gene therapy, and first oncolytic therapy – Imlygic® and has recently moved to Kite Pharma as the director of analytical development in the product sciences organization developing methods to support CAR-T and engineered TCR cell therapies.

Shihua Lin
MedImmune, A member of the AstraZeneca Group

Shihua Lin received his PhD in neuroscience and cell biology from Kyoto Institute of Technology, and postdoctoral training at Wayne State University School of Medicine. He has over 14 years of experience in pharmaceutical companies specializing in biomarker discovery, drug R&D and translational sciences in neuroscience, vascular biology, immunology and oncology fields. He is a group leader in analytical biotechnology development at MedImmune/AstraZeneca, responsible for bioassay development for early and late stage programs. Before joining MedImmune, he was a scientist at Teva Pharmaceuticals responsible for cell-based assay development and validation. During 2002 to 2011, he was a scientist and senior manager at Otsuka America Pharmaceuticals, where he was responsible for functional gene screening, cell therapy and therapeutic antibody discovery and development.

Martin Nemec
Health Canada

Martin Nemec is a senior biologist/evaluator at the monoclonal antibodies division of the Centre for the Evaluation of Radiopharmaceuticals and Biologics (CERB), Biologics and Genetic Therapies Directorate (BGTD), Health Products and Food Branch (HPFB), Health Canada. He received his BSc (Honours) in biochemistry (biotechnology) from the University of Ottawa, and subsequently a PhD degree in immunology from the University of Glasgow, Scotland. Upon his return to Canada, he worked as a post-doctoral fellow at the Hospital for Sick Children in Toronto. In 2001, he joined the ranks of Health Canada biologists responsible for the evaluation of quality data submitted in support of biological drugs.
Jyoti Velayudhan
Seattle Genetics, Inc.

Jyoti Velayudhan is a principal scientist and the potency assay group leader at Seattle Genetics. She is responsible for the development, qualification, and implementation of potency assays to support characterization and for lot release purposes. Dr. Velayudhan received her PhD degree from the University of Sheffield in the United Kingdom and has had previous industry experience with Amgen and Gilead leading the development of cell-based assays and binding assays to support product and process development activities from early research through late stage clinical and commercial stages of drug development.

Jenny Wang
Gilead Sciences

Jenny Wang is a senior research scientist and group leader in the department of analytical operations at Gilead Sciences. She is responsible for development, qualification, and implementation of biological potency assays to support the process development of biological drugs. Dr. Wang received her PhD degree from Medical College of Virginia with previous industrial experience at Bristol-Myers Squibb, Pfizer and Novartis leading the development and qualification of ligand binding assays, cell-based assays, and flow cytometry assays to support large molecule bioanalysis from preclinical through late stage of the clinical development.
Phase-appropriate Bioassay Strategy to Support a Novel Vaccine Product Development

Jenny Wang

*Gilead Sciences, Oceanside, CA USA*

Bioassays represent an essential part of the control strategy for assessing the safety and potency of the biological drugs to ensure the product quality and consistency. There are many potential challenges in bioassay development, qualification, and implementation. The development of a suitable assay for the potency measurement of novel cell-based vaccine products represents a particular challenge due to the high level product complexity of this class of biological drugs. This presentation will discuss the phase-appropriate potency strategy and the development and implementation of “suitable for intended purpose” bioassays to support the product development of a novel cell-based vaccine product.

NOTES:
Assessing and Controlling Potency of Vector and Drug Product for Chimeric Antigen Receptor T-cells

David Hambly

*Kite Pharma, Inc., Santa Monica, CA USA*

Abstract was not available at the time of printing

NOTES:
Antibody-drug conjugates (ADCs) represent an exciting and rapidly advancing class of targeted therapy for the treatment of cancer. The therapeutic concept of ADCs is to deliver an ADC to a target tumor cell via binding to a cell surface antigen, where the ADC may exert its effects through multiple mechanisms of action, including delivery of a potent cytotoxic drug to the target tumor cell. Among quality attributes that are monitored during ADC development, potency is considered essential as it can provide a direct link to *in vivo* efficacy. The potency assay plays a key role in establishing quality throughout the lifecycle of a drug product. The unique format of an ADC necessitates special considerations in the development of a potency assay strategy for the ADC. This presentation highlights the approaches to consider when developing a potency assay strategy for an ADC with multiple potential mechanisms of action, within the framework of current regulatory expectations. In addition to mechanism of action, factors that need to be taken into consideration in developing a relevant set of biological characterization and release potency assays are discussed.

Slides were not available at the time of printing.

NOTES:
Design and Development of Cell-Based Potency Assays for Biologics Targeting T-cell Co-stimulation and Co-inhibition Pathways

Shihua Lin

*MedImmune, A member of the AstraZeneca Group, Gaithersburg, MD USA*

T cells play a central role in cell-mediated immunity in the body. The specific interactions between MHC, T cell receptor (TCR) and other receptors/ligands on antigen presenting cells and T cells, direct T cell function, determine T cell fate, and regulate T cell anti-tumor response. Cancer immunotherapy that targets T cell co-signaling pathways (co-stimulation, co-inhibition) is now a promising approach for the treatment of cancer and autoimmune diseases. We have developed and optimized improved reporter gene bioassays for assessing the potency of therapeutic biologics targeting T cell co-signaling pathways. This presentation will highlight general consideration on the design and development of mechanism of action reflective potency assays, which are also simple to perform and amenable for use in a regulated environment

NOTES:
PANEL DISCUSSION – Questions and Answers

Chana Fuchs, CDER, FDA, USA
Denise Gavin, CBER, FDA, USA
David Hambly, Kite Pharma, Inc., USA
Shihua Lin, MedImmune, A member of the AstraZeneca Group, USA
Martin Nemec, Health Canada, Canada
Jyoti Velayudhan, Seattle Genetics, Inc., USA
Jenny Wang, Gilead Sciences, USA

The following questions will guide the panel discussion:

- What is the strategy employed for potency specification testing for therapeutics that interact with multiple targets?
- For living therapeutics, like chimeric antigen receptor therapies, what approaches are used to define release of each patient’s cells? What types of bioanalytics are used for characterization of the therapeutic?
- How is potency quantitated for chimeric antigen receptor therapeutics?
- What are the current requirements for evaluation of antibody drug conjugate therapeutics? Are both binding assays and cell-based functional assays required?
- What bioassay challenges do vaccines therapeutics pose compared to other types of modalities?

NOTES:
Exhibitor Partner Scientific Showcase

Session Chairs: Helena Madden, *Biogen* and Bruce Meiklejohn, *Eli Lilly and Company*

We all agree that the measurement of bioactivity of therapeutic drugs is necessary and challenging throughout all phases of a biopharmaceutical life cycle. What about the tools and logistics that allow us to get the job done? This session will highlight some of the forward thinking and scientific creativity needed to develop the tools we use now and the tools we will use in the future. Six exhibitor partners will give short presentations demonstrating unique and practical approaches to the measurement of potency. Advancement of scientific technology and vendor expertise will be emphasized.

NOTES:
Novel Cell-based Immune Checkpoint and Combination Bioassays Improve Drug Development in Cancer Immunotherapy

Mei Cong

*Promega Corporation, Madison, WI USA*

Immune checkpoint receptors (PD-1, CTLA-4) are promising immunotherapy targets to treat cancer by stimulating the immune system. Co-expression of multiple immune receptors on chronically activated T cells suggests that many immune pathways may synergistically modulate antitumor immune responses. Significant effort is now focused on developing combination immunotherapies that target multiple immune receptors. This focus is creating a need for functional cell-based bioassays that reflect the complexity of drug therapies that use multiple mechanisms of action. In this session, we will present development of bioluminescent reporter-based bioassays for immune checkpoint and combination bioassays for cancer immunotherapy drug development and research. Combination bioassays are able to quantitatively measure the synergistic effect of immune checkpoint combinatorial immunotherapies on T cell activation by co-blocking two immune inhibitory receptors. The assays are simple, specific, and robust with appropriate assay precision, accuracy and linearity required for potency testing and stability studies. Similar to immune checkpoint bioassays, which are being validated in immunotherapy, these combination bioassays could serve as valuable tools in antibody screening, characterization, as well as antibody development.

