Welcome to the CMC Strategy Forum

We are pleased to welcome you to the CMC Strategy Forum. The purpose of the CMC Strategy Forum is to provide a venue for biotechnology/biological product discussion. The meetings focus on relevant CMC issues throughout the lifecycle of a product and thereby foster collaborative technical and regulatory interactions. The Forum strives to share information with the regulatory agencies to assist them in merging good scientific and regulatory practices. Outcomes of the Forum meetings are published in an appropriate peer-reviewed journal.

Each meeting will focus on a CMC related issue such as product characterization, comparability, specifications, etc. The format of each meeting will consist of case studies and presentations by Industry and/or FDA experts to introduce the topic and the key issues of concern. Breakout sessions will then be conducted to allow for additional discussion on the technical and regulatory details of the topics. It is envisioned that the final outcome of the workshop discussions will be the development of a document to be submitted to the appropriate Regulatory Agency designees for their consideration in developing and/or clarifying good regulatory practice guidelines for biotechnology derived products.

The success of the CMC Strategy Forum will depend on your active participation in discussing and raising issues pertaining to development of biologics. We encourage you to participate wholeheartedly in the workshops that have been designed to stimulate exchange of ideas and information.

We would like to thank the speakers who are giving generously of their time and resources, and to you, for your attendance. We acknowledge the generosity of our program partners: AbbVie, Inc., Biogen, Eli Lilly and Company, F. Hoffmann-La Roche Ltd., Genentech, a Member of the Roche Group, Janssen Pharmaceutical R&D, LLC, MedImmune, A member of the AstraZeneca Group, Merck & Co., Inc., National Institute of Standards and Technology (NIST), Pfizer, Inc. We are grateful for the expert management from CASSS and the audio-visual expertise of Michael Johnstone from MJ Audio-Visual Productions. Their experience and guidance in the preparation of this Forum has been invaluable.
ACKNOWLEDGEMENTS

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As the biotechnology industry continues to mature, so has the fundamental understanding of the cell culture-based processes that are leveraged to manufacture biopharmaceutical products. Furthermore, new technologies are emerging that can provide additional insight through the characterization of these processes, specifically with respect to the cell substrate and cell culture process. This Forum will discuss considerations for the development of production cell lines including the choice of expression system, the characterization of cell populations and potential approaches to improve cell line performance through host engineering. Additionally, approaches to ensuring appropriate control of product quality throughout the cell culture process will be discussed including advancements in analytical control strategies. Overarching objectives will be in defining the myths and risks to cell line development and product quality associated with cell cultivation, including current best practices. Case studies will be used to illustrate the scientific and regulatory challenges and the approaches that help ensure regulatory expectations are met when assessing and assuring the appropriateness of cell lines used for production of biotechnology products during development and commercialization.
# CMC Strategy Forum Program Summary

**Production Cell Line Development and Control of Product Consistency during Cell Cultivation – Myths, Risks and Best Practices**

**Monday, January 23, 2017**

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<th>Time</th>
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<tr>
<td>07:30 – 17:00</td>
<td><strong>Registration</strong> in the Senate Room</td>
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<td>07:30 – 08:30</td>
<td><strong>Breakfast</strong> in the District Ballroom (previously the Colonial Room), Lower Level</td>
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<td>08:30 – 08:45</td>
<td><strong>CASSS Welcome and Introductory Comments</strong> in the District Ballroom</td>
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<td>Nadine Ritter, <em>Global Biotech Experts, LLC</em></td>
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<td><strong>CMC Strategy Forum Welcome and Introductory Comments</strong> in the District Ballroom (previously the Colonial Room), Lower Level</td>
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<td>Barry Cherney, <em>Amgen Inc.</em></td>
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<td>Dieter Schmalzing, <em>Genentech, a Member of the Roche Group</em></td>
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## Production Cell Line Development

**Workshop Session One** in the District Ballroom  

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| 08:45 – 09:10 | **Industry View on the Relative Importance of “Clonality” of Biopharmaceutical-producing Cell Lines**  
Anthony Lubiniecki, *Janssen R&D, LLC, Malvern, PA USA* |
| 09:10 – 09:35 | **Evolving of Biological Product Expression Systems with Host Cell Engineering**  
Lianchun Fan, *Bristol-Myers Squibb Company, Bloomsbury, NJ USA* |
| 09:45 – 10:15 | **Characterization of Production Cell Lines Part One: Industry**  
Luhong He, *Eli Lilly and Company, Indianapolis, IN USA* |
| 10:15 – 10:45 | **Networking Break** in the District Ballroom                          |
| 10:45 – 11:10 | **Characterization of Production Cell Lines Part Two: Health Authority**  
Rachel Novak, *CDER, FDA, Silver Spring, MD USA* |
| 11:15 – 12:30 | **PANEL DISCUSSION – Questions and Answers**  
Lianchun Fan, *Bristol-Myers Squibb Company, USA*  
Steffen Gross, *Paul-Ehrlich-Institut, Germany*  
Luhong He, *Eli Lilly and Company, USA*  
Michael Laird, *Genentech, a Member of the Roche Group, USA*  
Anthony Lubiniecki, *Janssen R&D, LLC, USA*  
Trent Munro, *Amgen Inc., USA*  
Rachel Novak, *CDER, FDA, USA* |
| 12:30 – 14:00 | **Networking Lunch** in the District Ballroom                          |
Monday, January 23 continued…

Control of Product Consistency during Cell Culture Cultivation
Workshop Session Two in the District Ballroom
Session Chairs: Dominik Gaser, Sandoz Biopharmaceuticals and Raghavan Venkat, MedImmune, A member of the AstraZeneca Group

14:00 – 14:10  Screening Approaches for Product Quality to Enable Attribute-driven Cell Line Development with an Eye towards Commercialization
Christopher Sellick, MedImmune Limited, Cambridge, United Kingdom

14:10 – 14:35  Advances in Product Characterization during Cell Cultivation
Jason Rouse, Pfizer, Inc., Andover, MA USA

14:35 – 15:00  Product Consistency during Cell Cultivation – Regulatory Expectations
Steffen Gross, Paul-Ehrlich-Institut, Langen, Germany

15:00 – 15:30  Networking Break in the District Ballroom

15:30 – 15:55  Regulatory Expectations and Case Studies for Product Cell Line Development
Juhong Liu, CDER, FDA, Silver Spring, MD USA

16:00 – 17:15  PANEL DISCUSSION – Questions and Answers
Fiona Cornel, Health Canada, Canada
Steffen Gross, Paul-Ehrlich-Institut, Germany
Juhong Liu, CDER, FDA, USA
Ilona Reischl, AGES-Austrian Agency for Health and Food Safety, Austria
Jason Rouse, Pfizer, Inc., USA
Christopher Sellick, MedImmune Limited, United Kingdom

17:15 – 17:45  Recap of Program
Summary Slide Presentation
Nadine Ritter, Global Biotech Experts, LLC

17:45 – 18:00  Invitation to CMC Strategy Forum July 2017

18:00 – 19:15  Networking Reception in the Chinese Room
Short Biographies

Barry Cherney
Amgen Inc.

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Fiona Cornel
Health Canada

Ms. Cornel is an acting senior biologist/evaluator in the cytokines division of the biologics and genetic therapies directorate at Health Canada. She earned her master’s degree in biology at Queen’s University in 1994 and joined the BGTD soon after as a research biologist. She has participated in a number of diverse research projects ranging from the expression of biological drugs in transgenic plants to the development of new analytical methods for the evaluation of vaccines. Fiona transitioned from research to the evaluation of biologic drugs in 2011. She joined the cytokines division in 2012 where she evaluates the chemistry, manufacturing, and controls of various biotherapeutic products from monoclonal antibodies to hormones and cytokines.

Lianchun Fan
Bristol-Myers Squibb Company

Dr. Lianchun Fan is a principal scientist and group leader in the Global Manufacture Supply (GMS) organization at Bristol-Myers Squibb Company, where he is leading several groups covering clinical cell line development, process automation and new technology development. Before joining BMS, Dr. Fan had spent seven years in Bioproduct Research and Development (BR&D) Department at Eli Lilly and Company where he has led the cell line generation and technology development groups. At Lilly, he established the GS-knockout based GS-CHO expression system, developed an engineered expression vector system, and initiated the application of host cell engineering concept to solve product quality related challenges. Dr. Fan got his PhD from the Chinese Academy of Science. He completed his postdoc training at Purdue University where he established the first zebrafish stem cell line. Dr. Fan has over 20 publications covering his research experience. His favorite hobby is volleyball.

Steffen Gross
Paul-Ehrlich-Institut

Steffen Gross has extensive experience in molecular and cell biology. Mr. Gross joined the Paul-Ehrlich Institute in 2005 and became head of the section monoclonal and polyclonal antibodies in 2012. He is involved in the assessment of the quality and preclinical issues for marketing authorization applications, scientific advices and clinical trial applications. The Paul-Ehrlich-Institut also performs official experimental batch testing independently of the manufacturer. Until 2012 Mr. Gross was laboratory head of the section monoclonal and polyclonal antibodies and involved in testing of immunoglobulins, immunsera and monoclonal antibodies, as well as in planning and performing research projects. Due to his experience, he also often supports inspections as an expert for certain products. Mr. Gross has participated extensively as a speaker at scientific and regulatory meetings. Before joining the Paul-Ehrlich-Institut, he worked in The Netherlands for three years as a postdoc. During this time, he had been working several months at the National Institutes of Health in Bethesda. After his return to Germany, he became a research group leader at the University of Frankfurt.
**Luhong He**  
_Eli Lilly and Company_

Dr. Luhong He is a senior research scientist at Eli Lilly and Company. She leads a molecular analytical group at the division of bioproduct R&D. She was an R&D scientist at Biolog, Inc. before joining Eli Lilly and Company. Dr. He received her doctorate degree in microbiology at Beijing Agricultural University and her postdoc training at the University of California, Berkeley and the University of Arizona.

**Michael Laird**  
_Genentech, a Member of the Roche Group_

**NEED BIO**

**Juhong Liu**  
_CDER, FDA_

Juhong Liu, PhD is currently an acting review chief of the division of biotechnology review and research II in the Office of Biotechnology Products of the FDA. After receiving his PhD in biochemistry and molecular biology in 1995, Dr. Liu joined the Section of Gene Regulation, Laboratory of Pathology at the National Cancer Institute in Bethesda, Maryland. He worked as a postdoctoral fellow and latterly as a staff scientist on characterization of transcriptional activators and repressors involved in the regulation of human c-myc oncogene. Dr. Liu joined the Office of Biotechnology Products as product quality reviewer for therapeutic proteins and monoclonal antibodies in 2008.

**Anthony Lubiniecki**  
_Janssen Pharmaceutical R&D, LLC_

Anthony S. Lubiniecki, ScD is senior scientific director & fellow, CMC Strategy, Pharmaceutical Development & Manufacturing Sciences at Janssen R&D, LLC. During his 41 years in industry, he worked on the development of over 40 recombinant derived investigational products using both microbial and eukaryotic expression systems, of which twelve have become marketed products, including Simponi, Stelara, Sylvant, and Darzalez during his 11 years at Janssen R&D LLC. He serves as CMC Strategist on a number of large molecule projects including daratumumab and several cell therapy projects, and also chairs the Large Molecule CMC Council. He is also a member of the Lean Early Development Core Team. Tony also has been active in shaping regulatory policy by serving as a Pharmaceutical Research & Manufacturers of America representative to the International Conferences on Harmonization (ICH) Expert Working Groups for 6 Q5-Q7 guidance documents and served as Rapporteur for two of them (ICH Q5D & Q5E). He earned his doctor of science degree in public health microbiology from the University of Pittsburgh in 1972, and his BS degree in biological sciences from Carnegie Mellon University in 1968.
Trent Munro  
*Amgen Inc.*

Dr. Munro joined Amgen in May 2013, where he is currently a scientific director and leads the cell line development group within process development. Dr. Munro obtained a BSc (hon) from James Cook University in 1997 and his PhD in protein biochemistry from the University of Queensland in 2000. He then went on to do postdoctoral training at Harvard Medical School and at the Gurdon Institute, University of Cambridge. In 2006, he moved to the Australian Institute for Bioengineering and Nanotechnology (AIBN) at the University of Queensland, where he was an associate group leader and a Queensland Government Smart Futures Fellow. Dr Munro has more than 30 peer reviewed publications, an H-index of 17 and over 1000 citations.

Rachel Novak  
*CDER, FDA*

Ilona Reischl  
*AGES-Austrian Agency for Health and Food Safety*

Ilona Reischl joined the Austrian Medicines and Medical Devices Agency AGES/MEA in March 2006, and is currently the head of the clinical trials unit in the Institute Surveillance. This unit is responsible for clinical trials with medicinal products and medical devices as well as GCP inspections. The transition to regulatory work from basic science was gradual. After an initial degree in pharmacy, a PhD in immunology/allergology and postdoctoral experience at an industrial research institute, post-doctoral positions followed at the University of Southampton (UK) and the National Institutes of Health (USA) in immunology. The experience of a dual research/regulatory position at the US Food and Drug Administration (FDA) led to the current regulatory focus. Among other activities, she is the Austrian member of the European Medicines Agency Biologics Working Party and the Committee for Advanced Therapies.

Jason Rouse  
*Pfizer, Inc.*

Jason Rouse manages the Mass Spectrometry and Biophysical Characterization (MSBC) laboratory at Pfizer, Inc., which is a branch of the Analytical Research and Development group in the greater Biotherapeutics Pharmaceutical Sciences process and product development organization. MSBC works collaboratively with research, development and manufacturing groups across Pfizer to carry out product characterization and comparability/biosimilarity exercises, as well as time-sensitive investigations and bioprocess support. Jason received his PhD degree in analytical chemistry from Michigan State University in 1993 and joined Genetics Institute (GI) as a post-doctoral research fellow. Jason was promoted into positions of greater responsibility over the years in GI, Wyeth and Pfizer, and currently he is the director of the MSBC group. Jason’s scientific interests include the elucidation and analysis of released N- and O-linked glycans by on-line and off-line MS techniques, as well as the top-down characterization of intact glycoprotein therapeutics by ultrahigh-resolution MS approaches. Jason is an active program committee member for the CASSS Mass Spec conference.
Dieter Schmalzing  
*Genentech, a Member of the Roche Group*

**NEED BIO**

Christopher Sellick  
*MedImmune Limited*

Chris has a PhD in biochemistry and molecular biology from the University of Manchester. He worked as a postdoc at the University of Manchester on a Bioprocessing Research Industry Club (BRIC) funded project using metabolite profiling to enhance our fundamental understanding of the molecular parameters that influence productivity in recombinant mammalian cell lines producing biopharmaceuticals. He joined MedImmune in 2011 to lead the BioProcess Analytics team within biopharmaceutical development. In this role he is responsible for the development and implementation of new high throughput analytical technologies to enable attribute-led cell line development and to increase understanding of bioreactor processes and their effects on biopharmaceutical production and quality.
Production Cell Line Development

Session Chairs: Fiona Cornell, Health Canada and Christopher Frye, Eli Lilly and Company

This session will explore scientific and regulatory considerations for the development of production cell lines including the choice of expression system, potential approaches to improve cell line performance through host engineering and the characterization of cell populations during product development and commercialization.

NOTES:
Industry View on the Relative Importance of “Clonality” of Biopharmaceutical-producing Cell Lines

Christopher Frye1; Rohini Deshpande2; Scott Estes3; Kathy Francissen4; John Joly4; Anthony Lubieniecki5; Trent Munro2; Reb Russell6; Tongtong Wang1; Karin Anderson7

1Eli Lilly and Company, Indianapolis, IN USA; 2Amgen Inc., Thousand Oaks, CA USA; 3Biogen, Cambridge, MA USA; 4Genentech, a Member of the Roche Group, South San Francisco, CA USA; 5Janssen R&D, LLC, Malvern, PA USA; 6Bristol-Myers Squibb Company, Pennington, NJ USA; 7Pfizer, Inc., Andover, MA USA

Recently, several health authorities have requested substantial detail from sponsor firms regarding the practices employed to generate the production cell line for recombinant DNA-(rDNA) derived biopharmaceuticals. Two possible inferences from these regulatory agency questions are that (1) assurance of “clonality” of the production cell line is of major importance to assessing the safety and efficacy of the product and (2), without adequate proof of “clonality”, additional studies of the cell line and product are often required to further ensure the product’s purity and homogeneity. We will address the topic of “clonality” in the broader context of product quality assurance by current technologies and practices, as well as discuss some of the relevant science and historical perspective. While the clonal derivation of a production cell line is one factor with potential impact, it is only one of many factors. Further, we believe that regulatory emphasis should be primarily placed on ensuring product quality of the material actually administered to patients, and on ensuring process consistency and implementing appropriate control strategies through the life cycle of the products.

NOTES:
Recent advancements in genome sequencing (Next Generation Sequencing) and gene-editing technologies (Zinc Finger Nucleases, Mega-nuclease, TALEN and CRISPR) have made modification of specific gene targets to change the performance of host cells very practical. New technologies have brought new capabilities and potential to impact cellular processes from transgene integration (e.g. Targeted Integration), transcription, translation, post-translational modification (Glycosylation), secretion, to manipulation of metabolic pathways. This presentation will provide a brief overview of factors to consider when deciding on choice of expression system. However the focus of the presentation will be on the evolution of current CHOK1 expression system with several recent successful case studies that showing the power of host cell engineering technology to drive the development of new host cells through improvement on cell line productivity, product quality and/or cell line development efficiency. The potential future application of some of these improvements such as development of targeted integration systems- (resulting in much more homogeneous bulk cell populations) will also be discussed.

NOTES:
Characterization of Production Cell Lines Part One: Industry

Luhong He

Eli Lilly and Company, Indianapolis, IN USA

As the biotechnology industry continues to mature so does our ability to understand and improve the processes used to manufacture biopharmaceutical proteins. Part of this understanding involves the recognition and ability to characterize production cell lines as populations of cells exhibiting various levels of genetic and phenotypic heterogeneity. It is also recognized that there is a growing need to characterize cell lines engineered for product quality improvement and other aspects of cell culture performance. Provided is a comprehensive, risk-based characterization strategy which includes characterization of production cell line populations and engineered cell lines in addition to more traditional genetic characterization methodologies. Case studies are presented, which demonstrate that the comprehensive characterization strategy enables the identification of production cell lines producing biopharmaceutical proteins consistently and of appropriate product quality even though the absolute genetic and phenotypic homogeneity of clonally-derived CHO production cell lines is not achievable.

NOTES:
Characterization of Production Cell Lines Part Two: Health Authority

Rachel Novak

CDER, FDA, Silver Spring, MD USA

A number of different cell substrates have been developed for use in the manufacturing of biotechnology products in response to considerations that include, but are not limited to, post-translational modifications, yield and safety. From bacterial cells to human cells, these substrates represent different levels of biological complexities and are more fully or less fully understood in terms of the risks they pose. In addition, a number of cell substrates have been genetically modified, for example to alter glycosylation profiles. The regulatory expectations for the characterization of cell substrate/production cell lines, as outlined in, e.g., ICH Q5D, ensure that identity, purity and safety are appropriately evaluated. These expectations are dictated by the origin of the cell substrate and cell line modifications and the inherent or introduced product quality or safety concerns. Increasing knowledge of complex cell substrates and the availability and capabilities of new technologies are also considered. This presentation will provide an overview of some of the current expectations regarding characterization of different types of cell substrates.

NOTES:
Panel Discussion – Questions and Answers
Lianchun Fan, Bristol-Myers Squibb Company, USA
Steffen Gross, Paul-Ehrlich-Institut, Germany
Luhong He, Eli Lilly and Company, USA
Michael Laird, Genentech, a Member of the Roche Group, USA
Anthony Lubiniecki, Janssen R&D, LLC, USA
Trent Munro, Amgen Inc., USA
Rachel Novak, CDER, FDA, USA

The following questions will guide the panel discussion:

1) Theme: Establishing Clonally-derived Cell Lines
   a) What do we know about the nature of the types of cell lines currently leveraged for the production of biopharmaceuticals and how does this impact bioprocess development?
   b) What is the basis for the importance of absolute demonstration of clonal derivation and what role does it play in the context of the holistic control strategy?
   c) If a cell line is discovered to be non-clonally derived late in development (or even during commercial production), how should the issue be addressed from both an industry and regulatory perspective?
   d) What are the new technologies available to improve the clonality of cell lines?

2) Theme: Host Cell Engineering
   a) What technologies and applications exist for cell line development?
   b) What are regulatory expectations (i.e. genetic characterization) for production cell lines which have been engineered to improve product quality (e.g. eliminate trace-level host antigen impurity)?
   c) How can Targeted Integration or Site-Specific Integration impact production cell line and process development (is it just timeline, is it PQ consistency, what else)?
   d) Are expectations evolving related to the availability of new genetic characterization technologies and applications? If so, how?

3) Theme: Using Cell Line “Pools” to Support Clinical Development
   a) What are the risks to the use of cell line “pools” to early-phase clinical development and if implemented how does one transition from “pool” to clonally-derived production cell line?
   b) What are industry and regulatory positions and/or experience on cell line “pools” for early-phase clinical development?
   c) What would be a practical approach to transition from a “pool” to a better defined cell line? How would this approach fit in for products with a relative short development cycle?

4) Theme: Addressing “Challenging” Cases: Cell line Stability
   a) If during genetic characterization of a production cell line, there are confounding data (e.g. Southern mapping data which are not as expected but are consistent from MCB to EoPC), what additional work might be needed to secure acceptability of the cell line for registration?

NOTES:
Control of Product Consistency during Cell Culture Cultivation

Session Chairs: Dominik Gaser, Sandoz Biopharmaceuticals and Raghavan Venkat, MedImmune, A member of the AstraZeneca Group

This session will explore the approaches to ensuring appropriate characterization and control of product quality throughout the cell culture process including advancements in analytical technologies and regulatory expectations for existing technology applications as well as new technologies.

NOTES:
Non-mAb drugs are representing an increasing percentage of the pipelines of biopharmaceutical companies. The successful development of these novel molecules into a commercial product represents a significant challenge given the increased complexity and wider range of post-translational modifications that are often observed. Defining the quality attributes early in the project lifecycle is essential to ensure the key product attributes for each molecule (e.g. attributes affecting activity and/or half-life) can be monitored during the development of bioprocesses. This in-process monitoring is crucial for controlling the product quality from early material supply through to toxicology batches and beyond to enable the rapid, and successful, progression of these molecules through the drug pipeline to commercialization. The limiting factors for obtaining this data with conventional techniques are often a combination of throughput, amount of sample required and data generation/analysis time. However, if product attribute information is to be used to drive decisions during early development activities, the techniques used must be able to generate the data for a large number of samples in a short timeframe.

At MedImmune, we have developed an analytical toolbox using state-of-the-art equipment to monitor product attributes (e.g. glycosylation, truncation, aggregation, terminal clips) throughout the bioprocess. These methods are high throughput, require low sample volumes and have a fast turnaround to enable timely decisions to be made. In most cases the techniques can be applied to crude supernatants for the analyses permitting screening during early cell line development stages as well as process development and process optimizations in bioreactors. This presentation will describe the implementation of these high throughput analytics into the CLD process to enable product attribute-driven cell line selection with consideration for the final commercial process. Case studies describing the use of these tools in the development of novel molecules will be described.
Advances in Product Characterization during Cell Cultivation

Jason Rouse; T. Jennifer Lin; Lisa Marzilli; Karin Anderson

Pfizer, Inc., Andover, MA USA

Traditionally, heightened product characterization via mass spectrometry (MS) and biophysical approaches confirms that drug substance contains the intended molecule with the expected primary structure, posttranslational modifications, and higher-order structure. The elucidation of structure, in combination with biochemical/functional release and stability testing, serves to establish structure-function relationships, identify product quality attributes, and build product and process knowledge, forming the basis of the control strategy, which helps ensure clinical safety and efficacy. Since the late 2000’s, the critical performance parameters of research grade mass spectrometers, such as resolution, mass accuracy, sensitivity, dynamic range, stability, and speed, have improved significantly, thereby providing more definitive elucidation of major, minor and trace level product proteoforms in a shorter time, as well as enabling new mainstream approaches to biotherapeutics characterization. As a result, the application of ultrahigh-resolution LC-MS/MS-based methods in biotherapeutics upstream and downstream process development has been growing steadily with respect to sequence variant (SV) analysis (1,2,3) and host-cell protein (HCP) analysis (4,5,6), respectively. At this time, both SVs and residual HCPs can be reliably detected, identified, and quantitated by LC-MS/MS (in combination with bioinformatics) down to a level of 0.001%, providing valuable insights as the final production process is developed, optimized, and scaled-up. Furthermore, the application of various ultrahigh-resolution MS-based methods in a multi-attribute format (7) at the clone selection stage and during cell culture process development has yielded vital product quality information at the molecular level for C-terminal lysine, trisulfides, N-glycosylation patterns, aglycosylation, signal peptides, misincorporations, etc. that overall helps steer process development, as well as affords more robust cell lines and increased process understanding/consistency. In the future, on-line LC/MS in the pilot plant is envisioned to help provide product attribute control in real-time producing greater batch-to-batch consistency with less off-line, in-process characterization testing (8). In this presentation, we will share our experiences and lessons learned in the application of ultrahigh-resolution MS-based methods for bioprocess development.

1. Unintended amino acid substitutions due to genetic mutations, mistranslation events, or misincorporations (i.e., amino acid depletion in cell culture medium).
4. Residual protein impurities secreted by the expression system or released by cell lysis.
5. Doneanu et al. mAbs 2012, 4, 24-44.
7. Rogers et al. mAbs 2015, 7, 881-890.

NOTES:
Different expression systems are used for the manufacture of therapeutic proteins. Currently mammalian cell culture systems especially Chinese hamster ovary (CHO) cells are predominantly used although other expression in bacteria, yeast and human cells are also in place. The expression host as well as the culture conditions strongly influence the protein quality attributes. Posttranslational modifications such as glycosylation are critical protein quality attributes that can modulate the efficacy of a commercial therapeutic glycoprotein. Obtaining a consistent profile in production is desired because a molecule can be defined by its structures. Posttranslational control in mammalian cell culture, through cellular, media, and process effects are well known. There is a close relationship between these variables and the resulting protein quality. Recently it became clear that not only posttranslational modifications might impacted but also the primary structure of recombinant proteins might change during cell cultivation, e.g. due to initial issue during cell bank establishment or genetic drift. Beside confirmation of genetic stability phenotypic stability studies should be performed in order to illustrate the effect of cell age on drug substance attributes. The extended characterization of functional parameters such as biological activity or pharmacokinetics should be considered. Depending on the outcome of these studies the inclusion of quality attributes such as sequence variants or glycoforms into the specifications with defined acceptance criteria might become necessary. Case studies will be presented in order to explain regulatory expectations with regard to the overall control strategy with regard to the cell cultivation process.

NOTES:
Establishing a well-characterized production cell line, while time-consuming, forms the foundation to ensure that critical product characteristics are maintained throughout product development and after post-licensure manufacturing changes. Recent biosimilar product development programs, where a great deal efforts have been undertaken in selecting an appropriate production cell line such that the critical quality attributes of the biosimilar product match the reference product, further illustrate the need for a well-characterized production cell bank early in product development. Therefore, the general expectation is that cell banks are well characterized during early IND phase and adequate data are available at the time of BLA submission. However, under certain circumstances, such as products under accelerated development programs, the cell bank may not be fully characterized, and data provided in a BLA may only be sufficient to suggest that the risk to product quality is relatively low. Under such situations, a well-designed, augmented control strategy to manage residual risk is expected to assure well-controlled manufacture of drug substance in the BLA. The control strategy should minimally include limits on in vitro cell age and comprehensive product characterization in combination with the end-of-product cell population or sub-population analyses. It is recognized that some parts of such strategies may require a substantial time commitment; the control strategy can therefore be accomplished as a combination of an interim plan to support licensure, and post marketing commitments to establish a well-controlled production cell bank and cell culture process as a part of product life-cycle management program.

NOTES:
Panel Discussion – Questions and Answers
Fiona Cornel, Health Canada, Canada
Steffen Gross, Paul-Ehrlich-Institut, Germany
Juhong Liu, CDER, FDA, USA
Ilona Reischl, AGES-Austrian Agency for Health and Food Safety, Austria
Jason Rouse, Pfizer, Inc., USA
Christopher Sellick, MedImmune Limited, United Kingdom

The following questions will guide the panel discussion:

1) Theme: Strategies for Monitoring and Controlling Product Quality and Consistency
   a. To ensure appropriate and consistent product quality for both clinical development and commercial use, what strategies are being pursued:
      i. During cell line development?
      ii. During cell culture process development?
      iii. Post-approval?
      iv. How do these strategies align with regulatory expectations and how should they be communicated?
      v. What considerations are appropriate for characterizing cells at the limit for in vitro cell age?

2) Theme: Current Trends in Monitoring and Controlling Product Quality and Consistency
   a. Low-level sequence variants are becoming an increasing topic of interest:
      i. What are the risks associated with sequence variants?
      ii. What methods should be used to evaluate sequence variants?
      iii. What is an appropriate limit of detection for sequence variants?
   b. There is a trend towards tighter control of CQAs such as glycosylation, sequence variants, and other product-related impurities:
      i. What is the current state of the industry in terms of understanding and controlling CQAs through the cell culture process?
      ii. How should control strategies evolve based on the continued improvement in the understanding of processes:
         1. During development?
         2. Post-licensure?
   c. What new technologies and existing technology applications exist in monitoring and controlling product quality and consistency?
      i. What are regulatory expectations for the use of these new technologies and applications?
      ii. How should application of emerging technologies and “updated” control strategies be transitioned into regulatory filings?

3) Theme: Addressing “Challenging” Cases
   a. If a cell line is discovered to be non-clonally derived late in development (or even during commercial production), how should product characterization (and the overall control strategy) be addressed from both an industry and regulatory perspective?

NOTES: