Annual Bioassay Conference

Presenters from:

Ablynx
Arlenda SA
Biogen Idec
BioOutsource
Catalent Pharma
Charles River Labs
Covance
Genentech
GSK Marburg
GSK Vaccines
ImmunXperts SA
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Public Health England
Quality Services
Sanofi Pasteur
Statistical Designs
Synthon
Teva Pharmaceuticals

The only constant about bioassays is that they are always changing. Regulatory requirements change, technologies improve and their uses during product development expand. It is critical for your company to know the latest; attend and find it all out.

Main Conference Topics Include:

- ***Preparing bioassay for late stage development and commercial use.*** **H**ints and case studies from Merck and Novartis
- ***Developing bioassays for biosimilar products.*** Case studies from Teva, Covance, BioOutsource and ImmunoXperts
- ***Stability indicating bioassays, how to development, how to use them and what to do with the data.*** Talks from Novartis, Ablynx, Novo Nordisk and Genentech
- ***Selecting the right number of runs and samples to support your purpose.*** Tutorial by Statistical design and Case study by GSK
- ***Dealing with outliers.*** Hints from Arlenda.
- ***Serious look at Bioassay kits-do they work?*** Case study by Covance
- ***Binding Assays as Bioassays.*** Case Studies by Catalent, Biogen-Idec and GSK Marburg
- ***Vaccine Potency Assays.*** PHE, Sanofi-Pasteur and Novatis.

For more information: www.bebpa.org

Annual Bioassay Conference
September 23-25, 2015

Bioassay Workshops September 23, 2015

Workshop 1

Bioassay Basics – Principles and Practice

This workshop provides an introduction to the field of biological assays for potency measurement of biopharmaceuticals and will include overviews of:

9:00: Opening Remarks
Dr. Jane Robinson, Consultant

9:05: Introduction to Bioassays
Dr. Jane Robinson, Consultant

9:35: Principles of Bioassay Design, Development & Validation
Dr. Jane Robinson, Consultant

10:45-11:15: Morning Break

11:15: Basic Tools & Bioassay Formats
Mike Merges, Catalent Pharma

12:00-1:30: Lunch

1:30: Reference Standards
Dr. Jane Robinson, Consultant

2:00: Cells & Regulatory Expectations
Mike Merges, Catalent Pharma

3:00: Case Study
Sian Estadale, Covance

3:30-4:00: Afternoon break

4:00: Transfer & Outsourcing
Mike Merges, Catalent Pharma

4:30: Case Study
Sian Estadale, Covance

5:00: Discussion

5:30: Conference Adjourns

Join Us for a Hosted Networking Reception Afterwards

Workshop 2

The Practical Aspects of Establishing a Biological Assay Monitoring Program

It's a commonplace to say that bioassays are expected to be robust ... robust in the sense of always performing as advertised, performing consistently, repeatably, reproducibly -- unaffected by changes in humidity, temperature, pH, identity of assayist, equipment manufacturer, plate manufacturer, etc. It's also a commonplace to say that bioassays don't always perform as expected, and it's important to know when this is happening so corrective actions can be taken. The purpose of the various "trending charts" is to give the bioassay a "voice" (though we have to listen to it visually) so the bioassay can tell us if it is behaving, or if it's misbehaving.

9:00: Opening Remarks
Dr. Stan Deming, President, Statistical Designs

9:05: Tutorial: Tracking and Trending -- Rockin' or Rollin'?
Dr. Stan Deming, President, Statistical Designs

10:45-11:15: Morning Break

11:15: Take it to the Limit One More Time: The Establishment and Use of Tolerance Intervals.
Mike Sadick, Catalent Pharma

12:00-1:30: Lunch

1:30: Tutorial Continued
Dr. Stan Deming, President, Statistical Design

3:30-4:00 Afternoon break

4:00: Continuous Bioassay Monitoring in QC – Impact of Reagent Quality on Assay Performance, 2 Case Studies
Steffen Pahlich, Lab Head, **Novartis Pharma AG**

4:30: Closing Remarks and Discussion
Dr. Stan Deming, President, Statistical Designs

5:30: Conference Adjourns

Join Us for a Hosted Networking Reception Afterwards

Bioassay Conference Main Day 1: September 24, 2015

9:00: Opening Remarks by Session Chair
Bassam Hallis, Manager, **Public Health England**

Session 1: Making Your Bioassay Ready for Real Life

9:10: Developing Commercial-ready Biopotency Assays: Experiences with the Optimization of Late Stage Potency Assays.
John den Engelsmen, Merck

9:40: A Statistics User's Toolbox for the Lifecycle of a Biological Assay
This talk will describe the application of different statistical tools through the development and validation of a Biological assay from a non-statisticians point-of-view.
Zeban Kolen, Synthon

10:10: The Challenges of Automating a Cell-Based Potency Assay–A Case Study
Marie Gottar-Guiller, Novartis

10:40-11:10 Morning Break

Session 2: The Bioassay, the Critical Method for Developing Biosimilar Product

11:10: The Role of Functional Assays in the Demonstration of Similarity for Biosimilars
Patrick Lui, Teva Pharmaceuticals

11:40: Importance of Understanding Structure-Function Relationships in Assessing Biosimilars
Sian Estdale & Stu Dunn, Covance

12:10-1:30 Lunch

1:30: The Application of Fingerprint-Like Methodologies to Establish ADCC Analytical Similarity during Biosimilar Development
Antibody-Dependent Cell Mediated Cytotoxicity or ADCC is often one of the mechanisms which monoclonal antibodies are effective in treating disease. This mechanism relies on innate constituents of the immune system being activated by the monoclonal drug and activation is a complex stepwise series of events which are slowly becoming more understood. The creation of biosimilars that match the Reference Product's ADCC activity is known to be one of the greatest challenges to developers. Establishing analytical similarity between these molecules at the earliest stages of product development is critical in order to minimise the risk of costly investigations, failure to extrapolate to additional indications or ultimately, failure of licensure as a biosimilar. The approval of the world's first monoclonal antibody, Remsima/Inflectra by the EMA and Health Canada, has provided a critical insight into how ADCC is viewed by regulators. The outcome of these considerations highlights the requirement to apply a wide variety of appropriate, orthogonal methodologies to study ADCC and its constituent components to define both analytical similarity and understand the physiological relevance of any observed differences. This presentation will provide an overview of the methodologies available within the "ADCC Toolbox", comparing the performance of Remicade and Remsima using a range of binding, bridging, functional and bioassay formats to define how we establish analytical similarity in the biosimilar context.
Andy Upsall, BioOutsource

2:00: The Use of In Vitro Cell-Based Bioassays in Immunogenicity Studies for Biotherapeutics and Biosimilars.
Biotherapeutics and biosimilars are a fast growing market providing treatments for a wide range of diseases. Almost all biotherapeutics will to some extend induce an unwanted immune response. The presence of these so called anti-drug antibodies can alter the efficacy and potency of the drug but can also have clinical consequences. Assessing this immunogenic potential early in the development phase can impact the patients' safety and the development costs. In vitro T cell proliferation assays using immune cells from healthy donors are a valuable tool to assess and compare the immunogenic potential of the lead candidates during the development and preclinical phase.
Dr. Séverine Giltaire, CTO, **ImmunXperts SA**

Session 3: Potency Assays are Stability Indicating …Aren't They?

Session Chair: Jane Robinson, Ph. D

2:30: Assessment of Different Bioassay Formats to Detect Stressed Variants of mAbs
An ideal bioassay should mimic the MoA and be able to detect changes in the integrity of the drug. Case studies of two mAbs, specific for membrane and soluble antigens respectively, will be presented. Temperature stressed and post-translationally modified samples were tested in multiple bioassay formats. Results of different bioassays suggested that regardless of antigen localization (membrane vs. soluble) binding bioassays were not sufficiently sensitive to detect stressed variants in these two case studies.
Natko Nuber , Principle Scientist, **Novartis**

Bioassay Conference Main Day 1: September 24, 2015

3:00-3:30: Afternoon Break

3:30: **Justifying the Use of Stability Indicating Surrogate Potency Assays for Nanobody® Development**
Nanobodies represent a novel class of therapeutic proteins based on the smallest functional fragment of heavy chain antibodies naturally occurring in Camelidae. By means of selected case studies, this presentation will cover the development of surrogate potency assays in support of release and stability testing of DS/DP batches for late stage clinical trials. Additionally, the justification of a surrogate potency assay compared to a cell-based bioassay will be discussed.
Philip de Decker, Associate Scientist, **Ablynx NV**

4:00: **Changing Potency Assays – Thoughts and Challenges**
What is required for changing a potency assay during clinical phase 2 studies, including ongoing drug stability programmes? What are the regulatory expectations as well as the scientific expectations - what is needed and how to do it? We asked ourselves these questions, as we were in the process of changing a potency assay from a growth assay to a reporter gene based assay. In this case study, different views and considerations about how to apply a statistical equivalence study will be discussed, including which sample types to address, and whether or not degraded samples should be contained within the study. Other considerations include if the two assays should be run in parallel during stability measurements, as well as timelines and strategy for registration.
Karen Dixen, Novo Nordisk

4:30: **Selection of Binding Assays to Establish Correlation with a Stability-Indicating Bioassay***
A MoA reflective bioassay is stability-indicating for oxidation stressed sample, but not for thermal stressed sample. The health agency requested a separate binding assay on the control system. This presentation aims to discuss 1) selection of the appropriate binding assay format; 2) correlation between the binding assay and bioassay; 3) strategies to influence the health agency that the bioassay is appropriate for measuring the product potency.
Guoying Jiang, Sr. Scientist, **Genentech**

5:00: **Poster Viewing Session**

5:30: Conference Adjourns

Bioassay Conference Main Day 2

9:00: **Opening Remarks by Session Chair, Dr. Hans-Joachim Wallny**

9:10: **Bioactivity Assays beyond lot release and stability testing**
Functional bioassays reflecting the drug's mechanism of action (MoA) are required by authorities when working with anticancer and anti-inflammatory drugs, such as therapeutic antibodies, to ensure efficacy and safety. Within the past years the requirement for bioactivity testing changed from later phase lot release and stability testing to additional applications like biocomparability testing for follow on biologics, accelerated stress condition testing and confirmation of successful production up scaling. Therefore a suitable bioassay is needed at a very early time point. In fact often even more than one assay is required since often drugs like therapeutic antibodies do follow more than one MoA. The assays used need to be reliable, reproducible and precise, which is reflected by the rising acceptance of surrogate approaches whenever primary MoA assays are tedious, time consuming and sometimes highly variable. Case studies for therapeutic antibodies are presented covering the classical immunological MoA assays antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), apoptosis and antibody dependent cellular phagocytosis (ADCP).
Ulrike Herbrand, Charles River Biopharmaceutical Services

Session 4: How Many Samples???

9:40: **A Mini-Tutorial on Determining the Number of "Runs" Required to Get the Necessary Precision Estimation**
Stan Deming, President, **Statistical Designs**

10:10: **Successful assay validation – the role of sample size calculations to ensure regulatory acceptance**
In recent years regulatory authorities have strongly increased demand for (bio-)analytical method validations. Two recurring themes are "variance component analysis" to assess intermediate precision and more generally the preference for equivalence tests over the classical null-hypothesis testing approach, as outlined in USP <1033>. The presentation combines both aspects and presents a pragmatic way of performing power and sample-size calculations for the total variance of general multi-factor mixed models. The approach allows to enter an assay validation with confidence to pass and yet satisfy the stringent requirements of regulatory authorities.
Walter Hoyer, GSK Vaccines

10:40-11:10: Morning Break

11:10: Effect of an Outlier on Quantitative Bioassays

In potency bioassays (e.g. ELISA), the aim is to compare the biological activity of a test product to the biological activity of a reference product. This comparison is usually made using a single measure: the relative potency (RP). In the case of parallel curve assay (PCA), the RP is only meaningful if the log(dose)-response function of the test product is an horizontal shift of the one of the reference. As biological activity is sometimes highly variable, the presence of an outlier in a log(dose)-response curve is a situation that might occur. In this work we present a simulation study that shows the effect of an outlier on the estimated relative potency of the test product. This is done by observing the bias in the estimation that is due to the presence of the outlier. Furthermore, we present its effect on parallelism testing. To that end, we assess the proportion of parallelism rejection due to the presence of an outlier as a function of the "distance" between the outlier and the curve. Finally, we compare several tests for the outlier detection.

Perceval Sondag, Arlenda SA

Session 5: We Have Come a Long Way….Bioassay Assay Kits….do They Work?

11:40: Kit Based Bioassays; Do they Do Exactly What They Say on The Tin?

There are several companies now marketing 'kit based bioassays' as alternatives to the traditional cell based assays. They are marketed as quicker, more reliable and even serum tolerant. We present data using biosimilars including anti-VEGFs, anti-RankL, anti-IL1R and anti-Her2 therapeutics that pit the 'kit based bioassays' against the traditional assays to see if they are worth the extra cost.

Paul Caldwell, Covance

12:10: Novel PD-1 Blockade Bioassay to Assess Therapeutic Antibodies in PD-1 and PD-L1 Immunotherapy Programs

Programmed death receptor-1 (PD-1) and its ligand (PD-L1) are among the few important immunotherapy targets for cancer. Current PD1 assays measure cell proliferation or cytokine production in primary T cells which are tedious, have high assay variation and small assay window. To enable quantitative potency measurement for key anti-PD-1 drugs in the market or in clinical trials such as pembrolizumab and nivolumab, as well as anti-PD-L1 drugs in clinical trials such as MPDL3280A and *BMS*-936559, here we report the development of a robust bioluminescent cell-based PD1 blockade bioassay. For this, we built a PD-1 effector cells in Jurkat cells which stably express human PD-1 and a NFAT-RE-luciferase reporter, and a PD-L1 positive artificial Antigen Presenting Cells (PD-L1$^+$ aAPC) in CHO-K1 cells which stably express PD-L1 and an engineered TCR activator. Once these two cell types were co-cultivated, transcriptional activation of NFAT pathway in PD-1 effector cells, mediated by binding of TCR complex with TCR activator in PD-L1$^+$ aAPC, is significantly suppressed by PD-1/PD-L1 engagement. This inhibition can then be specifically reversed by co-incubation of PD-1 or PD-L1 blocking antibodies in dose-dependent manner, but not by the antibody for other immune checkpoint receptors such as anti-CTLA4 ipilimumab. We further developed both PD1 effector cells and PD-L1$^+$ aAPC in Thaw-and-Use format so the cells can be plated for assay without the need of cell culture. The resultant PD1 assay using Thaw-and-Use cells brings the benefit of convenience, low day-to-day variation, and easy lab-to-lab assay transfer. We demonstrate the assay is able to measure relative potency for antibody biologics, and also can detect potency changes for stressed antibody samples. In summary, the reporter-based PD1 blockade assay provides a valuable tool for both drug screening and characterization in early drug discovery, and lot release and stability study in drug manufacture for therapeutic antibody drug candidates in PD-1 and PD-L1 immunotherapy programs.

Mei Cong, Director, **Promega Corporation**

12:40-2:00: Lunch

Session 6: Binding Assays are Bioassays Too

Session Chaired by: Dr. Bassam Hallis

2:00: Correlation Between Level of Glycan Species in Therapeutic Antibody Samples and FcγRIIIa-Dependent Activity

Glycosylation of Fc domains of antibodies has been shown to modulate their effector functions. Using high mannose afucosylated variants of human therapeutic antibody we demonstrate strong correlation between the level of these variants in the sample and FcγRIIIa-dependent functionality of the antibody. Correlations between several analytical assays are also established.
Marina S. Feschenko, Senior Scientist, **Biogen Idec**

2:30: Assessment of Monoclonal Antibody/Fc Receptor Interactions, using Bio-Layer Interferometry: Establishment of a Toolbox Panel for Characterization of Therapeutic mAb's and mAb Biosimilars
Characterization of originator and/or biosimilar IgG monoclonal antibody (mAb) therapeutic molecules requires, among other orthogonal assessments, measurements of the interaction of the Fc region of the mAb with all of the potential human IgG-Fc-binding cell-surface receptors. This list includes the low affinity $CD16_a$ and $CD16_b$ receptors, responsible for the majority of effector (antibody-dependent cellular cytotoxicity, or ADCC) function associated with mAb therapeutics (chiefly IgG_1). Also included are the low affinity receptors, CD32a and CD32b/c (FcgRIIA and FcgRIIB/C, respectively), as well as the high affinity receptor,CD64 (FcgRI), also responsible for mediating multiple immune responses. Finally, it is important to assess the interaction of the Fc region of IgG mAb's with the 'neonatal' FcRN, which is known to have great impact on the *in vivo* half-life of a therapeutic mAb. To this end, we have successfully developed and established a platform for characterization of IgG/Fc receptor binding at Catalent, Kansas City, utilizing a panel of appropriate Fc receptors and easy-to-use Octet instrumentation. Using Ni-biosensor tips and his-tagged recombinant receptors as the immobilized moieties for this panel, the analyte of interest (the mAb) is in no way derivatized, allowing objective analysis of the receptor panel with any mAb. With minimal additional optimization, the Fc receptor panel, established here, can be quickly adapted for pretty much any originator or biosimilar mAb.
Michael Sadick, Catalent Pharma

3:00: Development and Validation Approach of a Multiplex ELISA for the Evaluation of Vaccines Immunogenicity
Multiplex ELISAs on Luminex® platform are state of the art technologies that can be used to evaluate vaccines immunogenicity. They offer several advantages over conventional ELISA like increased sensitivity, broader working range, higher throughput and the need for lower sample volume. Here we present the development and qualification results of a microspheres based multiplex ELISA that will be used to evaluate immunogenicity of a multivalent vaccine product in clinical trials.
Rachid Marhaba, Head Assay Development, **GSK Marburg**

3:30-4:00: Afternoon Break

Session 7: Vaccine Potency Assays: Not Your Simple Methods

4:00: The Impact of Using Different Strategies for Assigning Value to Reference Serum during ELISA Development
During development of a quantitative bioassay, the value assigned to the reference material has a critical impact on the results reported for test samples. Where the reference material is a purified preparation of the protein of interest, it is usually possible to define what the concentration of the reference material is. Conversely, when a mixture of proteins is used as reference material, for example a serum sample, there is no standard method for defining what the concentration value should be reported as. This talk will discuss some different methods which can be used to define the reference material concentration and will investigate the impact this has on the assay readout.
Kelly Thomas, Public Health England

4:30: Development of a Novel Bioassay to Measure the Efficacy of Serum Antibodies to Cross-Neutralize Clostridium Difficile Toxin Activity from a Large Panel of Clinical Isolates.
Catherine Hessler, Sanofi Pasteur

5:00: From In-vivo Pyrogen Test to In-vitro Monocyte Activation Test: a Case Study Applied to a Vaccine
Barbara Capecchi, Novartis

5:30: Conference Adjourns

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