NOTES:
The Future of Science is Sound

Johanna Mylet

Labcyte Inc., Sunnyvale, CA USA

Every experiment performed in life sciences, molecular medicine, drug discovery and diagnostic testing involves liquid handling. Labcyte’s award-winning products use acoustic energy to transfer precise amounts of fluids without touching them. This capability significantly improves data quality and reliability, while eliminating or substantially reducing costs associated with pipette tips, reagents and waste disposal. Equally important, it conserves precious samples and enables highly accurate assay miniaturization, dramatically improving productivity.

Labcyte products offer cutting-edge solutions for:
• Genomics – Synthetic Biology, Next-Gen sequencing, RT-qPCR and genotyping • High throughput and secondary screening • Cell-based and biochemical assays and ADME-Tox • Proteomics – Protein/peptide arrays, biomarkers and protein crystallography

NOTES:
Evaluation of PathHunter® Assays for Biocomparability Assessment of Exendin-4, Bevacizumab and Cetuximab Biosimilar Candidates

Abhishek Saharia

DiscoverX Corporation, Fremont, CA USA

Assessment of the biological activity through relative potency testing with bioassays is a fundamental criterion to demonstrate biological equivalence between biosimilars and originators.

DiscoverX has developed innovative, ready-to-use kits and reporter cell lines for the characterisation of biological activities of various biologics, including biosimilars. SGS Life Sciences evaluated the bioassays for the potency measurement of Exendin-4, Bevacizumab and Cetuximab, using the appropriate marketed drugs. The evaluation involved the determination of the optimum dilution range to establish suitable dose-response curve with the marketed drugs together with investigation of position effect across the plate. This was followed by the determination of intra- and inter-run precision calculated on the relative potency and relative accuracy to assess assay performance. Here, we present data from this evaluation which demonstrates that the PathHunter bioassays for Exendin-4, Bevacuzimab amd Cetuximab are suitable for the biocomparability assessment, as required for the successful development of a biosimilars program.

NOTES:
Bioassay Method Transfer – A CRO Perspective

Maggie Bach¹; Anthony Trinh¹; Roanne Hynes²; Siobhan Flood²; Moira Elmore²; Manuela Grassi¹

¹PPD, Inc., Middleton, WI USA; ²PPD, Inc., Athlone, Ireland

Performance of bioassay methods to support demonstration of product potency in QC laboratories presents unique challenges compared to analytical testing, as a result of cell culture requirements and the extensive training required due to the complexity of each bioassay. Contract research organization (CRO) laboratories face additional challenges as a result of the extensive number and format variability in the methods performed, the variability of requirements across methods between clients and the high level of scrutiny placed on the work from clients and regulatory agencies. Strategies required to overcome these challenges in order to enable successful method transfer include extensive and consistent training requirements, critical reagent management including cell growth tracking, and appropriate protocol design and transfer acceptance criteria. Here we present a case study of a method transfer between US and EU laboratories.

NOTES:
Development of Equivalence Margins with PLA 3.0

Ralf Stegmann

Stegmann Systems GmbH, Rodgau, Germany

The successful development of equivalence margins is a challenging task. This scientific showcase presents the capabilities of PLA 3.0 for the development of equivalence margins from historic assay data. Case study 1 will show the development of 18 different equivalence margins for a non-linear 4 parameter curve fit system. Case study 2 has its focus on the behavior of those equivalence margins with regards to systematic response value effects.

NOTES:
Leverage Statistics to Reduce Bioassay Investment and Increase Assurance of Product Quality

Tara Scherder

Arlenda, Flemington, NJ USA

It is well recognized that the development and ongoing use of a bioassay can present challenges that have significant cost and time consequences. For instance, typical questions such as accuracy and precision, assay format, and method comparison often require substantial planning and resources. And as the complexity and/or cost of the bioassay increase, so does the potential resource investment, and hence the need for optimization.

The cost, timing and risk associated with a bioassay across its lifecycle can be optimized if all possible information is analytically leveraged. In this presentation, several examples of innovative approaches to analysis of bioassay information at different stages in the lifecycle will be presented, including: 1) reduction of samples to show parallelism for calculation of relative potency, 2) improvement of accuracy in bridging of standard lots, 3) reduction of overfill of product to compensate for dilution and measurement error, 4) optimization of assay format. The associated business benefit of each will be discussed.

NOTES:
Session Chairs: Camille Dycke, Genentech, a Member of the Roche Group and Xu-Rong Jiang, AstraZeneca

Parallelism is a prerequisite determined by United States Pharmacopeia (USP) and European Pharmacopeia (EP) for the determination of relative potency in biological assays. However, implementation of equivalence testing for non-linear curves is problematic because there are currently no commercially available software packages available for conducting equivalence testing in a laboratory setting. In addition the use of constrained or unconstrained models can be confusing. This session will cover several aspects of the statistical analysis and design of bioassays.

Efforts are being made by companies and vendors to find innovative ways of bringing automation to bioassays. Whether it is for research purposes, or for a QC environment, implementation of automation to bioassay can be a challenging task. This session will showcase some successes and challenges related to implementation of liquid handling systems for sample preparation and bioassay execution.

NOTES:
Evangelos Bakopanos
*Health Canada*

Dr. Bakopanos obtained his BSc in biochemistry and his PhD in experimental medicine from McGill University in Montreal, Canada. He joined the Biologics and Genetic Therapies Directorate in 2001 as a biologist/evaluator with the biotherapeutics division. In 2004, following the reorganization of the biotherapeutics division into three divisions, he was promoted to his current position of senior biologist/evaluator with the monoclonal antibodies division.

Dr. Bakopanos has over 14 years of experience in reviewing and evaluating chemistry and manufacturing information contained in biological drug submissions such as clinical trial applications, marketing authorization applications and post-market change applications. Furthermore, Dr. Bakopanos is a designated Health Canada inspector and has been the lead inspector on several on-site evaluations.

Since 2004, he has also been the supervisor of CERB’s (Centre for Evaluation of Radiopharmaceuticals and Biotherapeutics) potency laboratory which conducts potency testing of biotherapeutic products during the review of marketing authorization applications.

LeeAnn Benson-Ingraham
*Bristol-Myers Squibb Company*

LeeAnn Benson-Ingraham is a senior research scientist at the Bristol-Myers Squibb Bioassay and Immunoassay Center of Excellence. She joined the biologics department at BMS in 1992 after working at MedImmune for several years. LeeAnn has been a key contributor for Orencia, NuLogix, Ipilimumab, Opdivo and Empliciti cell-based assays. Her group is responsible for cell-based and immuno-assay development and validation, as well as sample analysis in support of the biologics pipeline. LeeAnn has been a driver for the use of automation for bioassays and immunoassays across the BMS network. As a result, automation has been successfully employed in the clinical and commercial settings since 2004.

Jill Crouse-Zeineddini
*Amgen Inc.*

Jill Crouse-Zeineddini received her PhD in biochemistry and molecular biology from the University of Southern California. She has worked at Amgen for over 20 years and is currently the leader of the potency and characterization assay group with responsibilities for the development and transfer of potency assays in support of Amgen’s products.

Stan Deming
*Statistical Designs*

Stanley N. Deming, PhD is president of Statistical Designs, Houston, TX, through which he consults and offers short courses in the areas of experimental design, optimization and the statistical analysis of laboratory data, with emphasis on bioassays. Dr. Deming received his BA degree in chemistry from Carleton College, Northfield, MN, and his MS and PhD degrees in analytical chemistry from Purdue
University, West Lafayette, IN. He has been on the chemistry department faculties of Emory University (1970-1974) and the University of Houston (1974-2001) where he is professor emeritus. Over his academic career he was the major research advisor for 16 PhD students and 19 MS students. Since 1975 he has taught (with Stephen L. Morgan) over 650 short courses.


Tsai- lien Lin  
CBER, FDA

Bio was not available at the time of printing.

Athar Masood  
CDER, FDA

Bio was not available at the time of printing.

Ayly Tucker  
Genentech, a Member of the Roche Group

Ayly received her bachelor degree in genetics from University of California, Davis. She has worked for Genentech for over 24 years. Her first 12 years was in research where she developed cellular and molecular assays. She has spent the next 12 years in quality control, QC, in various roles. In QC, she has developed rapid microbial detection methods using flow cytometry, led the construction of the PCR suits for in-process testing, and managed multiple QC groups. She is currently leading the efforts in developing automated potency assays for QC.
A Reviewer’s Perspective on the Use of Constrained Versus Unconstrained Models to Calculate Relative Potency

Evangelos Bakopanos

Health Canada, Ottawa, ON Canada

Relative potency estimates are based on the assumption that test samples and standard behave similarly in the assay system. This assumption is verified by assessing the similarity or parallelism of the dose response curves. As indicated in USP Chapter <1034> Analysis of Biological Assays, for those test samples in the assay that meet the criterion for similarity to the standard, relative potency estimates are calculated by analyzing the test and standard data together using a model constrained to have exactly parallel lines or curves, or equal intercepts. Nonetheless, a number of recent regulatory submissions have reported relative potency estimates based on calculations using an unconstrained model. This presentation will focus on the use of constrained 4-parameter logistic curve fitting model to calculate relative potency and will compare results generated by constrained and unconstrained methods.

NOTES:
Statistical Surprises When Averaging Across the Rows

Stan Deming

*Statistical Designs, Houston, TX USA*

Abstract was not available at the time of printing.

NOTES:
Qualification of a Liquid Handler in the Quality Control Environment and Application to a Commercial Bioassay Method

Ayly Tucker

*Genentech, a Member of the Roche Group, South San Francisco, CA USA*

Automation technology is frequently used in research and development areas for many years. Applying automation in Quality Control, QC, is very challenging due to GMP environment including the equipment qualification requirements.

Vendors have made more efforts to comply with the GMP requirements which make incorporating automation into the QC laboratory easier.

This presentation will cover benefits, infrastructure and challenge encountered when automating the dilution of a CDC bioassay.

Slides were not available at the time of printing.

**NOTES:**
Liquid handling is an integral part of the execution of potency assays. The product dilution component of assay execution is particularly challenging and can be both labor intensive and time consuming. This is also the step that typically contributes the most to assay imprecision. Different approaches to preparing the individual concentration points on the dose response curve have also attempted to mitigate some of the assay imprecision.

Over the years, advances have been made in liquid handling, from manual pipettes to electronic pipettes to automated liquid handling systems. The Echo® is an automated liquid handling instrument that transfers liquid without the need for any type of tip. The transfer technology employs acoustic droplet ejection based on sound waves to transfer liquid. The sound waves transfer a uniform, fixed amount of liquid to a desired destination. If larger volumes of sample are required to be added, multiple dispenses can be performed within seconds. Because the transfer is tipless, there is no sample cross-contamination and no tips to wash or dispose of.

This talk will outline the application of this technology to the preparation of the product dose response curve dilutions in potency assays and the impact it had on improving overall assay precision.

Slides were not available at the time of printing.

NOTES:
NOTES:
PANEL DISCUSSION – Questions and Answers
Evangelos Bakopanos, *Health Canada, Canada*
LeeAnn Benson Ingraham, *Bristol-Myers Squibb Company, USA*
Jill Crouse-Zeineddini, *Amgen Inc., USA*
Stan Deming, *Statistical Designs, USA*
Tsai-lien Lin, *CBER, FDA, USA*
Athar Masood, *CDER, FDA, USA*
Ayly Tucker, *Genentech, a Member of the Roche Group, USA*

The following questions will guide the panel discussion:

- What is the most appropriate statistical model for bioassay analysis? Linear regressions, 4-P, 5-P curve fitting? How to choose and justify the one chosen for the assay? How wrong can the data be if the “less than ideal” model is chosen?
- If carrying out parallel line analysis, should full curves be compared or straight lines?
- What are the approaches to determine response linearity in a parallel line (non-full curve) assay?
- What bioassay analytical software do people use and why?
- What are the practical challenges and opportunities in bioassay automation?
- Does it always make sense to automate the bioassay (e.g. in QC vs in R&D)?
- What are the drivers, the benefits, and the investment (in time and resource)?
- Should we establish comparability between the manual method and the automated method? i.e is the intent to use either manual or automated in the QC environment? What could be the criterion for such equivalency assessment?

NOTES:
NOTES:
Potency Assays: Cell-based versus Non Cell-based Formats
Session Abstract

Session Chairs: Thomas Anders Millward, Novartis Pharma AG and Sally Seaver, Seaver Associates LLC

Although it is clear that bioassays are required for product release and stability testing of biologics, the applicable regulatory guidelines provide relatively little advice with regard to number and type of bioassay to be employed. For example, 21CFR610.10 states that potency tests “shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency” where potency is “the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.” ICH Q6B lists animal-based biological tests, cell culture-based biological tests, biochemical tests and ligand and receptor binding tests as examples of biological tests. However, neither guideline provides any assistance on how to select the most appropriate bioassay format(s) for a given product.

In this session we wish to discuss the current practices and regulatory expectations regarding the potency test format, with particular emphasis on the question of cell-based versus binding tests for antibodies or other biologics that act as agonists or antagonists of a ligand-receptor interaction.

NOTES:
Katrin Buss  
*Federal Institute for Drugs and Medical Devices (BfArM)*

Bio was not available at the time of printing.

Kathleen Clouse  
*CDER, FDA*

Bio was not available at the time of printing.

Jan Hänisch  
*Boehringer Ingelheim Pharma GmbH & Co. KG*

After finishing my studies of biotechnology with the development of a patented system to immobilize enzymes on lipid bodies, I performed a cell biology-oriented PhD thesis at the Helmholtz Centre for Infection Research in Braunschweig, Germany. In this context, I identified novel protein signaling pathways driving pathogen uptake into gut cells.

After my PhD, I raised grants from the European Commission and the German Research Foundation in order to perform a postdoctoral research project at the Pasteur Institute in Paris, France. During this project, I characterized the functions of potential tumor marker proteins in the migration of primary brain tissue cells. This work also included the establishment and optimization of a range of novel cell-based bioassays in the lab.

In 2015, I started my current position as head of a bioassay lab at Boehringer Ingelheim Pharma in Biberach, Germany. My lab focuses on the development and optimization of cell-based bioassays for release and stability testing of therapeutic antibodies. In addition, I am responsible for coordinating the CMC analytics for individual antibody projects.

Marianne Hayes  
*Janssen R&D, LLC*

Bio was not available at the time of printing.

Vineetha Jayasena  
*Amgen Inc.*

Vineetha received her PhD in biochemistry and molecular biology from Lehigh University. She was a postdoctoral fellow at SRI International and the University of Colorado. Currently, she works as a principal scientist in the potency and characterization assay group at Amgen. She has over 12 years’ experience in potency assay development.
Martin Nemec
Health Canada

Martin Nemec is a senior biologist/evaluator at the monoclonal antibodies division of the Centre for the Evaluation of Radiopharmaceuticals and Biologics (CERB), Biologics and Genetic Therapies Directorate (BGTD), Health Products and Food Branch (HPFB), Health Canada. He received his BSc (Honours) in biochemistry (biotechnology) from the University of Ottawa, and subsequently a PhD degree in immunology from the University of Glasgow, Scotland. Upon his return to Canada, he worked as a post-doctoral fellow at the Hospital for Sick Children in Toronto. In 2001, he joined the ranks of Health Canada biologists responsible for the evaluation of quality data submitted in support of biological drugs.

Kathleen Shields
Pfizer, Inc.

Kathleen Shields is a principal scientist in the bioassay and impurity testing group at Pfizer Inc. in Andover, MA. She has more than 25 years of experience in the pharmaceutical industry and began her career as a discovery research scientist developing functional assays for lead candidate selection covering a range of therapeutic areas including cardiovascular and metabolic disease, as well as inflammation and hemophilia. Her current role within pharmaceutical analytical research and development has focused on the development of mechanistically relevant potency assays for monoclonal antibodies, antibody drug conjugates and hemostatic proteins.
Case Study: A Phase-driven Approach to the Development and Lifecycle Management of Potency Assays

Kathleen Shields

*Pfizer Inc., Andover, MA USA*

Potency assays play an important role in the development of monoclonal antibody therapeutics and are a regulatory requirement for drug product release and stability testing. Assay formats range from relatively simple, non cell-based, ligand binding assays to more complex cell-based assays designed to reflect the in-vivo activity of the drug. Non cell-based potency assay formats are typically employed at early stages in the development process and their design is guided by the primary proposed mechanism of action of the drug. Later in development, clinical data drives the design of a more mechanistically relevant cell-based potency assay. A case study is presented that illustrates the evolution of the potency assay strategy to support the development of a monoclonal antibody with a complex mechanism of action. In this example three potency assays were developed from Phase I to Phase III and include non cell-based and cell-based formats. Emphasis will be paid to the following; factors that guide phase appropriate potency assay selection, evaluation of stability-indicating method capabilities, comparison and bridging of potency assay formats and regulatory expectations that help guide decisions throughout the development process.

NOTES:
Potency Assays for Release and Stability Testing - Cell-based Assays vs SPR-based Formats

Jan Hänisch, Thomas Schwab, Markus Wendeler

Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

Cell-based assays and Surface Plasmon Resonance (SPR)-based methods are routinely used in the pharmaceutical industry to test the quality of biologics. When used as orthogonal methods, they provide comprehensive data on a drug’s potency. However, the need for balancing resources vs. regulatory requirements demands a thought-out strategy for a selective implementation of these methods during drug development and routine testing. It is therefore subject of intense discussions, whether both methods are needed during individual phases of drug development and which of them should be given the preference, when a single one is to be chosen. While both approaches give sound data on a drug’s potency, each option has its own advantages and disadvantages regarding reflection of the mode of action, robustness, costs and throughput.

Here, we compare the general principles and advantages of SPR- vs. cell-based assays with a focus on relative potency determination of biologics. In addition, we will discuss their suitability during different phases including drug development and routine testing. Based on case studies, we will discuss whether the two assay formats generate redundant data or are both required to sufficiently ensure the quality of therapeutic antibodies.

NOTES:
Strategies Used during Product Development for the Selection of Cell-based versus Binding Potency Assays

Vineetha Jayasena

_Amgen Inc., Thousand Oaks, CA USA_

The type of potency assay selected for product lot release and stability testing of biologics depends on many factors. Some of these are the availability of reagents such as the cell line, purified recombinant proteins, development phase and timeline, and knowledge of the drug’s mechanism(s) of action. Whereas all of these factors are important, it is critical that they are crafted into a strategy that ultimately supports method approval. For some therapeutics, a non cell-based receptor-ligand binding potency assay may be acceptable for product lot release and stability testing. A case study that resulted in the approval of a non cell-based receptor-ligand binding assay for release testing on a biologic will be presented.

Slides were not available at the time of printing.

NOTES:
Phase-based Potency Assay Development and Implementation

Marianne Hayes

Janssen R&D, LLC, Malvern, PA USA

Binding assays are generally used for potency assay for antibody therapeutics in early phase development and support rapid progress to FIH with cell-based assay(s) being phased in in later development to support full biological characterization for commercialization. Despite ongoing industry and regulatory forum discussions over the last several years it is still relatively uncommon for a binding assay to be the release assay for commercial products. Extensive biological characterization and knowledge of MOA, degradation pathways and structure function relationships is needed to support the choice of a binding assay as an appropriate potency assay and for where the outcome may not always support the choice of a binding assay. The use of platform assays to support rapid early development and full biological characterization in late development to build the case for the most appropriate potency assay, binding or otherwise and the challenges presented by rapid late development with breakthrough designation will be discussed.

NOTES:
Potency Assays: Cell-based versus Non Cell-based Formats
Workshop Session

PANEL DISCUSSION – Questions and Answers
Katrin Buss, Federal Institute for Drugs and Medical Devices (BfArM), Germany
Kathleen Clouse, CDER, FDA, USA
Jan Hänisch, Boehringer Ingelheim Pharma GmbH & Co. KG, Germany
Marianne Hayes, Janssen R&D, LLC, USA
Vineetha Jayasena, Amgen Inc., USA
Martin Nemec, Health Canada, Canada
Kathleen Shields, Pfizer, Inc., USA

The following questions will guide the panel discussion:

- When during product development do sponsors typically introduce a cell-based test for lot release and stability testing? When do they typically introduce a binding test? When and how do they make the transition from one test format to another?
- While it is not in any regulation, regulatory agencies would like the potency test to be stability indicating. Can any generalizations be made regarding the stability indicating properties of different potency test formats? Are any examples known where a cell-based test was less stability indicating than a binding test or vice versa?
- How do expectations for both binding and cell-based tests differ between development and licensure?
- For products designed solely to inhibit a ligand-receptor interaction, may a binding test alone be sufficient for lot release and stability testing if shown to be stability indicating and the cell based test is not more stability indicating?
- How do sponsors handle the differences in expectations for the two types of tests between regulatory authorities in the US, Europe and other parts of the world? How have these expectations changed over the last few years? In particular, how often are sponsors required to use both cell-based and binding tests when one is not stability indicating? Does it depend on which test is not stability indicating?

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Poster Abstracts

P-101

Potency Assessment of a Complexed Therapeutic via an “Ol Fashion” Proliferation Bioassay

Marla Abodeely; Craig Kaftan; Riddhi Dalvi; Kannappan VeeraRagavan

Shire, Lexington, MA USA

Analytical control strategies are essential to therapeutic drug program development to ensure the safety and efficacy of the patients we serve. The analytical method panel is designed to monitor the key critical quality attributes of the molecule by reproducibly and accurately assessing such qualities as identity, purity, safety and potency. In this poster, we will describe the development of a cell-based potency method to support the development of a late-phase complex protein therapeutic. In the method, dose titrations of the drug are applied to sarcoma cells. The drug via interactions with cell surface-expressed receptors initiates a signal transduction cascade resulting in cell proliferation that is directly proportional to drug concentration. The method was demonstrated to be both specific and stability indicating. The bioassay, which is reflective of the physiological mechanism of action, was implemented to assess potency for release and stability testing as well as to lend support for BLA-enabling, structure function studies.

P-102

Development of Electrochemiluminescence Sandwich Immunoassay for the Detection of Coiled-coil Domain Containing 47 in Human Serum

Liang Zhu; Wenfang Wu

Berg LLC, Framingham, MA USA

Following the “Guidance for Industry Bioanalytical Method Validation” published by USFDA, we have successfully developed an ultra-sensitive sandwich immunoassay for the measurement of a coiled-coil domain containing 47 protein in human serum. The development was performed on the electrochemiluminescence platform from Meso Scale Discovery (MSD-ECL), which was chosen after comparing several major immunoassay platforms in the current market. The development consisted of two major phases: assay optimization and validation.

Assay optimization included antibody pairing; optimization of concentrations for capture antibody, detection antibody and biotinylation of detection antibody; optimization of sample diluents and dilution factor via spike and recovery and dilution linearity tests.

In the assay validation, selectivity of the assay was validated by spike and recovery experiments in 10 individual normal serum samples. Specificity of the assay was investigated in interference study by spiking similar proteins and several common interference substances, although parallelism was not tested due to the lack of study samples. Accuracy and precision of the assay was demonstrated by repeatedly assaying QC samples in five days. In addition, the study for short term sample stability and long term assay stability were also conducted.
Unification of Charge Heterogeneity, Purity and Molecular Weight Analyses of mAbs into a Single Analysis Using CESI-MS

Bryan Fonslow; Olga V. Friese; K. Steven Cook; Robert Swart

1SCIEX, Brea, CA USA; 2Pfizer, Inc., Chesterfield, MO USA

Charge heterogeneity, purity, and molecular weight analyses are powerful CE-based methods for mAb characterization. Combining similar CE separations of intact mAbs with mass spectrometric (MS) detection could allow for unification of these three methods into one. Additionally, the MS detection would facilitate identification of unknown CE peaks and may also provide more accurate and sensitive purity and molecular weight measurements than with optical detection alone. The integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into one process (CESI) provides these possibilities while also lowering the sample mass analysis requirements which can be particularly useful in the mass-limited development phase. We describe the use of a single CESI-MS analysis that provides charge heterogeneity, purity, and molecular weight information. For such, the analyses of representative well-characterized mAb molecules were performed at the intact and reduced levels using both CESI- and CE-based methods. The CESI-MS results are compared to existing industry-accepted CE-based charge heterogeneity, purity, and molecular weight analyses. Notably, charge heterogeneity separations by CESI-MS using a CZE-based separation mechanism showed similar profiles to a cIEF-based method. Collectively, the results demonstrate the advantages of using MS as the detector for a charge heterogeneity analysis since it also provides molecular weight and purity information.

Considerations on Late-stage Potency Assay Strategy for a Bispecific mAb

Alexandre Briguet; Adelheid Rohde; Hermann Beck

F. Hoffmann-La Roche Ltd., Basel, Switzerland

For a new generation bi-specific therapeutic antibody targeting two soluble ligands, a bridging SPR-assay was applied for early clinical phases. To proceed to later clinical phases and BLA, two individual cell-based assays were developed addressing the two functionalities of the molecule independently. Here we present considerations on the bridging from one binding assay addressing two functionalities to two individual cell-based assays. Further we discuss the different control strategy options for late-stage and commercial potency testing.

NOTES:
A Reporter Gene Bioassay for Potency Assessment of a Therapeutic Monoclonal Antibody

Tianmeng Shao¹; Weimin Chen²; Victoria Bushman²; Jing Zhao²; Shihua Lin²

¹MedImmune, A member of the AstraZeneca Group, Frederick, MD USA; ²MedImmune, A member of the AstraZeneca Group, Gaithersburg, MD USA

We developed and optimized a novel reporter gene bioassay for quantifying the bioactivity of a therapeutic monoclonal antibody. The antibody blocks the binding of a ligand on the antigen-presenting cells to a receptor on T cells. The inhibition will sustain T cell activation and T cell immune function by blocking the negative regulatory signals generated by the binding of the ligand to its receptor. A T cell line has been engineered that expressed both the receptor and an IL-2 promoter driven luciferase reporter gene. The T cell activity is highly correlated with activation of the IL-2 transcription factor. The reporter gene bioassay can measure IL-2 activity in T cells that is proportional to T cell activation. The amount of luminescence that is proportional to the T cell activity is quantified in a luminometer after reaction with the Steady-Glo luciferase substrate. Test samples caused a concentration-dependent induction of IL-2 activity in T cells that is measured relative to a reference standard. The current bioassay format is highly robust, simple to perform, and amenable for use in a regulated environment.

Use of Quality by Design Principles in the Optimization of an ELISA Assay used for Dose/Potency Measurements

Silikhone Bouaraphan; Helen Yarovoi; Chris Roselle; Richard Peluso; Mary Shank-Retzlaff; Thorsten Verch

Merck & Co., Inc., West Point, PA USA

Immuonassays are commonly used to measure the dose and potency of subunit vaccines. These assays are critical for monitoring both product consistency and stability and as such, the assays must be robust, accurate and highly precise. We describe the use of Quality by Design (QbD) principles for the development and optimization of an ELISA used to release a polysaccharide conjugate vaccine. The tools used included development of an Analytical Target Profile (ATP), completion of a Cause and Effect (C&E) Matrix, Risk Ranking, and Design of Experiments (DOE). Through the use of these QbD tools, several assay pain points were identified for subsequent improvement, including variable sample preparation, lengthy data analysis, and overall assay complexity. Each of these issues was addressed, resulting in a final assay that is less prone to analyst error, more precise and highly robust.
NOTES:
Bioassays for an Agonistic Bispecific Antibody

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Anti-A/B is an agonistic bispecific antibody that binds both targets simultaneously to activate signaling. The anti-A arm binds with high affinity (KD= low nM) to Target A, and the anti-B arm binds with relatively low affinity (KD= high nM) to Target B. The potency strategy for anti-A/B features two ELISA-based binding assays: Target A binding and Target B binding. Binding to the extracellular domain (ECD) of both targets is saturable; however, binding to Target B (low affinity) by oxidatively stressed material showed hyperpotency that was attributed to aggregates present in the sample. When assessed in the presence of saturating levels of Target A, oxidation does not affect Target B binding. The resulting potency assay for A-bound anti-A/B binding to Target B additionally reflects the drug’s mechanism of action whereby both arms are engaged to activate the A/B signaling complex.

Getting to a Win-Win: Bispecific Antibody Potency Assay Strategy to Test 2 Unique Fabs

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Development of potency assays for bispecific antibodies involves special considerations associated with the unique format and MOAs of these molecules. We describe here the assay selection strategy for a bispecific antibody that weakly binds antigen A while binding to antigen B with much higher affinity. Initially, single-arm binding ELISAs were developed. While both methods detected differences between degraded bispecific and control, the low-affinity binding ELISA was hypersensitive to aggregate. To reduce the risk of aggregate-induced hyperpotency masking loss of binding due to degradation, additional assay formats were assessed. A 2-in-1 “bridging” ELISA (which requires simultaneous binding to both antigens) was found to be insensitive to aggregate hyperpotency when the high affinity antigen was immobilized on the assay plate. This bridging ELISA was therefore selected to support lot release testing, and the single-arm binding ELISAs were selected for characterization of binding to individual antigens.

Use of Design of Experiment (DOE) for Novel Molecule Potency Assay Optimization

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Cell-based potency assays are a critical lot release test for biologics. Relative potency is determined based on individual product characteristics and an assay reflective of the mechanism of action must be
developed for each product. Demonstrating biological activity of novel molecules (non-mAb) is challenging due to limited prior knowledge of novel molecules/formats and often multiple mechanisms of actions to characterize. The traditional ‘one factor at a time’ approach to assay development is not ideal for novel molecules as it can fail to identify the most optimal settings, gives little information on the impact of changing assay parameters and slows assay development impacting on project timelines. A design of experiment (DOE) study is a useful tool to evaluate multiple variables systemically. One can perform DOE at the assay development stage to identify optimal assay conditions allowing for the optimisation of robust fit-for-purpose potency assays. Here we show case studies for a bi-specific peptide and fusion protein molecule. We present data to show how key assay responses such as accuracy and assay window can be predicted within a multidimensional design space covering multiple assay factors at a range of settings with fewer assays. Furthermore we show how this data can be used to generate assays suitability for lot release testing with settings tailored to provide optimal performance for different product formats.

NOTES:
Development and Qualification of Hemolysis Bioassay for Therapeutic Anti-alpha Toxin Antibody Potency Determination

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*Staphylococcus aureus* (*S. aureus*) can cause a range of illnesses from minor skin infections to life-threatening diseases. It is also a common cause of hospital-acquired infections such as pneumonia. These infections are difficult to treat and can be complicated by a high prevalence of methicillin-resistant *S. aureus*. Alpha Toxin (AT) is a major *S. aureus* cytolytic virulence factor which can directly lyse cells leading to loss of epithelial integrity via cell death or degradation of junctional proteins resulting in alveolar edema and reduced lung function. Therapeutic antibodies against AT could provide immunotherapeutic approaches for prevention or treatment of invasive *S. aureus* infections.

MEDI4893 is an extended-half-life, high-affinity, AT-neutralizing monoclonal antibody under development for the prevention of *S. aureus* nosocomial pneumonia in high-risk patients. AT is known to cause hemolysis in erythrocytes. To determine the biologic activity of the antibody, we developed a red blood cell (RBC) hemolysis assay. In principle, anti-AT antibody (MEDI4893) inhibits the AT mediated hemolysis of RBCs, which release hemoglobin that has absorbance readings. The absorbance is inversely proportional to the concentration of antibody and thereby reflects potency of the antibody. In this assay, rabbit RBCs were incubated with anti-AT antibody and AT together. Afterwards, the amount of released hemoglobin was determined by measuring absorbance at 415nm. Qualification data shows that the assay is accurate, precise, robust and specific to the anti-AT antibody. The assay is stability indicating and can be used for lot release of drug substance, drug product and stability testing.

Potency Assay Platform for BiTE® Molecules

Kelli Matthies; Shane Nguyen; Kim Burkhardt; Jill Crouse-Zeineddini

Amgen Inc., Thousand Oaks, CA USA

Bispecific T cell engager (BiTE®) molecules are an exciting modality in Amgen’s therapeutic product portfolio. The canonical BiTE® molecule is derived from 2 single chain variable fragment (scFv) regions, one from an anti CD3 antibody and the other from an antibody to a specific target antigen, connected by a small linker region, thereby generating a bi-specific reagent. We have been able to establish the foundation of a potency assay that can be leveraged as a platform for all BiTE® molecules to date. The assay uses a transformed T cell line, HuT-78, as the effector cell, and target cells expressing the antigen specific for the disease being treated. The target cells have been engineered to express luciferase as a surrogate marker of cell viability, thereby enabling a homogeneous “add and read” assay format that is simple to execute. A case study of the development of the potency assay for blinatumomab (AMG 103) will be presented.
**P-113**

**Improved Cell-Based Assays to Assess Therapeutic Molecules Against Immune Checkpoint Receptors Such As PD-1, PD-L1 and PD-L2**

Sean Deacon; Jennifer Lin-Jones; Hanako Daino-Laizure; Mimi Nguyen; Jason van der Tuig; Abhi Saharia; Jane E. Lamerdin

*DiscoverX Corporation, Fremont, CA USA*

Regulation of immune responses is tightly controlled through a balance of co-stimulatory and inhibitory checkpoint receptors, often exploited by many cancers. Therefore, therapeutics that block inhibitory receptors have proved to be powerful agents to restore anti-tumor immune responses. One key inhibitory checkpoint receptor that is the target of several therapeutic agents in the clinic is programmed cell death 1 (PD-1). PD-1 is expressed on T-cells, while its ligands, PD-L1 or PD-L2, are expressed on the surface of tumor cells or antigen presenting cells. Like many other immunoglobulin receptors, PD-1 harbors immunoreceptor tyrosine inhibitory motifs (ITIMs) in its cytoplasmic tail that are important signaling motifs. When its ligand, e.g. PD-L1, binds to PD-1, Src family kinases phosphorylate the ITIM motif, resulting in the recruitment of SH2-domain containing phosphatases, SHP-1 and SHP-2, which are involved in inhibiting the T-cell response.

Here, we present the PathHunter® PD-1 signaling assay, using our proprietary enzyme fragment complementation (EFC) technology. Jurkat cells expressing the PD-1 and SHP-1 proteins, each fused to a fragment of our EFC system, are co-incubated with ligand-presenting cells. This results in PD-1 activation and SHP-1 recruitment to the PD-1 receptor, bringing together the two EFC fragments and generating a light signal. We demonstrate the suitability of the assay for quantifying pathway activation as well as inhibition of PD-1 signaling by both anti-ligand (anti-PD-L1) and anti-receptor (anti-PD-1) antibodies. The assay is rapid (<5 hours), extremely robust, and has an excellent assay window (>20-fold) with unparalleled sensitivity. In summary, the PathHunter® PD-1 assay provides a valuable tool for both drug screening & characterization assays, with a possible role in lot release testing and stability studies during drug manufacture. This also provides a proof of concept for developing assays for other therapeutically relevant checkpoint receptors, such as TIGIT and CD47.

**P-114**

**Automation of a Commercial Bioassay for Routine Lot Release and Stability Testing**

Jason Mango; Cindy Le; Wei-Meng Zhao; Camille Dycke

*Genentech, a Member of the Roche Group, South San Francisco, CA USA*

ACL TOP Hemostasis Testing System is a fully automated coagulation analyzer offering simplified user interface and standardization of method performance for Commercial Quality. System features include robotic dilution capability and concise data acquisition allowing for a highly resolved lysis curve. ACL TOP facilitated replacement of a manual potency by clot lysis method for routine product release and stability testing. The automated method demonstrates superior performance over the manual method including improved accuracy (increased from 96% to 101%) and precision (reduced from 5% to 2%), at minimum doubling of sample throughput, superior success rate (increased from 85% to near 100%) and a significant reduction in the potential for ergonomic risk.
Development and Optimization of an ADCC Assay for Measuring Critical Quality Attributes of an Anti-TNFα Therapeutic Antibody

Chih Kai Fang1; Satyajeet Haridas1; Dayong Qiu1; Will Bretzlaff2; Drew Shami2; Palanisamy Kanakaraj1; Scott Kuhns2

1Amgen Inc., Cambridge, MA USA; 2Amgen Inc., Thousand Oaks, CA USA

Measuring effector function activities such as antibody mediated cellular cytotoxicity (ADCC) is a critical analytical component in assessing the critical quality attributes of antibody therapeutics. ADCC is also an important functional activity evaluated for biosimilar drug development to demonstrate similarity to the innovator product. ADCC assays are known to be difficult assays to develop and perform due to their inherent variability. Recently, reporter gene methods have been used as a surrogate assay formats to provide an indication of ADCC potential. However, classical ADCC assays that measure target cell cytotoxicity are still considered to be a more relevant method required for product characterization and establishing biosimilarity to the innovator compound. Here we present the development, optimization and qualification of a calcein AM based classical ADCC assay for an anti-TNF antibody therapeutic using engineered NK92 expressing FcγRIIIa as effector cells. Key assay features that we have implemented to improve assay precision and variability will be shown. Final qualification data along with analysis of glycan variance will be presented.

Quantitative Cell-based Bioassays Ease the Development of Therapeutic Drugs Targeting Immune Checkpoint Receptors

Zhi-jie Jey Cheng; Jamison Grailer; Pete Stecha; Jun Wang; Jim Hartnett; Frank Fan; Mei Cong; Gopal Krishnan

Promega Corporation, Madison, WI USA

Immunotherapy harnesses the innate power of a patient’s immune system to fight cancer. Many immune inhibitory receptors and co-stimulatory receptors are promising drug targets for cancer immunotherapy. Current approaches to assay immunotherapy targets rely on primary cells and are highly variable, and therefore are not suitable for quality control environment in drug development. We have developed a panel of cell-based assays using a bioluminescent reporter platform that can quantitatively measure the potencies of the antibodies and ligand proteins targeting the immune checkpoint receptors PD-1, CTLA-4, LAG3, GITR, 4-1BB, OX40 and CD40. We generated multiple cell lines in a T cell background (Jurkat) where each cell line stably expresses an immune checkpoint receptor and a luciferase reporter driven by a response element either specifically responding to signaling mediated by TCR or directly from the immune receptor. The reporter-based bioassays reflect mode of action for each class of drug candidate, and each is able to measure the potency of on-market biologic drugs including PD-1 antibodies, pembrolizumab and nivolumab, and CTLA-4 antibody, ipilimumab. The assay signals are robust and specific, and exhibit good repeatability and linearity. Therefore any of the bioassays are valuable tools for drug screening, development and stability studies in immunotherapy drug development.
Does the Presence of Aggregates Affect FcRn Binding of Fc-conjugated Molecules? Case Study: Comparison of Different Formats of AlphaScreen®- and Biolayer Interferometry-based Assays

Adriana Bajardi-Taccioli; Marina Feschenko; Andrew Blum; Zoran Sosic; Svetlana Bergelson

Biogen, Cambridge, MA USA

Binding to FcRn protects antibodies and Fc-conjugated molecules from catabolism, increasing their half-life, therefore accurate and precise characterization of FcRn binding of Fc-containing biologics is very important. AlphaScreen® technology and Biolayer Interferometry (BLI) on Octet platform are often used to assess protein-protein interactions. AlphaScreen® technology is a very sensitive platform, but the presence of aggregates in the samples may interfere in the assay, rendering incorrect values. The selected assay format could also affect the results for these samples. In this study we show a clear correlation between levels of aggregates present in Fc-fusion protein samples and FcRn binding values measured using two different formats of AlphaScreen® technology. We also show that Biolayer Interferometry technology can be used as an alternative method for the assessment of FcRn binding activity in samples containing aggregates.

Transfer and Validation of a SPR Potency Bioassay

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Eurofins BioPharma Product Testing Munich GmbH (formerly BSL BIOSERVICE), Planegg / Munich, Germany

One requirement for potency assays is that the mechanism of action of a drug has to be mimicked. As this is not always possible one alternative is using binding assays instead of a functional cell based assays or a binding assay in addition to a functional cell based assay both acting as surrogate assays. For this purpose Surface Plasmon Resonance (SPR) has been shown to be an essential method as binding can be detected very sensitively and precisely. An example validation study will be presented demonstrating the accuracy and precision of a typical SPR potency bioassay.

Use of an Internal Standard to Reduce the Impact of Dilution Error in ELISAs

Thy Follmer; Thorsten Verch; Chris Roselle; Mary Shank-Retzlaff

Merck Research Laboratories, West Point, PA USA

Dilution bias is a major factor contributing to immunoassay variability. To reduce the impact of dilution error on the overall assay variability, we demonstrated that a fluorescent dye can be used as an internal control to measure the actual dilution factor and to correct resulting ELISA test results. An ELISA used to measure the dose of a developmental vaccine was used as the model system. Acridine-orange, a fluorescent probe, was added to samples and references at the very first dilution step and then monitored.
throughout serial dilutions. Sample dilution bias was measured by comparing the fluorescent signals from the reference and samples immediately after the serial dilution step. The measured dilution factor was then used to calculate the final ELISA result.

Using samples intentionally diluted to different target concentrations, we show that the dilution factor measured using the acridine data correlates with actual dilution performed and with the final ELISA results. We also demonstrate an improvement in method precision using the dilution correction method.

NOTES:
Bringing the Killer to the Target: ADCC Assay Formats for a mAb and its ADC

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Therapeutic antibodies rely on two types of functionalities to achieve clinical efficacy: target-specific binding by the Fab (antigen-binding fragment) domain and immune-mediated effector functions via interaction of the Fc domain with Fc receptors (FcRs) on various cell types and/or the complement system[1]. Simultaneous binding of Fab and Fc-domains causes antibody-dependent cell-mediated cytotoxicity (ADCC) which is the killing of antibody-coated target cells by effector cells for instance through secretion of cytotoxic granzymes.

With a growing number of antibodies in the pipeline, the need for flexible assay systems evaluating the biologic activity (potency) or binding capacity of these molecules are of increasing value. We envision three different ADCC assay formats for our mAb and its ADC which display either Fc-binding or ADCC activity against tumor cells overexpressing the targeted growth factor in vitro.

First, the ADCC AlphaScreen® (Perkin Elmer) assay is a competitive bead-based proximity assay to detect the binding capacity of the Fc domain. Second, the cell-based functional ADCC bioassay represents a fluorescent assay for quantifying biological activity on cytotoxic granzyme release induction by therapeutic antibodies reflecting the natural immune response. And finally the ADCC reporter bioassay (PROMEGA), which is a bioluminescent reporter assay for quantifying biological activity on pathway activation by therapeutic antibodies.

All three assay formats have been adapted and optimized for our mAb and its ADC. Furthermore, the ADCC AlphaScreen® assay is validated according to ICH guideline for the mAb and feasibility was shown for its ADC. For the ADCC reporter bioassay qualification efforts are ongoing for the mAb and its ADC to support extended characterization studies of the two antibodies as requested by the agencies.

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[1] Advances in the Assessment and Control of the Effector Functions of Therapeutic Antibodies
Xu-Rong Jiang; An Song; Svetlana Bergelson; Thomas Arroll; Bhavin Parekh; Kimberly May; Shan Chung; Robert Strouse; Anthony Mire-Sluis; Mark Schenerman

NOTES:
NOTES:
Scope of Revalidation When Introducing an Adjusted Dilution Series to a Previously Validated Growth Hormone Bioassay

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A growth hormone bioassay previously validated has been revalidated before transfer to QC laboratory. Revalidation was deemed necessary as distribution of the dilution points along the dose-response curve could be optimized. Typically, three to four dilutions points were located at the upper asymptote in the applied four-parameter-logistic (4PL) fit, with only two dilutions points at the hill slope of the dose-response curve. The dose-response curve was fitted from a total of 8 dilutions. The bioassay was subsequently revalidated with an adjusted series of dilutions with four dilution points at the hill slope. To decide the scope of the revalidation we used a two-step approach:

Step 1: Estimates of accuracy and precision from the original validation was recalculated excluding the two highest concentrations of sample from the 4PL fit resulting in a dose-response curve consisting of 6 dilutions. It was determined that excluding the two highest concentrations had no effect on estimates of accuracy and precision of drug product in the bioassay, as all estimates were comparable (<3% relative difference) to estimates generated in the original validation. Likewise, the relative difference for each recalculated potency was <1%.

Step 2: Since no significant effect was observed in the recalculation, it was decided to solely establish formal acceptance criteria for the revalidation of the precision parameters repeatability and intermediate precision, as the introduction of adjusted dilution points resulted in changes to the operational procedure of the bioassay. Revalidation was performed as six independent assays performed by two technicians in the range of 50%-150% of the reference material generating a total of 54 individual determinations used for estimation of repeatability and intermediate precision.

Result and conclusion: Repeatability and intermediate precision was improved from 7.74 %RSD to 3.41 %RSD and from 8.59 %RSD to 6.25 %RSD respectively. Accuracy was evaluated as being comparable to accuracy estimated in the previous validation and evaluation of residuals showed no deviations from the 4PL model. Hence, the growth hormone bioassay is concluded to be improved by introduction of the adjusted dilution series; and continues to be fully validated according to ICH guidelines Q2 (R1).

NOTES:
Exploring Universal Cytotoxicity Bioassays for Antibody Drug Conjugate Development

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Antibody drug conjugates (ADCs) are a novel class of biological therapeutics with promising indications for a wide range of cancers. ADCs are composed of monoclonal antibodies (mAb) conjugated to cytotoxic small molecules – known as “payloads” – via chemical linkers. The mAb component of an ADC is usually engineered to bind specifically to targets that are selectively expressed on cancer cells, with the goal of concentrating the conjugated cytotoxic payload at the cancer site through this mAb-target binding. Once bound to the cancer cells, ADCs are internalized into endosomes and then degraded by acidification, thereby breaking the conjugate chemical linker, releasing the payload into the cytosol, and initiating cell death.

Developing cell-based and/or non-cell based potency bioassays for ADC batch/lot release and for supporting stability studies is challenging, as the bioassays must reflect the multifaceted mechanism of action of ADCs (i.e. mAb binding and payload cytotoxicity). Moreover, the binding characteristics and the mechanisms of action of cytotoxicity can vary greatly across different ADCs, or even vary for the same ADC when different cell-lines are used for the same bioassay. Considering these complications, developing potency bioassays for any given ADC molecule can be a slow and laborious task.

We aimed to find a more ubiquitous cytotoxicity assay that measures the potency of various ADC payloads, enabling expediting of the development of pipeline ADC assays. The advantages and disadvantages of a variety of cytotoxic assay formats and their potential translation to future ADCs will be discussed.

Bioassay Strategy for a Bispecific Antibody

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Bispecific molecules combine antibodies that simultaneously address two different antigens or epitopes. This dual specificity allows design of molecules that can accommodate a mechanism of action (MoA) in one molecule to include interference with multiple signaling pathways in the same cell, mediated by cell surface receptors or ligands associated with diseases. Bispecific antibodies can also mediate activity by bringing two targets into close proximity, either to enhance protein-protein interaction on one cell, or to trigger targeted cell killing induced by immune effector cells such as cytotoxic T-cells as the primary MoA. In the current study, a bioassay strategy is presented for a molecule that utilizes the latter proposed MoA. This strategy employs single target binding assays as well as dual binding assays to support early development and a cell-based co-culture assay that measures T-cell mediated killing of a target cell to support late development for a bispecific antibody. The implications for this strategy are addressed.
Bioluminescent Reporter Bioassays with Lower Variability and Improved Reproducibility for Quantitative Measurement of Fc Effector Functions

Zhi-jie Jey Cheng; Denise Garvin; Rich Moravac; Frank Fan; Mei Cong

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Antibody Fc Effector function such as antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) contribute to clinical efficacy of a broad range of therapeutic antibodies. Binding affinities of the antibody to individual Fc receptors (e.g. FcγRI, FcγRII and FcγRIII) and the subsequent Fc receptor activation is critical for antibody Fc effector function. Here, we report the development of a platform of bioluminescent reporter-based assays to quantify Fc effector function for therapeutic antibodies. We engineered several Jurkat reporter cell lines to stably express an NFAT-RE driven luciferase reporter coupled with each of the Fc receptors. We demonstrate each of the Jurkat reporter cell lines expressing individual Fc receptor and show expected IgG isotype specificity, which aligns with binding affinities of each antibody isotype with each Fc receptor. The FcγRIIIa ADCC reporter bioassays exhibit good correlation in antibody biological activity with a PBMC-based ADCC cytotoxicity assay and with much better assay precision. The bioassays demonstrate performance characteristics for GMP lot release assay with appropriate precision, accuracy, linearity and robustness in assay qualification. The bioassays also can detect potency changes for heat-stressed antibody thereby demonstrating the potential application to stability studies. The reporter-based bioassay is a valuable approach to quantitatively measure Fc effector functions for therapeutic antibodies in drug discovery and development research.

Ready-to-Use Potency Assays for Anti-VEGF Drugs Such as Bevacizumab

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Cell-based bioassays often pose a hurdle during a rapidly moving biologics development program. High standards for assay accuracy, precision, reproducibility and robustness are additionally put to the test by the use of continuous culture cells that can add to variability and increase the cost and complexity of each assay. This is particularly challenging for anti-VEGF drugs, as the prevalent assay is the proliferation of human umbilical vein endothelial cells (HUVECs), which requires 72-96 hours to run, utilizes cells that are difficult to culture and introduces performance variability due to changes in passage number, culture conditions and operator. Here we describe the PathHunter® bioassay that has been developed as a fit-for-purpose QC Lot Release assay for anti-VEGF drugs. The assay quantifies inhibition of VEGF-A-induced VEGFR2 receptor activation by measuring receptor dimerization as an early event in the receptor activation cascade. With its shorter assay time (<24 hours), simple ‘add and read’ protocol and use of cryopreserved ready-to-assay cells, the PathHunter® assay has many advantages over the standard HUVEC assay. Data will be presented comparing the performance and reproducibility of the PathHunter® Bevacizumab bioassay to the standard HUVEC proliferation assay.
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Validation of Methods for the Measurement of Filgrastim and Pegfilgrastim Biopotencies

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As patents begin to expire on popular biologic medications, there is an emerging need for reliable testing capabilities in the biosimilar marketplace. Eurofins Lancaster Laboratories is currently focused on expanding its offerings of validated cell-based biopotency assays that are ready-to-use to test in-process drug substance and drug product biosimilar molecules. Here methods to evaluate the biological activities of Filgrastim and Pegfilgrastim were validated according to ICH guidelines by Eurofins Lancaster Laboratories, Inc. using an NFS-60 cell line. The methods are linear and accurate over the range of 50% to 150% of the nominal potency and demonstrated acceptable specificity, precision and robustness. These methods have been shown to be suitable for their intended applications for the measurement of the biological activity of both Filgrastim and Pegfilgrastim.

P-128

Appropriate Contamination Control and Environmental Monitoring of GMP QC Cell-based (Bio)assay Laboratories

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In vitro cell based potency assays are applied by highly trained scientists to support GMP quality determinations of biotherapeutics. Consistent responses require that cell culture be conducted so as to prevent contamination, and are generally conducted in laboratories designed, controlled, and monitored to conform to international aseptic manufacturing controlled support cleanroom regulations, with flexibility allowed based upon quality risk management of activities performed. PPD operates three such laboratories in the US and Europe with records demonstrating program effectiveness under routine use. Discussed are observations collected in response to recent program changes that impact laboratory efficiencies and have broader implications for GMP QC bioassay laboratories.

NOTES:
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**Using Simulation to Reduce Sample Costs of Parallelism Tests in Bioassays**

Perceval Sondag\(^1,2\); Pierre Lebrun\(^1\); Bruno Boulanger\(^1\); Eric Rozet\(^1\)

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In typical potency bioassays (e.g. ELISA), the biological activity of a product batch is measured relative to the biological activity of a reference product (“Standard”). This quantification is usually made using a single measure: the relative potency (RP).

The RP is estimated from a concentration-response function, usually a four parameter logistic (4PL) curve, and is computed by the horizontal distance between the functions of the reference and that of the test product expressed in log-concentration at inflexion point. Therefore, it is only meaningful if these functions are parallel to each other (Finney (1952)).

Several authors support the use of equivalence testing to prove parallelism. Equivalence tests require equivalence margins or an indifference zone. Within this zone, it can be stated that the curve behaviors are not different. These margins usually need to be derived using experimental data which might not be available during assay development. And obviously, the assay runs needed to create these data are costly. The goal of this work is the computation of these margins without the need of an extensive historical database. Instead, the data from preliminary experiments made during development, combined with simulation, can provide the information needed to define the equivalence margin.

Simulation of reference curves is performed using a Bayesian approach. It is shown through a case-study that equivalence margins derived as proposed are accurate and a useful alternative to performing expensive additional experiments.

P-130

**Optimizing Product Dilution and Assay Format Using a Bayesian Approach – Case Study**

Pierre Lebrun, Tara Scherder, Janik Adriaansen, Mark van Ooij, Bruno Boulanger

Arlenda, Flemington, NJ USA

A drug substance (DS) is diluted with a buffer to obtain the desired titer of the final drug product (DP) before being filled in vials. There is uncertainty in the titration of the DS and of the DP, and also in the physical dilution. To avoid the possibility that the DP does not meet the lower release specification, a “safety margin” is added to the amount of DP calculated for dilution. This deliberate overage has obvious cost consequences. Additionally, a single assay is costly (> $1100), so optimization of the assay format represents significant opportunity for cost reduction.

**NOTES:**
The goal was to assure product quality, while minimizing both product overage in the dilution, and the number of assays. Bayesian modeling was chosen to achieve this goal, because unlike other statistical methods, it permits incorporation of multiple variance components. Consequently, total uncertainty is properly estimated, resulting in less conservative outcomes. Specifically in the case, the total uncertainty in the unknown true amount of drug substance in the vial simultaneously incorporated: 1) error in titer of DS, 2) the dilution of the DS, and 3) titer of the DP. Not only did this modelling reduce the deliberate overage in dilution, it reduced the total number of assays required, without increasing risk to product quality.

NOTES: