USP WORKSHOP ON GLYCOSYLATION ANALYSIS FOR BIOPHARMACEUTICALS
August 25-26, 2015
USP Meetings Center, Rockville, MD USA

Final Agenda

DAY ONE: Tuesday, August 25, 2015

8:00 – 8:30 a.m. **Registration**

8:30 – 8:40 a.m. **USP Welcome & Workshop Introduction**
- Tina Morris
  Senior VP, Science Global Biologics, USP

SESSION 1
Opening Keynote Presentation
Introduction: Chris Jones, Ph.D.

8:40 – 9:20 a.m. **The Critical Features of Glycosylation for the Therapeutic Products**
- Pauline M. Rudd, Ph.D.
  National Institute for BioProcessing Research and Training in Ireland (NIBRT), University College, Dublin (Ireland)

SESSION 2
The Role of Glycosylation for Therapeutic Proteins
Moderator: Michael DeFelippis, Ph.D.

9:20 – 9:45 a.m. **The Use of Alternative Methods as Surrogate Measures of Biological Activity**
- Guoying Jiang, Ph.D.
  Genentech

9:45 – 10:10 a.m. **Break**

10:10 – 10:35 a.m. **Characterization and Impact of Glycosylation in an IgG fusion protein**
- Christopher Barton, Ph.D.
  MedImmune

10:35 – 11:00 a.m. **Case Study: Influence of Fab Glycosylation on The Pharmacological Properties of a Monoclonal Antibody**
- Bryan Harmon, Ph.D.
  Eli Lilly and Company

11:00 a.m. – 11:30 a.m.	**Panel Discussion**
11:30 a.m. – 12:30 p.m.	**Lunch & Poster Session**
	SESSION 3 **Current Technologies** Moderator: Trish Li, Ph.D.
12:30 – 12:55 p.m.	**Common Analytical Methods for Oligosaccharide and Monosaccharide Analysis** • Parastoo Azadi, Ph.D. *Member, USP Glycoproteins and Glycan Analysis Expert Panel*
12:55 – 1:20 p.m.	**The Common Challenges Faced Today When Performing Glycosylation Analysis** • Jeffrey S. Rohrer, Ph.D. *Member, USP Glycoproteins and Glycan Analysis Expert Panel*
1:20 – 1:45 p.m.	**Practical Glycoprofiling Approach for Measurement and Control of Glycosylation** • Tapan Das, Ph.D. *Bristol-Myers Squibb*
1:45 – 2:15 p.m.	**Panel discussion**
2:15 – 2:35 p.m.	**Break**
	SESSION 4 **Standardization** Moderator: Chris Jones, Ph.D.
2:35 – 2:55 p.m.	**Standard procedures: USP <1084>, <212> , <210>, and <129>** • Chris Jones, Ph.D. *Chair, USP Glycoproteins and Glycan Analysis Expert Panel*
2:55 – 3:05 p.m.	**USP Reference Standards** • Edith Chang, Ph.D. *USP*
3:05 – 3:30 p.m.	**The NIST mAb: A Reference Material to Supplement Biopharmaceutical Characterization** • John Schiel, Ph.D. *National Institute of Standards and Technology (NIST)*
3:30 – 4:00 p.m.	**Panel discussion**
4:00 – 5:00 p.m.	**Networking Reception & Poster Session**

DAY TWO: Wednesday, August 26, 2015

8:00 – 8:30 a.m.	**Registration**
8:30 – 9:10 a.m.	**SESSION 5** **Plenary Session: Regulatory Expectations** • Christopher Downey, Ph.D. *U.S. Food and Drug Administration (FDA)*
	SESSION 6 **Emerging Technologies** Moderator: Parastoo Azadi, Ph.D.
9:10 – 9:35 a.m.	**In Vitro Glycoengineering – A Useful Tool for Protein Characterization and Optimization** • Marco Thomann *Roche Diagnostics GmbH*
9:35 – 10:00 a.m.	**Bacterial Glycoengineering: from enzymes and pathways to human therapeutics and vaccines** • Matt DeLisa, Ph.D. *Department of Chemical and Biomolecular Engineering, Cornell University*
10:00 – 10:25 a.m.	**Break**
10:25 – 10:50 a.m.	**Relative and Absolute Quantitation of Glycoprotein Glycans Using Isotopically Labeled Standard Glycoproteins** • Ron Orlando, Ph.D. *Complex Carbohydrate Research Center, University of Georgia*
10:50 – 11:15 a.m.	**MAb Glycan Profiling by LC/MS Peptide Mapping** • Bhavana Shah, Ph.D. *Amgen*
11:15 – 11:45 a.m.	**Panel discussion**
11:45 a.m. – 12:45 p.m.	**Lunch & Poster Session**
	SESSION 7 **Regulatory Approaches on Glycosylation** Moderator: John Cipollo, Ph.D.
12:45 – 1:10 p.m.	**What are the Expectations from the Regulatory Agencies** • John Mark, Ph.D. *Health Canada*

1:10 – 1:35 p.m.	**Expectations for Glycosylated Biosimilars (manufacturer perspective)** • Catherine Srebalus Barnes, Ph.D. *Hospira*
1:35 – 2:00 p.m.	**Expectations for Glycosylated Biosimilars (regulatory perspective)** • Birgit Schmauser, Ph.D. *Federal Institute for Drugs and Medical Devices (BfArM) (Germany)*
2:00 – 2:30 p.m.	**Break**
	SESSION 8 **Post-approval changes registration** Moderator: John Schiel, Ph.D.
2:30 – 2:55 p.m.	**Modulation of Glycosylation Patterns by Bioprocess Techniques** • José M. Gomes, Ph.D. *Pfizer*
2:55 – 3:20 p.m.	**Post-approval Changes – Regulatory Perspective** • Maria-Teresa Gutierrez-Lugo, Ph.D. *OBP/CDER/U.S. Food and Drug Administration*
3:20 – 3:50 p.m.	**Panel discussion**
3:50 – 4:10 p.m.	**What Was Said & Next Steps** • Trish Li, Ph.D. *USP*
4:10 – 4:30 p.m.	**Closing Remarks** • Chris Jones, Ph.D. *Chair, USP Glycoproteins and Glycan Analysis Expert Panel*
4:30 p.m.	**Workshop Adjourns**

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Poster Presentations

1. Tomasz Baginski et al. Genetech	End-to-End HILIC UHPLC Glycoprofiling Method for Development, Process Characterization/Validation and Quality Control of Therapeutic Monoclonal Antibodies
2. Kudrat Goswami et al. Merck	Advanced analytical Technologies for N-Glycan Profiling: Assessment of InstantAB™ AssayMap® Automation and Glycoworks™ Rapifluor-MS™
3. András Guttman et al. Sciex Separations	Multi-Site N-Glycan Mapping by Capillary Electrophoresis
4. Akira Harazono National Institute of Health Sciences	New Japanese Pharmacopoeia General Test and General Information for Glycosylation Analysis of Glycoprotein
5. Jenifer L. Hendel et al. Ludger Ltd	A Fluorescent Labeling and Enrichment System for Glycopeptides Generated from Proteolytic Digestion of IgG mAbs; A System That Can Be Used as Part of the Peptide Mapping Workflow
6. Anne Kroll Kristensen et al. Novo Nordisk	Implementation of QbD Principles in Designing a Fast, Robust Method for Profiling of N-linked Carbohydrates in Antibodies
7. Jeremy Kunkel et al. Health Canada	Comparison of Orthogonal Chromatographic and Lectin-Affinity Microarray Methods for Glycan Profiling of a Therapeutic Monoclonal Antibody
8. Matthew A. Lauber et al. Waters Corporation	Wide-Pore Amide HILIC Separations for Assaying the Domain-Specific Glycosylation of mAbs
9. Matthew A. Lauber et al. Waters Corporation	Rapid Preparation of N-Glycans Using a Novel Fluorescence and MS Active Labeling Reagent
10. Leitão, O.L. et al. National Institute for Health Quality	New methods for Oligosaccharide Analysis from Recombinant Human Erythropoietin by UPLC/ESI/QTOF/MS in Brazil
11. Mark Lies et al. Sciex Separations	Rapid Level-3 Characterization of Glycoprotein Therapeutics BY CESI-MS
12. Paula Magnelli et al. New England Biolabs	Improving Sample Workflow: Rapid PNGase F for Accurate Antibody Characterization
13. Loretta Yang et al Glycosensors and Diagnostics	Development of a Glycoprofiling Method Using Multiplex Flow Cytometry
14. Jia Zhao et al. Merck	An effective LC-MS Based Method for On-line Characterization of N-linked Glycans in Biological Therapeutics

USP WORKSHOP ON GLYCOSYLATION ANALYSIS FOR BIOPHARMACEUTICALS

Speaker / Moderator / Planning Committee Information

(alpha order)

Parastoo Azadi, Ph.D.
USP Affiliation:
Member, USP Glycoproteins and Glycan Analysis Expert Panel

Technical Director
University of Georgia
Athens, GA, USA

Dr. Azadi received her B.Sc. in Chemistry in 1987 from University of North London, UK and her Ph.D. degree in biochemistry in 1991 from Imperial College of Science and Technology, University of London, studying structural characterization of carbohydrates and glycoproteins by mass spectrometry under the supervision of Profs. A. Dell and H.R. Morris.

In 1990 through to 1994 she was the senior scientist and the study director at M-Scan limited, an Analytical Mass Spectrometry Consultancy in UK where she was responsible for complete structural characterization of native and recombinant proteins and glycoproteins using mass spectrometry as a service to the pharmaceutical industry.

In 1994 she joined the Complex Carbohydrate Research Center as a postdoctoral fellow and studied the effect of the enzymes endohyrolase and endolyase on rhamnogalacturonan I, and characterization of the fragments produced by these enzymes by ESI-MS and ESIMS/MS. In 1996 she became the Associate Technical Director of plant and microbial Analytical Services at the Complex Carbohydrate Research Center where she was responsible for plant and microbial service program where polysaccharides and lipopolysaccharides were analyzed for other institutes.

Since 2001, Dr. Azadi has been the Technical Director of Analytical Service and Training at the Complex Carbohydrate Research Center. As the Technical Director, the she oversees and manages all analytical services and training conducted at the CCRC, which are supported by three federal resource centers of excellence that CCRC has been awarded: The Department of Energy-funded Center for Plant and Microbial Complex Carbohydrates, the National Institutes of Health Resources Center for Integrated Glycotechnology, and the National Institutes of Health for Biomedical Glycomics. The analytical service program offers two main areas of service: standard analyses and contract analyses. The samples submitted for these types of analyses come from academic, government, non-profit organizations and private companies, throughout the United States and internationally.

Dr. Azadi works closely with the research scientists in industry on developing carbohydrate based drugs and the need for out-sourcing prior to phase I and phase II clinical trials.

Her laboratory also conducts research in areas of structural characterization of plant, bacterial and animal polysaccharides, glycoproteins and glycolipids using MS and NMR techniques.

Planning Committee Member

Moderator
Session 6: Emerging Technologies
Wednesday, August 26, 2015, 9:10 a.m. – 11:45 a.m.

Presentation Abstract
Common Analytical Methods for Oligosaccharide and Monosaccharide Analysis
Tuesday, August 25, 2015, 12:30 p.m. – 12:55 p.m.

Production of high-quality pharmaceutical recombinant therapeutic glycoprotein with consistency in glycan quality is still challenging. Since glycans are responsible for bioactivity, solubility, immunogenicity, and clearance rate from circulation, it is vital to have detailed map of glycans in recombinant therapeutic glycoprotein. However, due to the enormous diversity of carbohydrate structures and their heterogeneity, this still remains one of the bottlenecks of full structural characterization. Detailed glycoprotein structural analysis has to be able to identify the peptide sequence where the glycans are attached, as well as the structure of the glycan portion, including oligosaccharide sequence and glycosyl linkages. We will detail methods for mass spectrometry (MS) experiments on both released glycans ("glycomics"), as well as on intact glycopeptides ("glycoproteomics") using EDT, HCD and CID fragmentation pathways that are needed to fully elucidate the structure of glycoproteins. Additional protocols will be shown where a combination of glycosyl composition and glycosyl linkage analysis using a combination of methylation analysis, MSn and exoglycosidase digestion will provide information on the glycan topology as well as detection methods for potential non-human modifications that could arise from mammalian expression systems such as Galα1-3Gal and N-glycolylneuraminic acid (NeuGc). Our consolidated experiments will outline all the necessary information pertaining to the glycoprotein, including glycan fine structure, attachment site, and glycosylation degree to be obtained pharmaceutical recombinant glycoproteins.

Christopher Barton, Ph.D.
Senior Scientist
MedImmune
Washington, DC, USA

Chris is a Senior Scientist at MedImmune, leading the novel molecules group within analytical biotechnology. He started working on carbohydrate analytics during a PhD on polysaccharides and glycoprotein synthesis at the University of Cambridge. He completed post-doctoral training working in a UK anti-doping laboratory, and developed expertise in mass spectrometry in the contract research sector. His role at MedImmune is focused on the development of analytical approaches for the testing and characterization of non-monoclonal antibody products. His research interests include developing tools for the analysis and understanding of protein glycosylation and post-translational modification in cell culture.

Presentation Abstract
Case Study: Characterization and Impact of Glycosylation on PK of an IgG fusion protein
Tuesday, August 25, 2015, 10:10 a.m. – 10:35 a.m.

Non-monocloncal antibody biotherapeutics often have multiple glycosylation sites occupied with a range of galactosylated and sialylated glycans. The nature and extent of these species can impact the protein's pharmacokinetic and pharmacodynamic profile, and is an important feature to monitor during biotherapeutic development.
We present a case study of the characterization of N-glycosylation in a dimeric IgG fusion protein with two Fc glycosylation sites and four non-Fc sites. The glycosylation of the protein was characterized by a series of tools of increasing detail. The first stage was compositional analysis of the level of component neutral monosaccharides and sialic acid species. Oligosaccharide profiling of enzymatically released glycans was then undertaken to characterize the global glycosylation profile of the protein. Finally analysis by peptide mapping was used to determine the site-specific glycosylation pattern.
The model protein was determined to be decorated with distinct glycans at each of the N-glycosylation sites. The Fc was occupied primarily by agalactosylated bi-antennary glycans. The non-Fc glycans were occupied by a broader range of species, with bi-antennary and tri-antennary cores being observed, decorated with variable levels of galactosylation and sialylation.

We further present an approach to understand the impact of glycosylation on protein function at an early stage in development, by producing samples of protein with specific glycosylation patterns and studying these in *in vitro* and *in vivo* model systems, including a non-human primate model to study the impact of

glycosylation on PK. This information can be combined with the glycosylation analysis tools described above to target process development on a protein with the most desirable glycosylation state.

Edith Chang, Ph.D.
Scientific Liaison, Global Biologics
U.S. Pharmacopeia (USP)
Rockville, MD, USA

Dr. Chang is a Scientific Liaison of Global Biologics with USP. She serves as a lead liaison for USP Proteins Expert Committee. Dr. Chang received her M.S. and Ph.D. in Biochemistry with a minor in Molecular Genetics from University of California, Riverside. Prior to joining USP, she worked most recently at Kansas City University of Medicine and Biosciences, Kansas City, MO, where she was Assistant Professor of Biochemistry and Principle Investigator for research projects to investigate the role of cyclooxygenase 2 in malignant transformation and cancer invasion. Earlier employment was at Thermo Fisher Pierce where she was a Research Scientist at R & D department.

Dr. Chang is the author or co-author of many peer-reviewed publications in the fields of biochemistry, molecular biology and cancer, and has served as an ad-hock reviewer for several peer-reviewed journals including Cancer Research, Molecular Cancer Therapeutics, Clinical Cancer Research, and The International Journal of Biochemistry & Cell Biology.

Planning Committee Member

Presentation Abstract
USP Reference Standards
Tuesday, August 25, 2015, 2:55 p.m. – 3:05 p.m.

USP Reference Standards (RSs) are highly-characterized physical specimens used in testing by pharmaceutical and related industries, and are closely tied with the documentary standards published in the USP–NF, Food Chemicals Codex, and Dietary Supplements Compendium. This presentation will provide an overview of USP reference standards, and discuss the biologics RSs that are associated with compendial procedures for analysis of protein glycosylation.

John Cipollo, Ph.D.
USP Affiliation:
Government Liaison, Vaccines for Human Use – Viral Vaccines

Senior Staff Fellow
U.S. Food and Drug Administration
Bethesda, MD, USA

Dr. Cipollo received his Ph. D. in 2000 from the State University of New York at Albany. He performed his post-doctoral work at Boston University School of Dental Medicine under Professors Catherine Costello in mass spectrometry and Carlos Hirschberg in biochemistry. He has published over thirty scientific articles in the areas of carbohydrate structural analysis and glycomics with strong emphasis in carbohydrate mass spectrometry. Dr. Cipollo has worked extensively in the glycomics of a series of organisms including human, *Caenorhabditis elegans, Entamoeba invadens*, *Entamoeba histolytica*, and several yeast species. His current interests include the function of glycosylation in protein carbohydrate interactions in adaptive and innate immunity and the impact of those functions in vaccine development and performance. Other interests include novel chemistries for improvement of polysaccharide conjugate and other glycoconjugate based vaccines. As there are few broadly accepted informatics platforms for glycomics analysis the Cipollo group has, and is currently developing, in house glycomics software for processing of mass spectrometry and glycan array glycomics data Dr. Cipollo serves as a Product Specialist for CBER FDA primarily as a product reviewer for bacterial polysaccharides and polysaccharide conjugate vaccines.

Planning Committee Member

Moderator
Session 7: Regulatory Approaches on Glycosylation
Wednesday, August 26, 2015, 12:45 p.m. – 2:00 p.m.

Tapan Das, Ph.D.
Director, Biologics Development
Bristol-Myers Squibb
New York, USA

Tapan is a Director in the Analytical Development group of Bristol-Myers Squibb. He leads the Mass Spectrometry and Biophysics Center of Excellence engaged in advanced characterization and analytics for biologics development.

Prior to joining BMS, he was at Pfizer Biotherapeutics R&D and built a world class state-of-the-art facility for Biophysical Center of Excellence.

Tapan is a member of AAPS (Amercian Association of Pharmaceutical Scientists) and served AAPS in various roles, most recently served as the Chair of the Biotechnology Section.

Presentation Abstract
Practical Glycoprofiling Approach for Measurement and Control of Glycosylation)
Tuesday, August 25, 2015, 1:20 p.m. – 1:45 p.m.

Glycan composition is a key quality attribute for many protein therapeutics. Glycans can influence the mode of action, pharmacokinetics, and safety. Robust analytical methods are critical for measurement and control of glycans during process development and for process control in manufacturing. Choice of glycan assay may depend on development phase, needed throughput, and characterization data sought. This presentation will include biotherapeutic case studies for (a) application in process development and (b) for extended characterization.

Michael DeFelippis, PhD
USP Affiliation:
Member, Monographs – Biologics and Biotechnology 1 Expert Committee; Member, Glycoproteins and Glycan Analysis Expert Panel; and Chair, Therapeutic Peptides

Senior Research Fellow
Eli Lilly & Company
Indianapolis, IN, USA

Michael R. DeFelippis, PhD joined the Lilly Research Laboratories of Eli Lilly and Company in 1990 after completing his doctorate in biochemistry. He is currently a Senior Research Fellow working in the Bioproduct Research and Development division. His work is focused on development of protein and peptide biopharmaceutical products with particular emphasis on characterizing physicochemical properties, defining delivery options, developing control strategies, executing technology transfers and preparing data packages to support worldwide regulatory submissions and post-launch registrations. Dr. DeFelippis has published manuscripts, review articles and book chapters on the subjects of protein and peptide structural characterization and formulation design/delivery strategies. He has given numerous presentations on these topics and is a named inventor on several patents related to these areas.

Planning Committee Member

Moderator
Session 2: The Role of Glycosylation for Therapeutic Proteins
Tuesday, August 25, 2015, 9:20 a.m. – 11:30 a.m.

Matt DeLisa, Ph.D.
William L. Lewis Professor of Engineering
Department of Chemical and Biomolecular Engineering, Cornell University
Ithaca, New York, USA

Professor DeLisa received a B.S. in Chemical Engineering from the University of Connecticut in 1996; a Ph.D. in Chemical Engineering from the University of Maryland in 2001; and did postdoctoral work at the University of Texas-Austin, Department of Chemical Engineering. DeLisa joined the Department of Chemical and Biomolecular Engineering at Cornell University as an assistant professor in 2003. He was promoted to associate professor in 2009 and to full professor in 2013. In addition, he recently served as a Gastprofessur at the Swiss Federal Institute of Technology (ETH Zürich) in the Institut für Mikrobiologie.

DeLisa has received several awards for his work including an NSF CAREER award (2005), a NYSTAR Watson Young Investigator award (2004), a Beckman Foundation Young Investigator award (2005), an Office of Naval Research Young Investigator award (2006), a NYSTAR Distinguished Faculty Award (2007), a Cornell Provost's Award for Distinguished Scholarship (2009), and the American Chemical Society BIOT division Young Investigator award (2010). He was also named as one of the top 35 young innovators (TR35) by MIT's Technology Review (2005), was selected as the Allan P. Colburn Memorial Lecturer at the University of Delaware (2009), and was chosen as the inaugural recipient of the Wiley-Blackwell Biotechnology and Bioengineering Daniel I.C. Wang award (2008), which honors a distinguished young researcher in this field. Most recently, he was selected to the IDA/DARPA Defense Science Study Group (2014-15) and elected to the American Institute for Medical and Biological Engineering (2014).

Professor DeLisa's research focuses on understanding and controlling the molecular mechanisms underlying protein biogenesis -- folding and assembly, membrane translocation and post-translational modifications -- in the complex environment of a living cell. His contributions to science and engineering include the invention of numerous commercially important technologies for facilitating the discovery, design and manufacturing of human drugs and seminal discoveries in the areas of cellular protein folding and protein translocation.

Presentation Abstract
Interest in using non-Mammalian cells
Wednesday, August 26, 2015, 9:35 a.m. – 10:00 a.m.

Carbohydrates add a level of diversity across all forms of life that is unparalleled by the information content of nucleic acids and proteins. The lack of a simple template to translate a glycan code into defined sugar structures contributes to this

complexity and impedes efforts aimed at the production of biologically important glycans and glycoconjugates. With the discovery of glycoprotein synthesis in bacteria and functional transfer of glycosylation pathways between species, Escherichia coli cells have become a tractable host for understanding glycosylation and the underlying glycan code of living cells. Moreover, efforts to manipulate the pathways from sugar nucleotides to glycolipids to glycoproteins have transformed E. coli into a living factory for scalable, bottom-up production of complex glycoconjugates by design. Here, I will discuss our efforts to develop E. coli for the biosynthesis of a diverse array of glycan structures, which can be used to tailor the activity, stability, half-life, and immunogenicity of an array of protein targets ranging from biopharmaceuticals to industrial enzymes. I will also discuss our efforts to unify protein glycosylation in E. coli with the advanced tools of protein engineering such as cell surface and phage display technologies. The result is a powerful new way to engineer the enzymes, pathways, end-products, and genomes of glycoengineered bacteria for creating the next generation of protein therapeutics and vaccines.

Chrisopher D. Downey, Ph.D.
Lead Chemist
U.S. Food and Drug Administration (FDA)/CDER Office of Biotechnology Products

Dr. Downey received his PhD in 2006 from the University of Colorado at Boulder, where he studied the conformational dynamics of RNA tertiary structures. From 2007 to 2011, he performed his postdoctoral work at the University of Colorado at Boulder, studying the molecular mechanisms of genomic DNA replication. Dr. Downey served as a Product Quality reviewer in FDA/CDER's Office of Biotechnology Products (OBP) beginning in 2012. He has extensive experience in the review of enzyme replacement therapies, hematologic growth factors, and digestive enzymes and in the inspection of facilities manufacturing therapeutic biotechnology products. Since 2014, Dr. Downey has served as a Lead Chemist in OBP's Division of Biotechnology Review and Research-II, providing training, technical and regulatory guidance, and secondary review for his team of product quality reviewers.

Presentation Abstract
Plenary Session: Regulatory Expectations
Wednesday, August 26, 2015, 8:30 a.m. – 9:10 a.m.

Protein glycosylation affects a wide-range of attributes that contribute to the safety and efficacy of protein therapeutics. These attributes include physicochemical stability, immunogenicity, bioavailability, and biodistribution. The complexity of the biological functions to which glycosylation contributes and the heterogeneous nature of glycoforms represent a distinct challenge in product development and in regulatory review. This presentation will provide a general overview, from a reviewer's perspective, of regulatory expectations for characterizing glycosylation, linking glycosylation attributes to function, and developing a control strategy. The talk will also discuss general concepts and expectations for establishing comparability before and after manufacturing changes and for analyzing analytical similarity data for glycosylation in the context of biosimilars.

José M. Gomes
Principal Scientist, Culture Process Development
Pfizer, Inc.
Andover, MA, USA

José is a biochemist & biophysicist with considerable experience in cell culture, chemical engineering, and molecular biology including 18 years of industry experience. He joined Pfizer through the acquisition of Wyeth and currently holds the position of Principal Scientist within the Culture Process Development group in Biotherapeutics R&D. His work focuses on development and support of upstream processes for a wide gamut of projects from early-stage to late-stage clinical products, biosimilars, commercial products, and next-generation products. He is currently leading the upstream process team for Pfizer's 1st biosimilar program. His particular areas of interest include understanding effects of process parameters on product quality, utilization of design of experiments & quality risk management, application of QbD, and understanding factors affecting process tech transfer & scale-up (including agitation and aeration effects). Prior to joining industry, José was involved in studying lipid and hormone metabolism at Tufts Medical School and the U.S.D.A.

Presentation Abstract
Modulation of Glycosylation Patterns by Bioprocess Techniques
Wednesday, August 26, 2015, 2:30 p.m. – 2:55 p.m.

The glycosylation of recombinant proteins may be a critical quality attribute dependent on mode of action. Variability in glycosylation patterns may alter an antibody's effector functions in patients, such as antibody-dependent cell mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and Fc-receptor binding. Therefore, the ability to modulate glycosylation patterns can enable structure/function studies to further product understanding and enable the development of optimized manufacturing processes capable of consistently delivering material of desired quality. This presentation will discuss the approaches and strategies of modulating glycosylation patterns through use of various bioprocess techniques such as process parameter control, cell culture media supplements, enzymatic inhibitors, and cell line selection.

Maria-Teresa Gutierrez-Lugo, Ph.D.
U.S. Food and Drug Administration
Silver Spring, MD, USA

Dr. Gutierrez-Lugo is a Product Quality Team Leader in the Office of Biotechnology Products (OBP), CDER, FDA. Prior to joining the FDA in 2008, Dr. Gutierrez-Lugo conducted postdoctoral research at the NIH and at the University of Arizona. She holds a Ph.D. in Chemical Sciences (Pharmacy) from the National Autonomous University of Mexico

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Presentation Abstract
Regulatory Perspective
Wednesday, August 26, 2015, 2:55 p.m. – 3:20 p.m.

Post-approval manufacturing changes of biopharmaceuticals could result in changes in product quality attributes which could impact safety and efficacy of the drug product. Comparability of pre-change and post-change product is needed to demonstrate that the manufacturing change did not adversely impact the safety and efficacy of the drug product. This presentation will provide a general background on the expectations of comparability and will present case studies of post-approval manufacturing changes of glycosylated products.

Bryan Harmon, Ph.D.
Research Fellow
Eli Lilly and Company
Indianapolis, IN, USA

Research Fellow, Eli Lilly and Company, Dr. Harmon received his B.S. in Chemistry from Rose-Hulman Institute of Technology and his Ph.D. in Analytical Chemistry from Purdue University under Prof. Fred Regnier. Following a post-doctoral position under Prof. Daniel I.C. Wang at the Biotechnology Process Engineering Center at MIT, he worked in the vaccine division of Merck Research Laboratories for 3 years. Since joining Lilly in 1999, Dr. Harmon has served as a group leader for the Bioproduct Structural Characterization Team. His team is responsible for detailed structural characterization of all peptide, protein, and monoclonal antibody candidates in Lilly's development pipeline, including the elucidation of post-translational modifications, such as glycosylation, as well as degradation products and mechanisms. At Lilly, Dr. Harmon has also played a leading role in establishing comparability strategies and in the development and application of Quality by Design principles for bioproducts. He has also served as the lead development scientist for several late phase projects.

Presentation Abstract
Case Study: Influence of Fab Glycosylation on The Pharmacological Properties of a Monoclonal Antibody
Tuesday, August 25, 2015, 10:35 a.m. – 11:00 a.m.

Recombinant monoclonal antibodies (mAbs) intended for use as therapeutic agents typically contain a conserved N-linked glycosylation site in the Fc region that can play an important role in the pharmacological properties of the mAb, including mediating effector functions for IgG1 mAbs. This case study involves a mAb that contains an additional N-linked glycosylation site in its Fab region. In order to understand the impact of the Fab glycosylation on the pharmacokinetics of the mAb, the mAb was isolated from time point samples obtained from a human clinical study, and LC-MS glycosylation analysis was performed in order to identify the oligosaccharide structures that are cleared more rapidly. This information was further utilized to establish a control strategy for ensuring consistent Fab glycosylation profiles and, thus, consistent pharmacokinetics, including the validation of the LC-MS method as a GMP specification test.

Guoying Jiang, Ph.D.
Senior Scientist | Biological Technologies
Genentech
San Francisco, CA, USA

Guoying Jiang, Ph.D., is a Senior Scientist and Group Leader in the Biological Technologies group within the Analytical Department & Quality Control Department at Genentech, a Member of the Roche Group. Guoying is leading a team of scientists and research associates focusing on potency assay development, optimization and validation in support of GMP lot release and stability testing. Her group is also supporting potency methods transfer and developing characterization methods for Fc effector function. Guoying obtained her B.S degree from Peking University in China, and Ph.D. degree from Columbia University in New York. She was a postdoctoral scientist in Mount Sinai school of Medicine in New York before joining in Genentech in 2008.

Presentation Abstract

The Use of Alternative Methods as Surrogate Measures of Biological Activity
Tuesday, August 25, 2015, 9:20 a.m. – 9:45 a.m.

Antibodies recognizing cell surface expressed antigens, are able to engage Fcγ receptors on effector cells such as monocytes, macrophages, natural killer cells, neutrophils, or bind to C1q, and elicit immune effector functions such as ADCP, ADCC, and CDC. Glycosylation represents one of the main sources of heterogeneity of antibody glycoforms and can greatly influence effector function with potential impact to safety or efficacy. Case studies will be presented describing the correlation between glycosylation and bioactivity, and the potential use of physicochemical or binding assays as a surrogate measures of CDC and ADCC activity.

Chris Jones, Ph.D.
USP Affiliation:
Chair, USP Glycoproteins and Glycan Analysis Expert Panel

Head of Division, Laboratory of Molecular Structure
National Institute for Biological Standards and Control
Potters Bar, Hertfordshire, United Kingdom

Dr. Chris Jones has worked at NIBSC since 1982, with 23 years as a Head of Division responsible for developing, implementing and validating sophisticated physico-chemical methods for the characterisation of complex biological products. He uses Nuclear Magnetic Resonance (NMR) spectroscopy, circular dichroism and other optical spectroscopy approaches, mass spectrometry and specialised HPLC approaches for glycan and peptide mapping.

Dr. Jones is a world expert on the physico-chemical characterisation of polysaccharide and glycoconjugate vaccines, widely used in infants to protect against meningitis, acute respiratory infections and invasive pneumococcal infections, and typhoid. He is also closely involved in writing regulatory guidance for these products and teaches courses.

Planning Committee Member

Moderator
Session 4: Standardization
Tuesday, August 25, 2015, 2:35 p.m. – 4:00 p.m.

Presentation Abstract
Standard procedures: USP<1084>, <212>, <210>, and <129>
Tuesday, August 25, 2015, 2:35 p.m. – 2:55 p.m.

The United States Pharmacopeia are developing a series of general chapters and reference standards to support the routine testing of glycoproteins, called <1084> *Glycoprotein and glycan analysis – general considerations*, <210> *Monosaccharide Analysis* and <212> *Oligosaccharide Analysis.* This talk will describe the aims, scope, structure and evolution of these chapters, the associated reference standards and relationship to product monographs and the product class chapter <129> *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies.*

Closing Remarks
Wednesday, August 26, 2015, 4:10p.m. – 4:30 p.m.

Trish Li, Ph.D.
Senior Science and Standards Liaison
U.S. Pharmacopeia (USP)
Rockville, MD, USA

Trish Li, Ph.D. joined USP in 2006. She is currently a Sr. Science and Standards Liaison working at Biologics & Biotechnology Department. She manages a portfolio of reference standards and monographs. Prior to USP, Dr. Li worked for Pfizer's Global Manufacturing Division, Quality Operation Department at Groton, Connecticut. She obtained BSc in Chemistry from Peking University, and Ph.D. in Organic Chemistry from University of Miami.

Planning Committee Member

Moderator
Session 3: Current Technologies
Tuesday, August 25, 2015, 12:30 p.m. – 2:15 p.m.

Presentation Abstract
What Was Said & Next Steps
Wednesday, August 26, 2015, 3:50 p.m. – 4:10 p.m.

This session will be a summary of the workshop's discussions.

John Mark, MSc, PhD
Health Canada

John Mark received his MSc in Physiology at the University of Manitoba developing magnetic resonance-based, non-invasive diagnostic methods at the National Research Council Canada - Institute for Biodiagnostics. Subsequently, he completed a PhD in Biochemistry at the University of Ottawa where he studied protein structure-function relationships in therapeutic target proteins.
John eventually joined Health Canada in 2008 as a Research Chemist in the Center for Vaccines Evaluation's Protein Chemistry laboratory where his work focused on the development of novel in-vitro methods to assess protein comparability, with a specific focus on Subsequent Entry Biologics. Currently, John is a Senior Biologist/Evaluator with the CMC Quality group in Health Canada's Center for the Evaluation of Radiopharmaceuticals and Biologics, Monoclonal Antibodies Division.

Presentation Abstract
What are the Expectations from the Regulatory Agencies
Wednesday, August 26, 2015, 12:45 p.m. – 1:10 p.m.

Protein glycosylation is one of nature's most widespread post-translational modifications. It is also one of the more difficult attributes to characterize by traditional analytical methods. Nevertheless, due to the potential effects that glycosylation can have on a biologic's potency, immunogenicity, and circulatory half-life; a comprehensive determination of the glycosylation is required in major regulatory filings.
But what are the expectations? There can be some confusion since the requirements described in ICH Q5E and Q6B are general, but the details requested by regulators are often much more specific and vary from region to region or even reviewer to reviewer. To provide some insight into what is expected, this talk will focus on the CMC Quality review process at Health Canada and the expectations from a Canadian perspective. Topics will include the theoretical risks related to changes in glycosylation, the challenges associated with producing product with acceptable glycan variability, and discussion of the regulatory expectations for glycan characterization and analysis.

Ron Orlando, Ph.D.
Professor of Biochemistry and Molecular Biology
Complex Carbohydrate Research Center, University of Georgia
Athens, GA, USA

Dr. Ron Orlando received his B.S. in natural science in 1983 from St. Mary's College of Maryland, his Ph.D. in chemistry in 1988 from the University of Delaware, and served as a post-doctoral fellow at the University of Maryland Baltimore County from 1988 until 1990. After serving for two years as a senior scientist at the Suntory Institute of BioOrganic Research (Osaka, Japan), he joined the faculty of the Complex Carbohydrate Research Center at the University of Georgia in January 1993, where he is currently a Professor of Biochemistry & Molecular Biology and Chemistry. Ron Orlando has over 27 years of experience with mass spectrometry, 23 of these years focused on the identification, characterization, and quantitation of proteins and their post-translational modifications. He has co-authored over 120 publications in peer reviewed journals, has given over 100 invited lectures at various conferences, and has served on over 40 NIH review panels. Dr. Orlando is the current Editor-in-Chief for the Journal of Biomolecular Techniques (JBT), and serves on a number of editorial boards. He has organized and taught short-courses on the Analysis of Glycoproteins by Mass Spectrometry at meetings of the American Society for Mass Spectrometry (ASMS), the Association of Biomolecular Resource Facilities (ABRF) and the International Symposium and Exhibit on the Separation of Proteins, Peptides & Polynucleotides (ISPPP), and is the founder and past-chair of the ABRF Glycoprotein Research Group. Dr. Orlando is also a serial entrepreneur having founded 3 spin-off/start-up companies: BioInquire in 2007, GlycoScientific in 2009 and Photochem Technologies in 2012. He was given the award of "Entrepreneur of the Year" from the Georgia BioBusiness Center, and was named a leader of tomorrow by Spectroscopy magazine.

Presentation Abstract
Relative and Absolute Quantitation of Glycoprotein Glycans Using Isotopically Labeled Standard Glycoproteins
Wednesday, August 26, 2015, 10:25 a.m. – 10:50 a.m.

The ability to accurately quantitate the glycan chains attached to glycoproteins has wide-ranging implications. Numerous studies over the past 40 years have demonstrated that abnormal glycosylation occurs in virtually all types of human cancers, and demonstrate the potential of using glycan markers in either a diagnostic or a prognostic manner. The glycosylation on recombinant protein therapeutics is also known to have profound effects, with one of the better known examples being the increased serum half-life of erythropoietin (EPO) resulting from glycoengineering. Hence, the quantification of glycoprotein glycans play important roles from the discovery of new diagnostic/prognostic markers to the development of therapeutic agents.

A current impediment for performing quantitative glycomics is the shortage of standard glycoproteins and isotopically labeled reagents to enable accurate quantitation. The difficulty with glycan quantitation has been highlighted by inter-laboratory studies conducted by the Human Proteome Organization (HUPO) and the Association of Biomolecular Resource Facilities (ABRF), which demonstrated that quantitative measurement of the glycans in a single sample deviated by more than 100%across participating laboratories.

The focus of this presentation is the evaluation of an IgG with isotopically labeled glycans as an internal standard for both the relative and absolute quantitation of N-linked glycans released from both human serum IgGs and whole human serum. These studies demonstrated that the use of the internal standard lead to a significantly improved accuracy, reduced the coefficients of variation (CVs) by over 10 fold, dropping the standard deviation to less than 10% from the over 100% deviation obtained without the labeled standard, and greatly reduced the inter-laboratory variability despite experiments being performed by different researchers in different locations. The improvements associated with the isotopically labeled IgG lead us to predict that this will be a valuable standard for the analysis of glycoprotein glycans.

Jeff Rohrer, Ph.D.
Director of Applications Development
Dionex Products for Thermo Fisher Scientific, Inc.
Sunnyvale, CA

Dr. Rohrer is the Director of Applications Development, Dionex Products for Thermo Fisher Scientific,. In this position he directs the work of the corporate applications laboratory in Sunnyvale California. He also advises and reviews the work of other chromatography labs at Thermo Fisher Scientific. These labs develop HPLC, IC, HPAE-PAD, and other LC-based assays. Dr. Rohrer is an author of 69 peer-reviewed publications and is a member of the United States Pharmacopeia (USP) Expert Committee on Monograph Development for Small Molecules #1, a member of the USP Expert Panel on Modernization of Identification Tests, and a member of the USP Expert Panel on Glycoprotein and Glycan Analysis. Between 2005 and 2010 he was a member of the USP Expert Committee for antibiotics monograph development. He is also the co-editor of a book titled Application of Ion Chromatography for Pharmaceutical and Biological Products that was published in 2012. Dr. Rohrer joined Thermo Fisher (then Dionex) in 1989 and has held positions that include, Marketing Field Chemist, Senior Biochemist in R&D, and Applications Lab Manager. Prior to joining Thermo Fisher he spent two years as a post-doctoral associate in Dr. Elizabeth Theil's lab at North Carolina State University studying the mechanism of iron deposition into the protein ferritin. Jeff received his BS in Chemistry from Franklin and Marshall College in 1981 and his Ph.D. in Chemistry from the University of Delaware working with Dr. Harold White III studying the role of glycosylation in the transport of chicken serum riboflavin-binding protein across the oocyte membrane.

Presentation Abstract
The Common Challenges Faced Today When Performing Glycosylation Analysis
Tuesday, August 25, 2015, 12:55 p.m. – 1:20 p.m.

While there are numerous methods/techniques for characterizing a protein's glycosylation, no single one can provide all the information desired, and no method is without its liabilities. For these reasons, few if any labs rely on just one method of glycosylation analysis. Three common glycosylation assays are monosaccharide composition, sialic acid composition, and oligosaccharide profiling. This presentation will look at some of the challenges faced for each of these three assays, with a focus on liquid chromatographic techniques. The chemical and enzymatic sample preparation required for each assay will be a major part of the discussion. For example, what are the problems/challenges of acid hydrolysis for monosaccharide analysis, should I choose acid hydrolysis or neuraminidase treatment for sialic acid analysis, and should I analyze native or labeled oligosaccharides? Accuracy, reproducibility, sensitivity, analysis time, and other factors will be discussed as they pertain to each method and typical analysis needs.

Pauline Rudd, Ph.D.
Research Professor of Glycobiology
National Institute for BioProcessing Research and Training (NIBRT),
University College, Dublin
Dublin, Ireland

Professor Pauline M. Rudd BSc, LRIC, MA (Oxon), PhD is the Research Professor of Glycobiology at University College, Dublin. She heads the GlycoSciences Research Group at the National Institute for BioProcessing Research and Training in Ireland (NIBRT) where she is a PI and consultant. In addition to her basic research interests, she has many links with pharmaceutical companies across the world because GlycoScience is a major area of specialised expertise required to ensure the safety and efficacy of biotherapeutic drugs, such as monoclonal antibodies for cancer and autoimmune disorders. The open access data bases and bioinformatics programmes developed in her lab have recently been incorporated into the Waters UNIFI software where they are tailored towards the need of BioPharma.

Professor Rudd obtained a BSc in Chemistry at the University of London and a PhD in Glycobiology at the Open University, UK. She was a Founding Scientist of Wessex Biochemicals (later Sigma London), Visiting Research Associate at The Scripps Research Institute, CA, Visiting Professor of Biochemistry at Shanghai Medical University PRC, Visiting Scientist at Ben Gurion University of the Negev, Israel and an Erskine Visiting Fellow, Canterbury University, Christchurch, New Zealand. She is a Fellow of the Royal Society of Medicine, London, visiting Professor at St. George's Hospital, London and an Adjunct Professor at North Eastern University, Boston, NUI Galway, Trinity College Dublin and at University College, Dublin. She is a VI at BTI, AStar, Singapore. In 2010 she was awarded the James Gregory Medal and an Agilent Thought Leader award and in 2012 she received a Waters Global Innovation award. In 2014 she was awarded an Honorary Doctorate at Gothenburg University, Sweden.

Professor Rudd has more than 280 scientific publications and has given over 350 lectures and seminars at international meetings. Before moving her group to Dublin in 2006, Professor Rudd was a member of the Oxford Glycobiology Institute for 25 years, where she was a Senior Research Fellow and a University Reader in Glycobiology at the University of Oxford.

Presentation Abstract
The Critical Features of Glycosylation for the Therapeutic Products
Tuesday, August 25, 2015, 8:40 a.m. – 9:20 a.m.

Alterations in glycosylation are common in physiological and pathological processes as well as in the bioprocessing of products such as MAbs and Etanercept.. Glycans give rise to critical features or GCQAs (Glycosylated Critical Quality Attributes) that can affect safety and efficacy, such as half-life, Fc

dependent downstream function and immunogenicity. Glycan structures are, in the first instance, controlled by genes, however the processing pathways also depend on the bioprocessing conditions and cell specific pathways. These regulate glycoprotein expression and provide further mechanisms for fine tuning and diversifying the glycans and the functions of the proteins to which they are attached. The profiling, characterisation,and detailed analysis of released N- and O-glycans and glycopeptides is challenging, therefore a platform has been designed to simplify the technology while preserving the integrity of the data. A robotic platform to release and label glycans from glycoproteins in a 96/384 well plate format has been developed as a front end to glycan separations technologies including on line-HILIC/MS/MS and capillary electrophoresis. Data bases for these technologies are open source and also incorporated into Waters UNIFI software aswll as being available on the NIBRT web site (http://glycobase.nibrt.ie/tools.html). A sensitive label (ADC) has recently been developed by NIBRT and Waters also have developed Rapifluor. The platform has been optimised for the rigorous, detailed and quantitative analysis of biopharmaceuticals at each stage of the manufacturing process and is also applicable to basic research. The plate format makes it convenient for large sample sets; it is relatively cheap, robust and quantitative. The bioinformatics programmes enable the less experienced researcher to handle the data and the possibility of integrating the programmes with other –omics data is now on the horizon.

References

Automated, high-throughput IgG-antibody glycoprofiling platform. Stöckmann H, Adamczyk B, Hayes J, Rudd PM. Anal Chem. 2013 Sep 17;85(18):8841-9.
Genomics meets glycomics-the first GWAS study of human N-Glycome identifies HNF1α as a master regulator of plasma protein fucosylation. Lauc G, Essafi A, Huffman JE, Rudd PM, Rudan I. et al., PLoS Genet. 2010;6 (12)
.Automated, high-throughput serum glycoprofiling platform. Stöckmann H, O'Flaherty R, Adamczyk B, Saldova R, Rudd PM. Integr Biol (Camb). 2015 Jul 20. [Epub ahead of print]
Ultrahigh Throughput, Ultrafiltration-Based N-Glycomics Platform for Ultraperformance Liquid Chromatography (ULTRA3).Stöckmann H, Duke RM, Millán Martín S, Rudd PM. Anal Chem. 2015 Jul 31. [Epub ahead of print]
In press: Justin M. Prien, Henning Stöckmann, Simone Albrecht, Silvia M. Martin, M. Varatta, M. Furtado, S. Hosselet, Meiyao Wang3 Trina Formolo, Pauline M. Rudd, and, John E. Schiel ACS publications Ch8. The Elucidation of Co and Post-Translational Modifications Orthogonal Technologies for NIST mAb N-Glycan Structure Elucidation and Quantitation

John Schiel, Ph.D.
Research Chemist
National Institute of Standards and Technology (NIST)
Gaithersburg, MD, USA

Dr. Schiel received his BS (2004) and Ph.D. (2009) in chemistry from the University of Nebraska-Lincoln. Dr. Schiel joined the National Institute of Standards and Technology in 2009 to begin work on glycoanalytical method development, and is currently a research chemist in the NIST Biomolecular Measurement Division with an appointment at the Institute for Bioscience and Biotechnology Research. He is leading LC- and MS-based biomanufacturing research efforts at NIST/IBBR; developing a suite of fundamental measurement science, standards, and reference data to enable more accurate and confident characterization of product quality attributes. Dr. Schiel is also the project coordinator for the recombinant IgG1κ NIST monoclonal antibody Reference Material (NISTmAb) program and a co-editor of the associated ACS book series "State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization". He is an author of over 20 peer reviewed publications, 4 book chapters, and recipient of numerous Awards, including the ACS Division of Analytical Chemistry Fellowship, *Bioanalysis* Young Investigator Award, and UNL Early Achiever Award.

Moderator
Session 8: Post-approval changes registration
Wednesday, August 26, 2015, 2:30 p.m. – 3:50 p.m.

Presentation Abstract
Providing Well-characterization Standards (NIST)
Tuesday, August 25, 2015, 3:05 p.m. – 3:30 p.m.

Monoclonal antibodies (mAbs) are the fasted growing class of therapeutics, whose safety and efficacy have been directly linked to glycoprofile content. The complex, multi-antennary nature of glycans has resulted in development of a tiered, lifecycle stage-appropriate toolbox of analytical approaches for glycoanalysis. As a rule of thumb, more information-rich methods require more complex sample handling and analysis. Evaluation of evolving glycoanalytical methods has been supplemented by a widely available representative test material. This presentation will discuss the establishment of an IgG1k Reference Material expected to more firmly underpin regulatory decisions and facilitate the development of originator and follow-on biologics. The RM is intended for a variety of uses including, but not necessarily limited to: system suitability tests, establishing method or instrument performance and variability, comparing changing analytical methods, assisting in method qualification, etc. The NIST mAb glycoanalytical characterization and certification will be discussed. Conclusions drawn from the unprecedented industry-wide book collaboration "State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization" will also be presented.

Birgit Schmauser, Ph.D.
Federal Institute for Drugs and Medical Devices (BfarM)
Bonn, Germany

Dr. Birgit Schmauser is a senior assessor of pharmaceutical quality at the German Regulatory Authority (BfArM) in the Unit "Pharmaceutical Biotechnology, Biologicals, Inspections". She studied pharmacy at the University of Munich and holds a PhD from Berlin Free University. She has scientific and regulatory experience of many years. Her expertise and profound knowledge in glycobiology optimally apply in evaluation of biotechnology-derived medicinal products including investigational medicinal products. For several years she has been a temporary adviser for the World Health Organisation (WHO) in a prequalification programme.

Presentation Abstract
Expectations for Glycosylated Biosimilars (regulatory perspective)
Wednesday, August 26, 2015, 1:35 p.m. – 2:00 p.m.

A similar biological medicinal product is developed based its own, unique manufacturing process. At the same time the claim to be biosimilar to an existing biological medicinal product requires adaptations of process design and performance in such a way that the biosimilar´s quality target product profile as well as its quality attributes and characteristics are highly similar to the reference medicinal product. Glycosylation of a biological substance may exhibit a high level of complexity and inherent molecular variability per se including diversity in type and site of glycosidic linkages, in type of monosaccharides with site-specific modifications and in the extent of branching and branch elongation. The manufacturer of a biosimilar is consequently challenged to demonstrate that there are consistent ranges of similarity between the glycostructures of the biosimilar and reference product to a sufficient extent and that these consistent similarity ranges allow reducing the panel of non-clinical and clinical studies to be performed with the biosimilar. The level of detail that can be achieved upon analysis of glycosylation profiles has tremendously increased in the past years. Thus it is one of the main regulatory expectations that manufacturers of biosimilars provide a thorough, comprehensive comparison of biosimilar and reference product with the necessary level of structural detail to picture the glycosylation profiles of both products and derive the similarity claim thereof. Evaluation of similarity should include a thorough discussion of all structural results obtained as well as a sound justification for the representativeness of results based on number and choice of batches for comparison. It is considered essential to distinguish acceptable and unacceptable differences taking into account the critical quality attributes defined. Nevertheless a regulatory decision will always include the quality of data provided as well as interpretation and justification provided to exclude that potential differences observed may have an unexpected impact on safety and efficacy.

Bhavana Shah
Senior Scientist
Amgen Inc.,
Thousand Oaks, CA, USA

Bhavana is a senior scientist in the product attributed science group of the process development department at Amgen Inc. She has worked as an analytical chemist in various Amgen departments including research, quality, and process development for more than 25 years. She has developed a method of glycan profiling for glycoprotein and antibody products by LC-MSMS peptide mapping. She has extensive experience in site-specific glycan characterization as well as reference standard characterizations of glycoprotein and monoclonal antibodies, using various LC and mass spectrometry techniques. As a part of quality department, she developed, validated, and implemented routine lot release methods, such as glycan profiling and sialic acid estimation for glycoproteins. She is also involved in method development for the estimation of post-translation modifications and misincorporation of proteins, as well as monoclonal antibodies.

Bhavana has an M.S. in Analytical and Medicinal Chemistry from the University of Bombay, India. She has authored several technical research articles in peer-reviewed publications.

Presentation Abstract
MAb Glycan Profiling by LC/MS Peptite Mapping
Wednesday, August 26, 2015, 10:50 a.m. – 11:15 a.m.

Traditionally N-glycan analysis of a glycoprotein is done by high pH anion exchange chromatography (HPAEC), reversed phase liquid chromatography (RPLC) or recently developed hydrophilic interaction liquid chromatography (HILIC). These methods require release of N-glycans by enzyme followed by fluorescent labeling of N-glycans. The protocol is labor intensive and time consuming, and does not provide site-specific glycosylation information. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) peptide mapping is routinely used for protein structural characterization and, as a bonus, can potentially provide glycan profile on each individual glycosylation site. In this work, a recently developed glycopeptide fragmentation model is used for automated identification of glycan, based on their MS/MS (fragmentation pattern) of N-glycopeptides from proteolytic digestion of monoclonal antibodies (mAbs). LC-MS conditions were optimized for accurate glycan profiling. Glycan profiles obtained from LC-MS/MS were compared with the profiles obtained by conventional methods like HPAEC, RPLC and HILIC. Robustness of the LC-MS/MS method is also evaluated for accuracy, reproducibility, and linearity. Based on the same concept a fully automated fast high throughput method is also developed for at-line profiling of mAb glycans directly from cell culture media. The LC-MS/MS peptide mapping method with fully automated data analysis requires less sample

preparation and also provides site-specific information. It can serve as an alternative method for routine profiling of N-glycans on immunoglobulins as well as other glycoproteins with simple N-glycans.

Catherine Srebalus Barnes, Ph.D.
Senior Director, Global Biologics – Analytical Sciences
Hospira Global Biologics R&D
Lake Forest, IL, USA

Dr. Srebalus Barnes is currently the Senior Director for Hospira Global Biologics, Bioanalytical Sciences. Her current responsibilities include management of Hospira's Global Bioanalytical Sciences groups located in the U.S, Asia and Australia. These groups are responsible for bioanalytical and bioassay method development and validation, characterization of Hospira and originator biologic products as part of analytical biosimilarity assessments, bioanalytical support for cell line and process development/optimization and testing or oversight for outsourced release/stability testing of products for use in clinical trials. In her role, Cathy is responsible for definition and implementation of strategies for demonstration of analytical biosimilarity between Hospira and originator products and providing oversight for development and implementation of analytical control strategies for Hospira's biologic products. She actively participates in authoring/review of Regulatory submissions and meetings with Regulatory agencies in support of Hospira's biologics programs.

Prior to joining Hospira in 2012, Cathy held a number of leadership roles with increasing levels of responsibility in the Bioproduct Development organization at Eli Lilly and Company, including Director, Bioproduct Analytical Development and Director, Bioprocess Purification Development and Viral Safety. Cathy has a B.S. in Chemistry (West Virginia University, U.S.A.) and a Ph.D. in Analytical Chemistry (Indiana University, U.S.A).

Presentation Abstract
Expectations for Glycosylated Biosimilars (manufacturer perspective)
Wednesday, August 26, 2015, 1:10 p.m. – 1:35 p.m.

FDA guidelines for biosimilar products require that sponsors demonstrate there are no clinically meaningful differences in the safety, purity and potency of the proposed biosimilar product and reference product to support approval. The development of glycoprotein biosimilar products requires a comprehensive comparison of glycosylation attributes for the proposed biosimilar product and the reference product. The analytical biosimilarity assessment should start with an assessment of the product attributes that are potentially clinically meaningful (i.e., Critical Quality Attributes or CQAs). The criticality of specific glycosylation sites and glycan structures depends on the mechanism of action for the specific glycoprotein. The glycosylation CQAs should be considered to define the analytical methods and glycosylation attributes that are the focus of the biosimilarity assessment.

A proposed framework for defining the glycosylation CQAs for biosimilar glycoprotein products and the application of the proposed CQAs to define the analytical methods and strategy for the analytical biosimilarity assessment will be discussed. Case studies for therapeutic monoclonal antibodies and erythropoietin biosimilar products will be used to demonstrate the approach. In addition, the use of supportive in vitro and in vivo studies to refine preliminary CQA assignments for monoclonal antibodies and erythropoietin biosimilar products will be discussed.

Marco Thomann
Groupleader Development Analytics
Roche Diagnostics GmbH
Munich, GERMANY

Marco Thomann has been working with Roche Diagnostics in Penzberg, Germany, for eleven years now. He finished his study of Biotechnology/Bioinformatics at University of Applied Sciences in Weihenstephan, Germany, with a German "Diploma Engineer" degree.

He started at Roche with his diploma thesis in the field of Bioinformatics, continued as an analytic specialist, where he worked on the characterization of proteins using different HPLC, spectroscopic and mass spectrometry techniques in a GMP environment.

In 2008, Marco became group leader in the same department, responsible for the characterization of therapeutic proteins during clinical phase II and III including preparation of regulatory filings. Recently, he got also involved in CQA assessment for a late stage project. His group also focuses on oligosaccharide analytics as well as glycoenzyme characterization for in vitro glycoengineering and their application on therapeutic proteins.

Presentation Abstract
In Vitro Glycoengineering – A Useful Tool for Glycan Structure-Function Characterization and Molecule Optimization
Wednesday, August 26, 2015, 9:10 a.m. – 9:35 a.m.

Glycosylation of therapeutic proteins is of special interest since it can significantly impact biological activity. Glycosylation is heterogeneous and subject to batch-to-batch variability, thus its impact on protein function is not easy to investigate. However, assessment of e.g. antibody Fc glycan structure – function relationship becomes more and more of interest. In this talk the in vitro glycoengineering (IVGE) technology and its application will be introduced. Multiple examples will be shown where IVGE adds a clear benefit to glycan characterization as well as in vitro structure-function analysis. Sample preparation workflows for in vitro galactosylation and sialylation of IgG1 Fc glycans will be explained. And its effect on the Mode-of-Action, Fc receptor binding and ADCC activity, as well as the impact on pharmacokinetic properties will be addressed. Also IVGE has been used to support CQA assessment of a late stage project. In summary, the technology can be applied to increase specific molecule knowledge as well as to optimize molecular properties of clinical candidates.

Earl Zablackis, Ph.D.
USP Affiliation:
Member, Monographs – Biologics and Biotechnology 2 Expert Committee Panel; Member, Vaccine Polysaccharide NMR Identity Testing Expert Panel; Member, Vaccines for Human Use – Viral Vaccines Expert Panel; Member, 1041 Biologics Revision Expert Subcommittee; and Member, 210 Monosaccharide Analysis Subcommittee

Director, Method Validation
Sanofi Pasteur
Swiftwater, PA, USA

I am a biologist/botanist who developed a keen interest in cell wall polysaccharides and thus became carbohydrate biochemist. My education took me through programs at the University of California, Berkeley to the University of Hawai'i and finally the University of California, Santa Barbara, followed by a post-doctoral fellowship at the Complex Carbohydrate Research Center at the Univ. of Georgia.

My career spans 30 years working as a carbohydrate chemist in academia and the biopharmaceutal industry. My carbohydrate work was focused on the isolation, purification and characterization of fine structure of polysaccharides using multiple techniques employing various separation technologies to various analytical tools such as mass spectroscopy and NMR. My industrial experience covers glycan analysis from recombinant glycoproteins, monoclonal antibodies and bacterial polysaccharides for vaccines. I have additional expertise in validation of analytical methods.

In my current position at Sanofi Pasteur (vaccines) in the Manufacturing Technology department, I am the Director-Principal Scientist in the Analytical Process and Technology platform responsible for method qualification, validation and transfer programs as well as compliance with quality systems.

I am currently a member of the Biotechnology Advisory Board (BioAB) of the PDA as well as a member of the USP Biologics and Biotechnology 2 Expert Committee. I have contributed to PDA Technical Reports 57 and 57-2 covering analytical method development, qualification, validation and transfer; and to USP chapter <1238> *Bacterial Vaccines*. I am also currently active on USP expert panels developing chapters <210> *Monosaccharide Analysis*, and <198> *Identification of Bacterial Polysaccharides Using Nuclear Magnetic Resonance Spectroscopy*

Planning Committee Member
Not Presenting

Rebecca Zangmeister, Ph.D.
USP Affiliation:
Member, USP Glycoproteins and Glycan Analysis Expert Panel

Science Advisor
Biomolecular Measurement Division
National Institute of Standards and Technology
Gaithersburg, MD, USA

Rebecca A. Zangmeister is the Science Advisor for the Biomolecular Measurement Division of the Material Measurement Laboratory at the National Institute of Standards and Technology (NIST) in Gaithersburg, MD. Rebecca graduated from the University of Arizona, Tucson, AZ, in 2001 with a Ph.D. in Analytical Chemistry, specializing in organic thin films and surface characterization. The common theme of her subsequent research projects at NIST have involved the deposition and analytical characterization of biomolecules at surfaces (planar or nanomaterial based). Her most recent research efforts have focused on the development of measurement methods for biologics with an emphasis on rapid glycosylation assays for use in the biomanufacturing of therapeutic proteins.

Planning Committee Member
Not Presenting