THE HUMAN PROTEIN ATLAS – IMPLICATIONS FOR HUMAN BIOLOGY, DRUG DEVELOPMENT AND PRECISION MEDICINE

Matthias Uhlén, KTH Royal Institute of Technology and Karolinska Institute, Sweden
mathias.uhlen@scilifelab.se

Key Words: Human Protein Atlas, bioproduction, drug development, precision medicine

The Human Protein Atlas (HPA) is a Swedish-based program with the aim to map of all the human proteins in cells, tissues and organs using integration of various omics technologies, including genomics, transcriptomics, antibody-based imaging, mass spectrometry-based proteomics and systems biology. A Tissue Atlas was launch in 2015 (1) followed by a Cell Atlas in 2016 (2) and a Pathology Atlas in 2017 (3). This open access knowledge-base can be used to explore targets for next generation antibody therapeutics, as well as a discovery tool to find potential biomarkers and drug targets for disease (4,5). A focus has been to use a new drug development platform based on the affibody molecule developed in our group and use this concept for applications in cancer, autoimmune diseases and neurodegenerative diseases. Recently, we have set-up an animal cell factory using CHO cells with the aim to produce full-length proteins representing all the 2,000 secreted proteins encoded in human genome. The Human Protein Atlas program has already contributed to several thousands of publications in the field of human biology and disease and it was recently selected by the organization ELIXIR as a European core resource, due to its fundamental importance for a wider life science community. All the data in the knowledge resource is open access to allow scientists both in academia and industry to freely access the data for exploration of the human proteome.

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MOVING BEYOND CHO: ALTERNATIVE HOST SYSTEMS MAY BE THE FUTURE OF BIOTHERAPEUTICS

Christina Alves, Biogen
christina.alves@biogen.com
Chapman Wright, Biogen
Hanxiao Jiang, Amyris
Carl Co, Biogen
Matthew Westoby, Biogen
Venkatesh Natarajan, Biogen
Christopher Love, MIT
Bill and Melinda Gates Foundation
Heather Saforrian, Biogen

Key Words: Non-mammalian hosts, antibody expression, increased productivity

CHO cells are the primary expression system for recombinant proteins with significant investment over the last three decades resulting in robust cell lines and processes. The flexible nature of CHO has lent itself to multiple process formats, such as fed batch, perfusion and continuous cultures, and advances in omics technology has enabled customization of media formulations and targeted engineering of CHO cells. This knowledge has led to large gains in protein productivity that can be captured with culture duration and/or scale. Despite this, constant pressure exists to reduce cost of manufacturing and improve per batch productivity to meet the needs of increased patient populations and increase accessibility of these therapeutics. Biogen has partnered with MIT to take a holistic view of the potential future of biomanufacturing to identify technologies that can make step changes in productivity and cost reduction. This effort has identified the host system as the most important factor to enabling this vision. Specifically, a non-mammalian host could be the key to realizing the most significant gains in productivity and reduction in cost of manufacturing.

Through this initiative, we sought to take a more comprehensive approach to investigate alternative hosts for recombinant antibody production. Eight non-mammalian hosts were selected based on several properties, including proven secretion of recombinant protein products, ability to glycosylate proteins, established genome or molecular biology toolkit, amongst others. The final panel of organisms included yeast, filamentous fungi, a diatom, and a trypanosome. In collaboration with Amyris, we evaluated these eight non-mammalian host cell lines to compare their suitability as a potential primary host for the biotechnology industry. Only non-engineered, wild-type strains were used as a starting point for this evaluation, which assessed the ability of each host to express the same IgG1 model antibody. The outcome of this comparative analysis demonstrated that several of the alternative hosts could express full length antibody with acceptable glycoforms. Additionally, the ease of culture, ability to engineer the genome, and flexibility of carbon source were assessed. As an output of this work, the most productive strains will be made available for use without restrictions to allow others in the community to freely work with these hosts. Based on this initial assessment, a strategy to further investigate the potential of the most promising hosts will be shared.

INSECT CELL PLATFORMS FOR PRODUCTION OF PSEUDO-TYPED VLPs FOR DRUG AND VACCINE DEVELOPMENT

António Roldão, iBET/ITQB-NOVA, Apartado 12, 2780-901 Oeiras, Portugal
aroldao@ibet.pt
Marco Patrone, San Raffaele Scientific Institute, Italy
Manuel Carrondo, iBET, Portugal
João Vidigal, Barbara Fernandes, Ana Teixeira and Paula Alves, iBET/ITQB-NOVA, Portugal

Key Words: Insect cells, VLPs, Flipase-mediated cassette exchange, Bioprocess engineering, Drug and vaccine development

Conformational-complex membrane proteins (MPs) are vaccine/drug targets in many diseases, but drug and vaccine development has been slowed down by the lack of efficient production tools. Co-expression of MPs with matrix proteins from enveloped viruses is a promising approach to obtain correctly folded proteins at the surface of ordered nanoscale architectures such as virus-like particles (VLPs), preserving their native lipidic environment.

In this work, we implemented an innovative site-specific recombination strategy based on flipase-mediated cassette exchange technology to establish reusable insect cell platforms for fast production of enveloped VLPs pseudo-typed with target MPs. Influenza M1 and HIV Gag proteins were evaluated as scaffolds, and proof-of-concept (PoC) demonstrated using two membrane proteins, the influenza HA protein (e.g. for vaccines) and the human beta-2 adrenergic receptor (e.g. for drug screening or antibody discovery). Bioprocess engineering schemes were designed (adaptive laboratory evolution to hypothermic culture conditions and supplementation with productivity enhancers), allowing to improve HIV Gag-VLPs production in the developed stable insect cells. Under hypothermic culture conditions, adapted cells expressed up to 30-fold more HIV Gag-VLPs than non-adapted cells. Noteworthy, the element driving such increase in productivity is the adaptation process and not the temperature shift as the later alone leads to lower production yields. A more modest increase in productivity (up to 7-fold) was observed when supplementing non-adapted cell cultures with productivity enhancers NaBu and DMSO. PoC was successfully demonstrated in 0.5 L stirred-tank bioreactors.

Profiting from the platforms developed above, a modular system comprising stable and baculovirus-mediated expression in insect cells was established for the production of a multi-HA influenza VLP as vaccine candidate that otherwise could not be obtained due to baculovirus vector instability. By combining stable with transient expression systems, we could rationally distribute the number of genes to be expressed *per* platform and thus generate the target VLP for subsequent animal studies. In addition, a tailor-made refeed strategy was designed based on the exhaustion of key nutrients during cell growth resulting in a 4-fold increase in HA titers *per* mL. PoC was successfully demonstrated in 2 L stirred-tank bioreactors.

Overall, the insect cell platforms and bioprocess engineering strategies herein assembled have the potential to assist/accelerate drug and vaccine development.

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PRODUCTION OF BIOPHARMACEUTICALS IN AN INTENSIFIED PERFUSION PROCESS OF HEK 293 CELLS

Johan Rockberg, KTH ; Wallenberg Centre for Protein Research (WCPR) ; NovoNordisk Center for Biosustainability (NNF CFB), Sweden
Caijuan Zhan, KTH ; Wallenberg Centre for Protein Research (WCPR)
Magdalena Malm, Wallenberg Centre for Protein Research (WCPR)
Hubert Schwarz, KTH ; Wallenberg Centre for Protein Research (WCPR)
Magnus Lundqvist, KTH ; Wallenberg Centre for Protein Research (WCPR) ; NovoNordisk Center for Biosustainability (NNF CFB), , Sweden
Atefeh Shokri, KTH ; Wallenberg Centre for Protein Research (WCPR) ; NovoNordisk Center for Biosustainability (NNF CFB), , Sweden
Ray Field, BioPharmaceutical Development, MedImmune, Cambridge, UK
Richard Turner, BioPharmaceutical Development, MedImmune, Cambridge, UK
Veronique Chotteau, KTH ; Wallenberg Centre for Protein Research (WCPR) ; AdBIOPRO, Competence Centre for Advanced Bioproduction by Continuous Processing

CHO cells are the workhorses of the biopharmaceutical field with many success stories. However human cell-based systems might bring important advantages. These can provide production systems resulting in proteins with more human-like posttranslational modifications and potentially alleviate the production of difficult-to-produce molecules. HEK 293 cells are well known and used today for the production of two biopharmaceuticals and for viral vectors. The purpose of the present study is to evaluate the potential of this system for its ability to produce biopharmaceuticals, benchmarking against CHO cells.

We are currently exploring the possibility to secrete human proteins in CHO cells by systematically addressing all the human proteins naturally secreted in the human body. So far, we have covered around half of the human secretome (N = 3000) with an overall success rate around 65%. To address the need for a host capable of expressing difficult-to-produce proteins, different HEK 293 strains have been investigated for the production of 30 selected proteins in comparison with CHO cells, revealing a higher success rate in HEK 293 system. This expression has been studied in flask system and includes comparative transcriptomics analyses.

To evaluate the potential of HEK 293 cells for the production of biopharmaceuticals, a high cell density perfusion process using Alternating Tangential Flow filtration has been developed for the production of EPO. In this process, the cells are stably maintained at a density of 80 to 100 x 10^6 cells/mL while the EPO cell specific productivity is comparable to low cell density (e.g. 20 x10^6 cells/ml) in perfusion mode. The cell metabolism is slowed down by lowering the temperature, allowing a reduction of the perfusion rate down to 1 reactor volume per day at this high cell concentration. This process has been developed in our new scale-down perfusion bioreactor of 200 mL working volume. In this system, the effect of shear stress on the HEK 293 cells resulting from their passage in the hollow fibre filter has been characterised by transcriptomics analysis helping to decipher why HEK 293 cells are more sensitive than CHO cells and a systematic feeding strategy for perfusion has been developed.

The ability to express difficult-to-produce proteins and to achieve very high cell densities with productivity comparable to low density processes make HEK 293 cells an attractive system for the production of biopharmaceuticals which are challenging for CHO cells.

BEYOND CHO CELLS: CELL-FREE PROTEIN SYNTHESIS FOR BIOTHERAPEUTICS

Robert Kiss, Sutro Biopharma
bkiss@sutrobio.com
James Zawada, Sutro Biopharma
Robert Hoffman, Sutro Biopharma
Dawei Yuan, Sutro Biopharma
Maureen Bruhns, Sutro Biopharma

Key Words: cell-free protein synthesis,

The production of recombinant proteins and antibodies using a cell-free synthesis step can now deliver industrially-relevant quantities of the target biologic. The technology is ideally suited to the rapid generation of antibody-drug conjugates (ADCs), bispecific antibodies, and other protein formats that do not require glycosylation for functionality. A proprietary technology allows for incorporation of a non-natural amino acid at the desired sequence location(s) that can subsequently be combined with site-specific conjugation chemistry to deliver highly precise drug to antibody ratio (DAR). This presentation will describe this alternative production technology in terms of the underlying technology, the technical challenges that have been overcome and the milestones achieved, and the benefits that can be delivered using this approach. In particular, a cell-free protein synthesis step enables extremely rapid (e.g, days) generation of candidate molecules once plasmid DNA is available. This allows for rapid screening of multiple candidate molecular sequences and formats without the need to utilize or generate any living cells (CHO, or otherwise) on the critical path to product expression. The applications of this technological approach will be highlighted and contrasted with the CHO workhorse of our industry.

ENGINEERING CHO CELLS FOR THE PRODUCTION OF "HARD-TO-PRODUCE" PROTEINS

Bjørn Voldborg, the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark. bgrv@biosustain.dtu.dk
Stefan Kol, the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.
Johnny Arnsdorf, the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.
Anders Holmgaard Hansen, the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.
Tune Wulf, the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.
Hooman Hefzi, the Novo Nordisk Foundation Center for Biosustainability, University of California San Diego
Nathan Lewis, the Novo Nordisk Foundation Center for Biosustainability, University of California San Diego
Marianne Decker, the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.
Johan Rockberg, the Novo Nordisk Foundation Center for Biosustainability, Kungliga Tekniske Högskolan, Stockholm.
Helene Faustrup Kildegaard, the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark
Bernhard Palsson, the Novo Nordisk Foundation Center for Biosustainability, University of California San Diego

Key Words: CHO, Engineering, Product Quality, Hard-to-produce Proteins

Over the past decades, the CHO cell has become increasingly popular as the favorite host cell line for the production of protein based therapeutic drugs. In comparison with the popularity of the CHO cells and the frequent use of these cells to produce a large part of the bestselling blockbuster drugs, less intensive efforts have been done to understand the machinery used by the CHO cells during growth and production. The main approach has (broadly speaking) been to approach the CHO cell as a "black box" where one could insert the gene of interest, perform a number of amplifying steps, like gene amplification, selection for stable clones, intense screening for stably expressing high producers, and massive efforts to optimize a specific bioprocess for the selected cell line(s).

Since 2013, the Novo Nordisk Foundation Center for Biosustainablity at the Technical University of Denmark has embarked on a large CHO program to open up the "black box", to get a deeper understanding of the available machinery inside the protein producing "cell factory" that is CHO cells. We are using this understanding to engineer new CHO cell lines having significantly improved features for the production of therapeutic proteins. We are not only doing this by improving the titer, quality, downstream processing and speed of development for already well-known proteins (e.g. Ab), but also for the production of therapeutic proteins that cannot be produced in CHO cells today, due low titer, wrong post translational modifications, and/or low activity.

By combining the competences embedded in the CHO program, we are able to exploit the combination of genome scale modelling, high throughput protein expression, deep understanding of both the glycosylation machinery as well as the secretory and metabolic pathways involved in the expression of secreted proteins. This knowledge is being used as input to a high throughput CHO cell line engineering pipeline, able to engineer up to 10 cell lines and 25 gene targets in parallel.

This has resulted in a large number of new CHO cell lines enabling the production of proteins with specific tailor-made glycoprofiles, higher quality, less degradation, improved bioprocess, higher viable cell density and better cell viability.

We have made a cell lines where we have removed a number of naturally expressed host cell proteins (HCP) from CHO, which has resulted in higher titer and higher VCD, cell lines showing increased resistance to viral infections, cell lines displaying homogenous glycoprofiles, reduced degradation, and drastically changed cell lines that does not produce lactate. These features are currently being combined to engineer CHO cells able to produce proteins that have not been possible to produce with adequate product quality and titer using CHO cells to date.

CRISPR/CAS9 MEDIATED KNOCKOUT OF MICRORNAS FOR PECISE CELL ENGINEERING

Nadja Raab, University of Applied Sciences Biberach
raab@hochschule-bc.de
Kerstin Alt, Genetikum Neu Ulm
Sven Mathias, University of Applied Sciences Biberach
René Handrick, University of Applied Sciences Biberach
Vaibhav Jadhav, BOKU Vienna
Kerstin Otte, University of Applied Sciences Biberach

Key Words: CRISPR/Cas, microRNA, CHO production cells, multiplexing, apoptosis

Recent advances in the development of molecular tools available for cell line engineering has opened avenues for precise modulation of production cell lines to optimize cellular process relevant parameters. Among those, the CRISPR/Cas9 system represents a rapid and straightforward cell line engineering tool which allows for precise gene editing of host genomes to modify relevant signaling pathways. In addition, microRNAs (miRNAs) have proven to serve as versatile molecular tools to improve production cells regarding protein production and growth characteristics. These small non-coding RNA molecules are regulators of gene expression and regulate process relevant cellular pathways as growth, apoptosis, protein expression or stress. With an individual microRNA being able to regulate up to several hundred target genes, the modulation of miRNA expression may mimic simultaneous modification of several hundred target genes. Therefore, miRNAs may offer great potential to serve as targets for CRISPR/Cas9-mediated genome editing, as an individual microRNA can regulate up to several hundred target genes and therefore mimics multiplexing of hundreds of target genes in one single step. While the overexpression of miRNAs to improve the performance of biopharmaceutical production hosts has received major interest in the past years, effects of precise knockout of unfavorable miRNAs in Chinese hamster ovary (CHO) production cells have not been reported yet.

Therefore, the current study aimed at applying a novel strategy to increase product yields by enhancing viability and culture longevity of CHO cells using CRISPR/Cas9-mediated deletion of a miRNA causing adverse effects. In a previous high content screen with more than 1000 miRNAs we were able to identify miR-744 as being involved in the regulation of apoptosis. After the apoptosis-inducing capacity of miR-744 in monoclonal antibody-producing CHO-mAb1 cells was revalidated, the genomic precursor sequence of miR-744 was deleted by two sgRNA-Cas9-mediated DNA double-strand breaks flanking the miR-744 gene in the CHO genome. A plasmid encoding both required sgRNAs in a tandem array, Cas9 and GFP was used to allow for simultaneous delivery of all required components and thereby reducing the screening effort for potential *knockout* (KO) cell lines. After fluorescent-activated cell sorting (FACS) seven putative, clonal miR-744-KO cell lines could be recovered. From these, three were identified and confirmed as miR-744-KOs by sequencing of their miR-744 locus respectively. In a subsequent growth experiment over a period of nine days considerable differences in the investigated parameters viable cell count, viability, apoptosis, necrosis and antibody titers of the miR-744-KO cell lines in comparison to untreated cells and non-targeting sgRNA transfected cells could be observed. Further experiments are conducted to analyze the observed effects.

In summary, the present study elucidates the role of microRNAs, which bear adverse effects on production cells, as targets for the novel CRISPR/Cas9 genome editing technique in the context of cell line engineering. This approach contributes to the idea of a rational design of CHO production hosts with controlled modulation of signaling pathways or metabolic characteristics to enable the generation of optimized production cells.

miR-CATCH IDENTIFIES BIOLOGICALLY ACTIVE miRNA REGULATORS OF THE PRO-SURVIVAL GENE XIAP, IN CHINESE HAMSTER OVARY CELLS

Paul S Kelly, National Institute for Bioprocessing Research and Training
paul.kelly@nibrt.ie
Alan Griffith, National Institute for Cellular Biotechnology
Sebastian Vencken, Department of Clinical Microbiology
Nga T Lao, National Institute for Cellular BiotechnologyCatherine M Greene
Martin Clynes, National Institute for Cellular Biotechnology
Niall Barron, National Institute for Bioprocessing Research and Training

Key Words: miR-CATCH, microRNAs, Targeted Cell Engineering, Recombinant Proteins, XIAP

Genetic engineering of mammalian cells, in particular Chinese hamster ovary (CHO) cells, is of critical interest to the biopharmaceutical industry as a means to further boost the yields of therapeutic proteins. Complimentary to already in place advanced bioprocesses, stable overexpression of the pro-survival X-linked inhibitor of apoptosis (XIAP) is one example of the successful manipulation of CHO cell genetics resulting in prolonged culture survival, ultimately increasing recombinant protein productivity. However, saturation or burdening of the cells translational machinery can occur in instances of forced expression of a trans-gene thereby achieving the anticipated cellular phenotype without the associated improvement in productivity. Ribosomal footprint sequencing has demonstrated that ~15% of an IgG-producing CHO cell translatome is occupied by the Neomycin selection marker. microRNAs (miRNAs) have the ability to fine tune endogenous gene expression thereby achieving elevated gene levels without the excess that could negatively impact global gene expression. Additionally, not only does a single miRNA have the capacity to regulate multiple mRNA transcripts simultaneously but individual mRNAs can be regulated by a multitude of miRNAs at the post-transcriptional level. This can facilitate the maximal translation of an endogenous gene without surpassing the super-physiological threshold associated with diminished productivity. The promiscuous nature of miRNA represented by the variety of binding patterns associated with mRNA targeting limits the predictability of high confidence miRNA regulators of attractive engineering candidates. This results in a lengthy list of falsely predicted *in-silico* miRNA regulators for a single gene. We explored the identification of direct miRNA regulators of the pro-survival endogenous XIAP gene in CHO-K1 cells by using a miR-CATCH[1] protocol. A biotin-tagged antisense DNA oligonucleotide was designed for an exposed predicted secondary structure loop of endogenous CHO XIAP. This mRNA anchor resulted in the pulldown of XIAP and all associated RNA/protein complexes thereby enriching for all bound miRNAs. Two miRNAs were chosen out of the 14 miRNAs identified for further validation, miR-124-3p and miR-19b-3p. Transient transfection of mimics for both resulted in the diminished translation of endogenous CHO XIAP protein whereas their inhibition increased XIAP protein levels (*Fig. 1).*

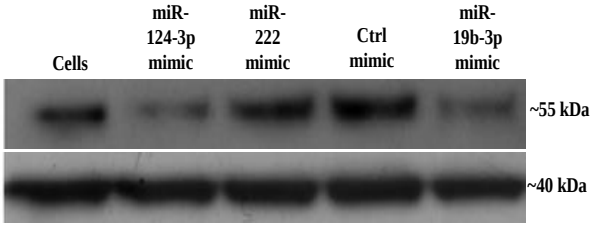

Fig. 1 P

Furthermore, simultaneous inhibition of the miRNAs further enhanced XIAP translation beyond what was achieved independently. A 3'UTR reporter assay confirmed miR-124-3p to be a bona fide regulator of XIAP in CHO-K1 cells. Despite miR-19b-3p inhibiting XIAP protein translation, no repression was observed in 3'UTR assays suggesting binding sites

sites to be within the coding sequence itself. This method demonstrates a useful approach to finding miRNA candidates for CHO cell engineering without competing for the cells translational machinery. The miRNAs identified may be suitable for CRISPR-directed genomic knockout to ensure translational derepression of XIAP followed by the predicted extended survival phenotype.

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GLYCOENGINEERING OF CHO CELLS FOR PRODUCTION OF RECOMBINANT THERAPEUTICS WITH ENHANCED EFFICACY

Zhiwei Song, PhD, Bioprocessing Technology Institute, A*STAR, Singapore
song_zhiwei@bti.a-star.edu.sg

Key Words: Glycoengineering, CHO cells, Glycan structures, Recombinant therapeutics, Antibodies

Glycosylation can significantly affect the efficacy of recombinant therapeutics. Glycoprotein drugs, such as EPO, require a high degree of sialylation on their N-glycans in order to have a longer circulatory half-life. Mannose-terminated N-glycans can target the protein to dendritic cells and macrophages cells via cell surface mannose-binding receptors. Removal of core fucose from human IgG1 antibodies has been shown to significantly enhance its affinity to Fc□RIIIa and thereby dramatically improves its antibody-dependent cellular cytotoxicity (**ADCC**). Cancer cells generally express glycoproteins with shortened O-glycans. Therefore, recombinant anti-cancer vaccines carrying these short tumor-associated O-glycans are more ideal for triggering specific anti-tumor immune responses.

With cytotoxic lectins and genome editing tools, namely ZFNs, TALENs and CRISPR-Cas9, we have so far created 29 CHO glycosylation mutant cell lines (CHO-gmt1 to CHO-gmt29; gmt = glycosylation mutant). In some of these mutant lines, only one gene has been inactivated (CHO-gmt1, 2, 3 4 cells), whereas in others more than 10 glycosylation genes have been knocked out in order to obtain a particular glycan structure. With these mutants, we have been able to produce EPO with highly sialylated N-glycans for extending *in vivo* half-life. We have developed stable cell lines to produce recombinant human β-glucocerebrosidase with mannose-terminated N-glycans (mainly Man5). This product is similar to Cerezyme, but the *in vitro* glycan modification is not needed. We have also developed stable cell lines to produce fucose-free rituximab and GA101 with high titers. In a cell-based ADCC assay, these fucose-free rituximab and GA101 outperformed their commercial counterparts, Rituxan and Gazyva, respectively.

Furthermore, rituximab produced by more than a dozen of these CHO-gmts were purified and the glycans attached to the antibody were analyzed by MALDI-TOF MS and LC-MS. The results showed that many of these mutants were able to produce the antibody with one major N-glycan which represents 90-97% of the total N-glycans attached to the antibody. These antibodies are invaluable tools for studying the impact of glycans on antibody functions including PK/PD characteristics. Rituximab produced by these CHO-gmt cells have been compared in a cell-based ADCC assay. Their binding affinities for Fc□RII, Fc□RIIIa and the neonatal Fc receptor (FcRn) were also determined. As expected, fucose-free glycans bind Fc□RIIIa much tighter than others, and are most effective in activating ADCC. While most glycans did not affect the binding to Fc□RII and FcRn, two glycans significantly enhanced the binding for Fc□RII and FcRn. The *in vivo* cell killing ability and circulating half-life for the rituximab antibodies that carry different N-glycans are being investigated in mouse models.

Taken together, we have generated a large panel of CHO glycosylation mutants. Each of these mutants produces a unique set of N- and O-glycans. They can be used to study the impacts of glycans on therapeutic efficacies of biologics. Our study on the rituximab carrying different homogeneous N-glycans represents a first systematic investigation on the impact of glycans on antibody functions.

ELIMINATION OF THE "ESSENTIAL" WARBURG EFFECT IN MAMMALIAN CELLS THROUGH A MULTIPLEX GENOME ENGINEERING STRATEGY

Nathan E. Lewis, Novo Nordisk Foundation Center for Biosustainability at the University of California, USA
n4lewis@ucsd.edu
Hooman Hefzi, University of California, USA
Soo Min Noh, KAIST
Iván Martínez Monge, Autonomous University of Barcelona
Nusa Pristovsek, Novo Nordisk Foundation Center for Biosustainability, DTU
Marianne Decker, Novo Nordisk Foundation Center for Biosustainability, DTU
Elham Maria Javidi, Novo Nordisk Foundation Center for Biosustainability, DTU
Kristian Jensen, Novo Nordisk Foundation Center for Biosustainability, DTU
Anders Holmgaard Hansen, Novo Nordisk Foundation Center for Biosustainability, DTU
Stefan Kol, Novo Nordisk Foundation Center for Biosustainability, DTU
Johnny Arnsdorf, Novo Nordisk Foundation Center for Biosustainability, DTU
Zulfiya Sukhova, Novo Nordisk Foundation Center for Biosustainability, DTU
Karen Kathrine Brondum, Novo Nordisk Foundation Center for Biosustainability, DTU
Patrice Ménard, Novo Nordisk Foundation Center for Biosustainability, DTU
Sara Petersen Bjorn, Novo Nordisk Foundation Center for Biosustainability, DTU
Helle Munck Petersen, Novo Nordisk Foundation Center for Biosustainability, DTU
Lars K. Nielsen, Novo Nordisk Foundation Center for Biosustainability, DTU
Helene Faustrup Kildegaard, Novo Nordisk Foundation Center for Biosustainability, DTU
Gyun-Min Lee, Department of Biological Sciences, KAIST
Bjorn Voldborg, Novo Nordisk Foundation Center for Biosustainability, DTU

Key Words: CRISPR, metabolism, lactic acid, metabolic engineering, combinatorial genome engineering.

The Warburg effect has posed a constant challenge in mammalian bioprocessing since the field began. Indeed, the predisposition of mammalian cells to secrete large quantities of lactic acid through the Warburg effect leads to premature cell death, reduced product yields, and often lower quality products. Thus, over the past decades, numerous innovations in the mammalian cell culture field have focused on mitigating lactate secretion, including through media optimization, feeding control, chemical inhibition, etc. Despite extensive efforts from many researchers, complete elimination of lactic acid production has not yet been obtained. Specifically, several independent efforts to knock out lactate dehydrogenase (the enzyme responsible for producing lactic acid from pyruvate) have been unsuccessful, as it has seemed essential for immortalized cell growth.

Here I present our work in which we discovered a panel of genes involved in a genetic feedback circuit that controls lactic acid secretion in mammalian cells. Knocking out individual genes in serial was unsuccessful since LdhA and other targets are essential for CHO cell growth. However, we knocked out these genes simultaneously and overcame the "essentiality" of these genes, leading to the successful elimination of lactic acid secretion in Chinese hamster ovary cells.

Since many hypotheses have been proposed regarding the essentiality of lactic acid secretion for rapid cell proliferation in cancer, immune cell activation, and embryonic development, we were interested to study how the complete elimination of the Warburg effect impacts CHO cells. Surprisingly, the cells show improved metabolic and growth phenotypes, despite the elimination of this fundamental metabolic activity. To understand how immortalized mammalian cells can cope without this seemingly essential metabolic process, we conducted a comprehensive analysis of these cell lines using time-course RNA-Seq, metabolomics, and analysis with a genome-scale metabolic network model developed for Chinese hamster ovary cells[1]. We further characterized its impact on recombinant drug production yields and quality. Thus, through a multiplex metabolic engineering effort and comprehensive systems biology analysis, we have been able to engineer out a leading challenge in protein biotherapeutic development and begin to understand now a cell can survive without a seemingly essential process.

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LIMITATIONS OF SUBCLONING AS A TOOL TO CHARACTERIZE HOMOGENEITY OF A CELL POPULATION

Hedieh Barkhordarian*, Nicole Tejeda*, Tharmala Tharmalingam*, Pheng Yam, Sam Yaghmour, Trent Munro, Chetan Goudar, Jennitte Stevens
* = These authors contributed equally.
Cell Line Development, Drug Substance Technologies, Process Development
Amgen, Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA

Cloning, or the derivation of a cell line from a single cell is a critical step in the generation of a manufacturing cell line. The expectation is that the process of cloning will result in a uniform and homogeneous cell line that will ensure robust product quality over the lifetime of the product. Regulatory guidelines require the sponsors provide assurance of clonality of the production cell line and when such evidence is not available, additional studies are required to further ensure consistent long-term manufacturing of the product. One approach to characterize homogeneity of a cell line is subclone analysis where clones are generated from the original cell line and an evaluation of their similarity is performed lines. To study the suitability of subclone analysis to provide additional assurance that a production cell line is clonally derived, an antibody producing CHO Master Cell Bank (MCB), which was cloned by a validated FACS method and with a clear documented day 0 image was characterized. Specifically, this MCB was subcloned and imaged to assure each of the subclones were derived from a single cell. A total of 46 subclones were analyzed for growth, productivity, product quality, as well as copy number and integration site analysis. Despite demonstration of clonality for both the MCB and the subclones, significant diversity in cell growth, protein productivity, and product quality attributes was observed between the 46 subclones. The diversity in protein productivity and quality were reproduced across bioreactor scales, suggesting that albeit different, the subclones were stable populations that varied from the parental clonal cell line. Additionally, while ~2-fold shifts in copy number were seen, no significant integration site changes were observed. Our data suggest subcloning induces changes (genetic or epigenetic) outside the region of the transgene which result in the subclones exhibiting a wide diversity in cell growth protein productivity, and product quality. Transcriptomic and genomic characterization studies are underway to further characterize the differences between subclones and the MCB. Importantly, the subclones do keep their individual characteristics as they mature and stabilize, suggesting that the resulting population that grows out of a single cell is stable but with unique properties. Overall, this work adds to the growing body of work on CHO cell plasticity and suggests that subcloning is not an effective approach to demonstrate homogeneity of a cell bank.

REGISTRATION ENABLING CAMPAIGN FOR ACCELERATED DEVELOPMENT: A PPQ STRATEGY WITH MINIMAL EARLY INVESTMENTS TO ENABLE FAST TO MARKET DEVELOPMENT FOR A PROMISING MONOCLONAL ANTIBODY

Jessica Wuu, Genentech, Inc.
wuu.jessica@gene.com
Kristen Koterba Douglas, Ph.D. Genentech, Inc.

Clinical development programs are increasingly designing trials and timelines with extra degrees of flexibility to allow for acceleration based on early positive data. It is becoming more common that a program in Phase I can plan for filing a Biological License Application in less then five years given a certain set of aggressive assumptions. This often places CMC activities on a critical and potentially risky path requiring a large resource commitment at an early stage of clinical development when the likelihood of commercial launch remains low.

In this presentation we explore a case study for one such program that demonstrated both a low supply demand and limited clinical data, but also has the potential for a fast-to-market strategy gated to positive clinical results. We introduce the concept of a Registration Enabling Campaign (REC) conducted in parallel with a traditional Phase III supply campaign that would supply the Registration Batches for a Biological License Application, but also eliminate the need for a traditional PPQ campaign. While this initially requires an early investment prior to Phase III, it significantly reduces the resources required for a traditional qualification campaign that would be otherwise discarded for a low volume product.

We will present the strategy for required prospective studies needed to support a Phase III-REC, rationales for study deferrals and overall risks associated with analytical quality control systems that must be leveraged at a very early phase in development.

DERIVATION OF PROCESS CONTROL STRATEGY FOR BIOSIMILAR – IS IT DIFFERENT FROM THE WAY CONTROL STRATGEY IS DERIVED FOR A NOVEL BIOLOGIC?

Dinesh Baskar, Biocon Research Limited
dinesh.baskar@biocon.com
Chandrashekhar KN, Biocon Research Limited
Saravanan Desan, Biocon Research Limited
Ankur Bhatnagar, Biocon Research Limited
Anuj Goel, Biocon Research Limited

Key Words: Biosimilar, process characterization, control strategy, Quality target product profile

Quality based development (QbD) has become the preferred choice for developing manufacturing process for any biologic drug. A proponent for this approach has been the US Food and Drug Association (FDA). Recently, the first QbD applications have been successfully filed with FDA. Biosimilars have also gained popularity in the recent past. Development of these drugs are very different from the way a novel biologic is developed. In the last five years, many companies around the world have started working on Biosimilars of which some companies have been able to successfully develop and get approvals for Biosimilars in both FDA and European Medicenes agency (EMA).

Application of QbD for a Novel and a Biosimilar drug is quite different. By nature of the requirement for developing a Biosimilar, quality of the 'reference product' against which the biosimilar is being developed is considered while making decisions during process development. Though the same concepts applies for a novel drug, the target quality profile is not as defined as one can write for a Biosimilar. This is because product quality information regarding the reference product is well-known and can be thoroughly analyzed and characterized. While the targets can be easily derived for a Biosimilar, deriving a process control strategy is tough.

Critical Process Parameter (CPP) is defined as a process parameter that has significant impact on the safety and efficacy of the drug. While this definition for CPP is applicable for a Bisomilar also, another aspect which requires consideration for a Biosimilar drug is the impact of process parameters on 'fingerprint biosimilarity'. Hence the classification of process parameters as those that are critical and those that are not is not as straight forward like for a Novel drug. Derivation of acceptance range for these parameters also is different – The acceptance range for CPPs when compared to that for a novel biologic is generally found to be narrow. This is because the desired range for the outputs (such as aggregates, glycan, charge, size variants etc.) is narrow owing to the product quality ranges observed for the reference product and not just the levels of the outputs which has an effect on safety and efficacy.

These subtle differences make deriving the process control strategy for a Bisomilar different from a novel biologic. In this presentation, a detailed overview of scale down model qualification, process characterization experiments, and the control strategy for Biosimilar manufacturing processes is provided. A case study will be presented which showcases some of these concepts of deriving control strategy as how it is applied for a Biosimilar process.

CONFRONTING THE ANALYTICAL CHALLENGES OF CHIMERIC ANTIGEN RECEPTOR T CELL

Heidi Zhang
Juno Therapeutics
USA
Heidi.Zhang@junotherapeutics.com

Intensive research and development effort has been devoted to Chimeric antigen receptor T (CAR-T) cell therapies. CAR-T presents unique challenges for analytical development due to its complex mechanism of actions, large heterogeneity of the starting material, the need for appropriate reference material, and the limited lot size and testing material. This presentation will use Lisocabtagene maraleucel as a case study to illustrate how an integrated analytical strategy enables the development of a late phase CAR-T program.

LARGE SCALE PRODUCTION AND CHARACTERIZATION OF EXOSOMES

Konstantin Konstantinov, Codiak Biosciences, USA
konstantin.konstantinov@codiakbio.com

Exosome-based therapeutics are rapidly evolving as a new modality with a promising potential in multiple clinical areas. Successful implementation requires development of robust large-scale processes for the manufacture of highly purified material. However, the complexity and heterogeneity of exosomes pose significant R&D challenges. Here, we present the successful development of a manufacturing process using immortalized human cells, currently operating at commercial scale. Contrary to traditional ultracentrifugation approaches for exosome production, our process is based on modern biochemical engineering principles, resulting in a robust and scalable operation. The related analytics and characterization methods are also discussed.

CELL CULTURE BIOPROCESS LEARNINGS – PAST SUCCESSES AND FUTURE CHALLENGES

Günter Jagschies, GE Healthcare Life Sciences, Freiburg im Breisgau, Germany
Guenter.Jagschies@ge.com

Key Words: manufacturing technologies, cost, future challenges

Therapeutic proteins, vaccines and – further away – cell and gene therapies are considered essential to find efficacious interventions and preventive measures to a rather long list of prioritized needs in healthcare. The challenge is developing into a 'tsunami' of sorts as the global demographic transition causes populations to age, dramatically accelerating the growth of non-communicable disease (NCD) in all parts of the world. India and China are the markets with the largest patient population for almost any NCD, or they are going to be in that position soon.

Competition in this field is ramping up with an intensity that was previously unknown. Not only are there typically five to ten molecules from western originator companies for the same medical indication, but now there is finally also a huge wave of biosimilars entering the global markets. Asia is a hotspot in three ways: as the largest future market, as a location for manufacturing, and as an area with growing significance for the development of biologics.

These developments all focus management teams onto financial performance of their businesses, namely via pricing of drugs and vaccines and cost of operations, not the least manufacturing cost. Different approaches to think about setting up manufacturing technologies such as continuous processing and incorporation of single-use equipment into routine large scale production are all part of this economic improvement discussion.

Individualized therapies at the extreme for just one patient are an exciting medical route but process scientists and regulatory affairs must have nightmares over cost and approval challenges. Healthcare payers are already alarmed looking at annual treatment costs between $10's to 100's of thousands per patient with current therapies produced with the best of todays' manufacturing tools. Regulators find proteins complex enough, cells must look like 'mission impossible' to them with today's approaches. The discussion about future strategies and technical approaches goes far beyond the science and the engineering of bioprocesses.

This presentation aims to describe the future scenario with its challenges, extract what was truly successful in the past, but then also de-mystify some of the hype topics in technology often considered as the holy grail of future manufacturing. Finally, there will be more questions than answers and these shall be the framework for this conference the ones to come.

INTENSIFICATION OF A MULTI-PRODUCT PERFUSION PLATFORM – MANAGING GROWTH CHARACTERISTICS AT HIGH CELL DENSITY FOR MAXIMIZED VOLUMETRIC PRODUCTIVITY

Shawn Barrett, Continuous Manufacturing Skill Center, BioPharmaceutics Development, Sanofi
shawn.barrett@sanofi.com
Wenqin He, Continuous Manufacturing Skill Center, BioPharmaceutics Development, Sanofi
Jared Franklin, Continuous Manufacturing Skill Center, BioPharmaceutics Development, Sanofi
Mara Stangl, Continuous Manufacturing Skill Center, BioPharmaceutics Development, Sanofi
Aleksandar Cvetkovic, Continuous Manufacturing Skill Center, BioPharmaceutics Development, Sanofi

Key Words: Perfusion, process intensification, volumetric productivity, growth rate

Integrated Continuous Biomanufacturing (ICB) provides many important strategic advantages for therapeutic protein production through process intensification, simplification and integration. Dramatic reductions in cost of goods manufactured can be achieved by pushing perfusion culture towards high productivity at moderate perfusion rate and integrating with multi-column capture. We have demonstrated that an in-house chemically defined medium designed for high volumetric productivity (VPR) can support clones producing different monoclonal antibodies in perfusion bioreactors at cell densities >100 million viable cells/mL and VPR from 4 to 6 g/L/day. However, for other cell lines tested productivity could not be consistently sustained due to declining growth rate at high cell density. It was demonstrated that increased bleed rates could extend the culture duration for these clones but only with substantially lower cell density and productivity, and reverting to a less productive perfusion medium improved culture longevity to a certain degree. It was shown that continuous addition of a concentrated supplement to this medium could improve productivity to levels comparable to the high-VPR medium, but this appeared to be less effective for clones with lower specific productivity. Some clones producing the same biologic were observed to exhibit either sustained or declining growth rate at high cell density, indicating clonal variability should be considered as another factor that can affect this growth phenotype. Potential strategies to mitigate declining growth rate in high cell density perfusion culture will be discussed and additional case studies on the application of intensified perfusion will be examined.

EVOLUTION OF TFF-BASED PERFUSION: A PATH TOWARDS NON PRODUCT SIEVING AND DIRECT CHROMATOGRAPHY INTEGRATION

Nuno D.S. Pinto, Biologic & Vaccines, Bioprocess Research & Development, Merck & Co., Inc., Kenilworth, NJ
nuno.pinto@merck.com

Key Words: Monoclonal antibody, CHO cell culture, microfiltration, tangential flow filtration, product sieving.

Manufacturers of therapeutic proteins are becoming increasingly interested in continuous bioprocessing. Via uninterrupted medium exchange, perfusion cell culture yields higher volumetric productivity than traditional batch/fed-batch processes with 2 to 5 fold higher cell density and cultivation duration. In addition, an uninterrupted removal of therapeutic proteins promotes a desired alignment with integrated continuous purification processes to eliminate large hold tanks.

Hollow fiber tangential flow filtration (TFF) is commonly used for cell retention in perfusion cell culture. In these perfusion systems the major limitation can be inefficient or decaying product sieving. This increases product's retention time in the bioreactor, which may detrimentally affect the target protein quality attributes and decrease yield.

Herein, three commercially available TFF membranes are employed in high cell density CHO perfusion for more than 21 culture days. This work highlights the learnings obtained from interrogating various filter characteristics (e.g., pore size, chemistry) as well as operating conditions. The resulting understanding led to a solution with high mass transfer (>90% sieving) and particle free permeate stream (turbidity <10 NTU) to allow direct loading of a chromatography capture step. This work not only showcases a TFF perfusion toolbox, but also demonstrates that continuous processing is possible with TFF-based perfusion cell culture.

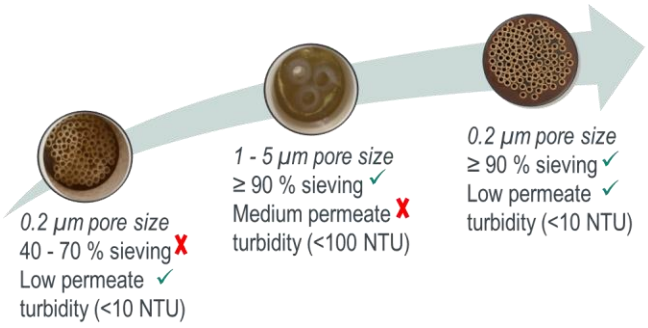

Figure 1 – TFF perfusion evolution to overcome product sieving.

DEVELOPMENT TOWARDS A HIGH-TITER FED-BATCH CHO PLATFORM PROCESS YIELDING PRODUCT TITERS > 10 g/L

Laurel Zhang, Late Stage Cell Culture, Pharma Technical Development, Genentech, USA
zhangg34@gene.com
Brian Wong, Late Stage Cell Culture, Pharma Technical Development, Genentech, USA
Robert Shawley, Late Stage Cell Culture, Pharma Technical Development, Genentech, USA
Martin Gawlitzek, Late Stage Cell Culture, Pharma Technical Development, Genentech, USA

Key Words: Global Cell Culture Platform, chemically-defined media, lactate lever, high titer, “traditional” fed-batch

Roche’s current Global Cell Culture Platform (GCCP) using chemically-defined media was implemented in 2012 and has been successfully used in process development and clinical manufacturing for numerous molecules. Several minor version changes have been implemented since its inception mainly to further optimize product quality requirements. However, high lactate levels have been observed in several projects using our CHO-K1-M GS host cell line (Random Integration host), resulting in sub-optimal culture performance when not addressed by process modifications (e.g., off-platform pH changes, etc.). Understanding the “triggers” for undesirable lactate metabolism and identifying levers to control lactate metabolism are keys in improving process robustness and enabling further advances in platform process optimization towards higher titers required for high-demand products.

Using a lactogenic model cell line, we examined numerous potential lactate levers including starting osmolality of production media and other factors that can mitigate the buildup of in-process osmolality (e.g., media components, media powder concentration, feed strategies, and process parameters). The results from these studies were then used to further optimize our existing platform media and process to develop a high titer proof-of-concept fed-batch process yielding > 10 g/L. We also investigated the optimization of media solubility and stability of our proprietary liquid media, thus enabling the development of new highly concentrated liquid media which are required for high titer processes. Case studies that demonstrate the applicability of the newly developed high titer process with numerous mAb producing cell lines including our new Targeted Integration host will be discussed.

The optimized “traditional” fed-batch process may ultimately lead to our next generation platform process, which still fits within our current manufacturing network, but will significantly reduce cost of goods and runs required to support clinical and commercial production of our biopharmaceutical proteins.

DEVELOPMENT OF A NOVEL AUTOMATED PERFUSION MINI-BIOREACTOR 'AMBR® 250 PERFUSION'

Barney Zoro, Sartorius Royston, UK
barney.zoro@sartorius.com
Andrew Tait, Sartorius Royston, UK
Melisa Carpio, Sartorius Bohemia, USA
Kevin McHugh, Sartorius Bohemia, USA

Key Words: automated, high throughput, single-use, perfusion, bioreactor,

In recent years a strong trend towards continuous biopharmaceutical processing has gathered momentum, driven by the promise of process intensification, reduced cost of goods, and more consistent and better controlled product quality. Key technologies in upstream cell culture (ATF, TFF) have enabled the start of a shift towards process intensification/continuous processing in the seed train (N-1 perfusion) and main production culture (concentrated fed-batch, perfusion) for biopharmaceutical production processes. While these technologies are now available for large scale bioreactor operations, small-scale application is limited to traditional benchtop bioreactor scales and formats. Benchtop bioreactors do provide a route to developing this new wave of intensified/continuous cell culture processes, however this approach is manually intensive, relatively low throughput and cost-intensive to operate. In the last 5 years, fed-batch cell culture process development has been significantly accelerated by wide spread implementation of the ambr 15 and ambr 250 fully automated, single-use, micro and mini bioreactor systems. Case studies will be presented on the utility of the ambr 15 as a perfusion mimic, and we also present here novel performance data of a new version of the ambr 250 system 'ambr 250 perfusion'. Technical description and operating data and cell culture results presented for the novel 'ambr 250 perfusion' system outline the capacity and capability of this technology. As established with ambr 250 for fed-batch processes, ambr 250 perfusion has the potential to provide the industry with a step change in perfusion process development capacity, enabling implementation of DoE based approaches for process optimization and characterization. It is envisaged that 'ambr 250 perfusion' can therefore facilitate and significantly accelerate an industry wide transition to upstream cell culture perfusion processes for novel biopharmaceuticals currently in early development.

INTENSIFIED CELL CULTURE USING A LINKED BIOREACTOR SYSTEM

Matthew Gagnon, Pfizer
Matthew.Gagnon@Pfizer.com
Gregory Hiller, Pfizer

Key Words: intensified cell culture, perfusion, continuous-flow stirred-tank bioreactor, CSTR, steady-state

As manufacturing capacity becomes limited and demand and competition increases in the biologics space, the need for more flexible, cost effective, and productive biomanufacturing processes grows. We will describe a novel, intensified cell culture process that uses the cell-bleed of an N-1 (or seed) perfusion bioreactor to continuously feed a production-stage continuous-flow stirred-tank bioreactor. This continuously-linked bioreactor system effectively separates cells into two cultures, one a high growth phase, and the second a highly productive stationary phase. The design of the system allows it to operate indefinitely under steady-state conditions with volumetric productivities exceeding 1.0 gram/liter/day, regardless of any cell-specific productivity loss due to cell line instability. Multiple modes of system start-up will be discussed as part of the optimization work performed to date. Due to the unique design of the linked bioreactor system it may be effectively operated at very large scales typically deemed impractical for more conventional perfusion processes, potentially enabling more efficient use of installed production capacity. The linked bioreactor system attained productivities up to 2.4-fold those achieved in the respective commercial-ready, fed-batch processes, while drastically reducing overall process media consumption. Finally, since the production bioreactor operates as a continuous-flow stirred-tank bioreactor the system creates additional flexibility for downstream operations. Cell culture could be harvested continuously, or intermittently on a potentially variable cycle, depending upon facility fit and maximum efficiency using existing large-volume disc-stacked centrifuges. The resulting integrated continuous biomanufacturing system could operate under very nearly true steady-state conditions uninterrupted for months at a time.

CAR T CELL THERAPY: FIFTEEN YEARS OF ACADEMIC DRIVING

Isabelle Rivière, Ph.D, Michael G. Harris Cell Therapy and Cell Engineering Facility, Memorial Sloan Kettering Cancer Center, USA

Key words: CAR T cells, manufacturing, cell therapy, vector production, clinical trials

We have developed platforms to manufacture T lymphocytes expressing specific chimeric antigen receptors (CARs) that have enabled the successful implementation of multiple phase I/II CAR T cell clinical trials at MSKCC -including 2 multi center trials-. Subjects with leukemia, lymphoma, myeloma, breast cancer, mesothelioma, ovarian cancer and prostate cancer have been enrolled. Over 300 CAR T cell products have been successfully manufactured and more than 200 subjects have been infused across 13 phase I/II clinical trials. In order to support these trials, we established early on a robust platform using magnetic beads coated with agonistic anti-CD3 and anti-CD28 antibodies for the selection and activation of T cells, and the Wave/Xuri bioreactor for CAR T cell expansion. We also established our own process to manufacture replication-defective gammaretroviral vectors encoding CARs. This manufacturing platform consistently allows the generation of clinical doses in less than two weeks. Using this platform, the US Food and Drug Administration granted MSK Breakthrough Therapy Designation and Orphan Drug Designation in late 2014, for its CD19-targeted CAR therapy in patients with relapsed or refractory acute lymphoblastic leukemia yielding more than 85% complete remission. This vast experience provides many insights into addressing the substantial challenges that still remain to be resolved in order to broaden the usage of CAR T cells at the Point of Care and enable the commercialization of this therapeutic modality.

ENGINEERED CAR T CELL THERAPY FOR SOLID TUMORS

Pradip Bajgain, Sujita Sukumaran, Alejandro Torres, Norihiro Watanabe, Malcolm Brenner, Ann Leen, Juan F. Vera

Center for Cell and Gene Therapy, Baylor college of medicine, Texas Children's Hospital
jfvera@txch.org

Key Words: Cell Therapy, CAR-T

The adoptive transfer of T cells redirected to tumor-associated antigens via transgenic expression of chimeric antigen receptors (CARs) has produced impressive clinical responses in patients with hematologic malignances. However the successful extension of this therapy to solid tumors has proven challenging due to i) the paucity of target antigens that are tumor selective, leading to a heightened risk of "on-target, off-tumor" toxicities and, ii) the suppressive tumor microenvironment, which subverts T cell effector function. Therefore, to overcome these limitations we have programmed T cells with a combination of receptors that recognize a gene expression pattern that is unique to the tumor site and whose endodomains deliver intracellular signals 1, 2 and 3 (antigen, co-stimulation and cytokine) required for optimal T cell activation and protection from suppressive factors present at the tumor site. The current presentation will not only highlight our T cell engineering improvements but also our process optimization, including the incorporation of the G-Rex device, to facilitate the clinical and commercial development of potentially curative therapies.

iPSC-DERIVED NEUROSPHEROIDS RECAPITULATE DEVELOPMENT AND PATHOLOGICAL SIGNATURES OF HUMAN BRAIN MICROENVIRONMENT

Catarina Brito, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
anabrito@ibet.pt

Ana Paula Terrasso, Daniel Simão, Francisca Arez, Marta M Silva, Marcos F Sousa, Patrícia Gomes-Alves, Paula M Alves, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
Nuno Raimundo, Universitätsmedizin Göttingen, Institut für Zellbiochemie, Göttingen, Germany
Neus Bayó-Puxan, Neural Commitment and Differentiation Department, The Institute of Biomedicine of the University of Barcelona (IBUB), Barcelona, Spain; Institut de Génétique Moléculaire de Montpellier, CNRS UMR 5535, Montpellier, France; Université de Montpellier, Montpellier, France
Sophie Creysells, Eric J Kremer, Institut de Génétique Moléculaire de Montpellier, CNRS UMR 5535, Montpellier, France; Université de Montpellier, Montpellier, France,

Key Words: 3D culture, Induced Pluripotent Stem Cells, neurospheroids, extracellular matrix, Mucopolysaccharidosis type VII

Brain microenvironment plays an important role in neurodevelopment and pathology, where extracellular matrix (ECM) and soluble factors modulate multiple cellular processes. Neural cell culture typically relies on the use of heterologous matrices that poorly resemble the brain ECM or reflect its pathological features.
We have previously demonstrated that perfusion stirred-tank bioreactor-based 3D differentiation of human neural stem cells (NSC) - pSTR-neurospheroids, sustains the concomitant differentiation of the three neural cell lineages (neurons, astrocytes and oligodendrocytes) and the establishment of physiologically relevant cell-cell interactions. Here, we hypothesized that if the pSTR-neurospheroid strategy would also allow the deposition of native neural ECM components and diffusion of secreted factors, it would be possible to: (i) mimic the cellular and microenvironment remodeling occurring during neural differentiation without the confounding effects of exogenous matrices; (ii) recapitulate the pathological phenotypes of diseases in which alteration of homotypic and heterotypic cell-cell interactions and ECM components are relevant.
To demonstrate the first point, we analyzed pSTR-neurospheroid differentiation by quantitative transcriptome (NGS) and proteome (SWATH-MS). Data showed that neurogenic developmental pathways were recapitulated, with significant changes at cell membrane and ECM composition, diverging from the 2D differentiation profile. A significant enrichment in structural proteoglycans typical of brain ECM, along with downregulation of basement membrane constituents was observed. Moreover, higher expression of synaptic and ion transport machinery in pSTR-neurospheroids suggest higher neuronal maturation than in 2D.
Having shown recapitulation of neural microenvironmental dynamics in pSTR-neurospheroids, we used Mucopolysaccharidosis VII (MPSVII) as a disease case study. MPS VII is a lysosomal storage disease caused by deficient β-glucuronidase (β-gluc) activity, which leads to accumulation of glycosaminoglycans (GAGs) in many tissues, including the brain. In pSTR-neurospheroids generated from hiPSC of a MPS VII patient, the main molecular disease hallmarks were recapitulated, namely accumulation of GAGs. Notably, MPS VII neurospheroids showed reduced neuronal activity and a disturbance in network functionality, with alterations both in connectivity and synchronization, not observed in 2D cultures. These data provide insight into the interplay between reduced β-gluc activity, GAG accumulation, alterations in the neural network, and its impact on MPS VII-associated cognitive defects.
Overall we demonstrate that neural cellular and extracellular developmental and pathological features are recapitulated in healthy and diseased pSTR-neurospheroids, respectively. These can be valuable *in vitro* models to address molecular defects associated with neurological disorders that affect neural microenvironment homeostasis. Moreover, the 3D neuronal connectivity assay developed is a new tool with potential to assess other lysosomal storage diseases and neurodegenerative diseases that have variable phenotypes.

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BIOMANUFACTURING OF PLATELET-LIKE CELLS AND CELL MICROPARTICLES FOR CELL-THERAPY APPLICATIONS

Chen-Yuan Kao, Department of Chemical & Biomolecular Engineering, University of Delaware
cykao@udel.edu
Christian Escobar, Department of Biological Science, University of Delaware
Eleftherios T Papoutsakis, Department of Chemical & Biomolecular Engineering, Department of Biological Science, University of Delaware

Key Words: Biomanufacturing, Extracellular Vesicle, Platelet, Megakaryocyte, Megakaryocytic Microparticle

Megakaryocytes (Mks) are large polyploid cells derived from hematopoietic stem/progenitor cells (HSPCs) triggered by thrombopoietin (Tpo). During differentiation and maturation from HSPCs, Mks migrate from bone marrow toward blood vessels, and give rise to proplatelets (PPTs) and platelets (PLTs) released into blood circulation. We have shown that Mks also shed megakaryocytic microparticles (MkMPs) (1), which are 0.1 to 1 micron extracellular vesicles (EVs). These MkMPs specifically target HSPCs *in vitro* and induce them into Mk differentiation in the absence of Tpo by delivery of cargo such as proteins and RNA (1,2). PLTs (collected from donated blood) are an expensive cell product in limited supply due to their short life time (4-5 days at room temperature; freezing is not possible) and large needs in Transfusion Medicine for patients with thrombotic deficiencies. Culture-derived PLTs has been shown to have functional activity as PLTs and hold a great potential for providing abundant PLT supply. In this study, we examined biomanufacturing issues of cells or cell derived particles for potential use in lieu of collected PLTs.

First, we examined the possibility that MkMPs may be used in lieu of collected PLTs. To examine this, we tested the hypothesis that human MkMPs (huMkMPs) might interact with murine HSPCs and promote Mk and PLT biogenesis *in vivo*. If this hypothesis is correct, it would suggest that huMkMPs can be used in Transfusion Medicine in lieu of PLTs, especially because huMkMPs would interact more efficiently with huHSPCs than with muHSPCs and also because huMkMPs can be stored frozen. To test this hypothesis, we investigated the interaction of huMkMPs with huHSPCs, both *in vivo* and *in vitro*. Injection of huMkMPs to wild-type mice enhanced PLT levels by up to 49%, while reticulated (newly synthesized) PLTs increased from 11.8 % to 15.9 % (a substantial and statistically significant increase). Furthermore, huMkMPs were able to rescue the PLT levels of antibody-induced thrombocytopenic mice by up to 52% Taken together, these data show that huMkMPs target murine HSPCs to enhance PLT biogenesis *in vivo*.

How would one then optimize Mk, PLT and MkMP biomanufacturing for practical applications at large scale? Difficulties regarding PLTs yield per Mk or per input HSPC and PLT functionality remain unsolved. We have shown that shear forces enhance Mk maturation, and the production and function of PPTs, PLTs and MkMPs (1). To achieve metrics suitable for biomanufacturing of PLTs and MkMPs, we improve at the late stage of Mk culture. Cultures under mixing conditions imposing increased biomechanical forces in different culture vessels were carried out. PPTs, PLPs, and MkMPs production under mixing condition were enhanced by ≥ 4-6 fold. Furthermore, PLTs and MkMPs generated under increased biomechanical forces maintained their biological functionality. These data suggest that biomanufacturing of these PLTs and MkMPs (and other cell types and EVs) produced under optimized culture condition engaging optimal biomechanical forces show great potential for serving as PLTs substitutes in Transfusion Medicine, and, more broadly, as agents for novel cell therapies.

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CAR T MANUFACTURING: PROCESS MODIFICATIONS FOR A TRANSFORMATIONAL AUTOLOGOUS PRODUCT ON A RAPID PATH TO LICENSURE

Thomas Brieva, Celgene
tbrieva@celgene.com
Eric Law, Celgene
David Hsiung, Celgene
Seth Jones, Celgene
Shiv Misty, Celgene
Sherry Zhou, Celgene
Ryan Glussi, Celgene
Ryan Shorr, Celgene
Kevin Friedman, bluebird bio
Molly Perkins, bluebird bio
Shannon Grande, bluebird bio
Holly Horton, bluebird bio
Kruti Shah, Celgene
Jaymes Fuller, Celgene
Christopher Wiwi, Celgene
Greg Russotti, Celgene

Key Words: Cell therapy, comparability, process change

The transformational impact of CAR T cell therapies on serious diseases demands a rapid path to licensure in order to establish widespread availability to desperate patients. In addition, the complex, labor intensive, and costly patient-specific manufacturing processes for CAR T cell therapies demand process modifications that enable scalability and affordability to maximize availability to patients. There are many options to improve CAR T processes ranging from automation to improved medium composition to simplified closed-system tubing sets. However, the dramatic dose-dependent safety and efficacy activities of these therapies amplifies the need for maintaining product comparability across process changes. This assessment of comparability is challenged by limited knowledge of product Critical Quality Attributes as well as limited availability of patient cells for process development studies. We have developed a comprehensive analytical toolbox that enables the assessment of product impact of process changes along with a risk-based approach to applying a matrix of appropriate tools for each change. This risk-based approach involves the most extensive product analysis for high-risk changes and a relatively restricted product analysis for low-risk changes. In all cases, the product analysis includes assessments of product characteristics that can hypothetically be impacted by the process change. We describe our approach to identifying, prioritizing, and assessing feasibility of process changes along with generating a suitable product comparability dataset to implement the most impactful process changes on an expedited timeline to licensure. We share examples of comparability data and its application to decision making.

DEVELOPMENT OF A LARGE SCALE GMP COMPLIANT SUSPENSION CELL CULTURE SYSTEM FOR THE MANUFACTURING OF ALLOGENIC EXOSOME-BASED BIOTHERAPEUTICS

Agata Villiger-Oberbek, Codiak BioSciences
agata.villiger-oberbek@codiakbio.com
Andrew Grube, Codiak BioSciences
Tik Yan (Emily) Chan, Codiak BioSciences
Scott Estes, Codiak BioSciences
Konstantin Konstantinov, Codiak BioSciences

Key Words: exosomes, extracellular vesicles, chemically-defined media, suspension culture, GMP.

As efforts accelerate to translate exosome biology into new medicines, clear technology gaps have emerged between the current state of the art for producing extracellular vesicles (EVs), comprising exosomes, and the capabilities necessary to support large scale clinical and commercial manufacturing. An allogenic EV production system is necessary to make the technology viable for a broad range of therapies and large patient populations. To this end, Codiak BioSciences has leveraged cutting edge bioprocessing methods developed through decades of recombinant protein manufacturing to create a >1,000-fold scalable exosome production platform based on an immortalized human cell line growing in suspension and chemically defined media.
Until recently, EVs have been produced largely in discovery labs where process scale up and manufacturability were not of prime concern for this novel biotherapeutic modality. Using an established human cell line, we first developed a lab scale production process, which we then scaled up to bench scale and pilot scale through optimization of key process parameters, such as pH, gassing, and agitation. To expediently produce clinical material for our first program, we implemented a fed-batch process. However, with the goal for this platform to support a portfolio of clinical programs in the future, parallel efforts were focused on the development of a second generation production process using continuous technology. Importantly, our studies revealed a direct, positive correlation between bioreactor cell mass and EV productivity. Moreover, improvement in culture health was as important as the increase in biomass, since higher culture viabilities minimized contamination of the harvest with microvesicular and other membranous impurities, proteinaceous material, nucleic acids, and other small molecules—a positive outcome for downstream processing. Our results indicate that EVs can be efficiently produced in stirred-tank bioreactors in a fed-batch and continuous process representative of large scale manufacturing under GMP conditions. Due to process comparability at different platforms, the intermediate scales can be used to provide uniform batches of development material for discovery research. The EVs produced are strongly positive for canonical exosome markers such as tetraspanins, have classical exosome morphology by TEM, and are capable of fusing with recipient cells, although the effect of process manipulations on EV quality has not yet been fully understood. Lastly, our current process is robust, relies on standard bioprocessing infrastructure found at most CMOs and is compatible with single use disposable technology.

PROBING LACTATE METABOLISM VARIATIONS IN LARGE-SCALE BIOREACTORS

Sen Xu, Upstream Process Development and Engineering, Biologics Process Development & Clinical Manufacturing, Merck & Co., Inc., Kenilworth, NJ, USA
sen.xu@merck.com
Rubin Jiang, Upstream Process Development and Engineering, Biologics Process Development & Clinical Manufacturing, Merck & Co., Inc., Kenilworth, NJ, USA
Roland Mueller, Biologics Process Development & Clinical Manufacturing, MSD Werthenstein BioPharma GmbH, Schachen, Switzerland
Nadja Hoesli, Biologics Process Development & Clinical Manufacturing, MSD Werthenstein BioPharma GmbH, Schachen, Switzerland
Thomas Kretz, Biologics Process Development & Clinical Manufacturing, MSD Werthenstein BioPharma GmbH, Schachen, Switzerland
John Bowers, Bioprocess Technical Operations, Biologics Process Development & Clinical Manufacturing, Merck & Co., Inc., Kenilworth, NJ, USA
Hao Chen, Upstream Process Development and Engineering, Biologics Process Development & Clinical Manufacturing, Merck & Co., Inc., Kenilworth, NJ, USA

Key Words: Scale-up, Mixing, Chinese Hamster Ovary cells, CO_2 removal, Single-use.

Lactate metabolism variations are frequently encountered in mammalian cell culture processes, especially during process scale-up. In this work, we took a multipronged approach to investigate the impact of pH, pCO_2, osmolality, base addition, and mixing conditions on the observed lactate variations in a Chinese Hamster Ovary (CHO) fed-batch process at 2,000 L scale. Two cultivating methods, CO_2-controlled and pH-controlled, were used to decouple the individual and synergistic effects from those factors. The individual effects from pH, pCO_2, and osmolality on lactate consumption/reproduction in the stationary phase were insignificant in the ranges studied though the initial lactate production rates varied. In contrast, lactate metabolism was found to be impacted by an interaction between mixing conditions and CO_2 accumulation. High CO_2 accumulation and poor mixing led to lactate reproduction, whereas either low CO_2 or improved mixing were sufficient to result in lactate consumption. Base addition was not required for pH control in the low CO_2 conditions, and therefore lactate reproduction was correlated with base addition under poor mixing conditions and high CO_2. Under good mixing conditions, CO_2-triggered base addition did not pose significant impact on lactate reproduction. It is reasonable to postulate that increased mixing times promoted lactate production during base addition. As lactate reproduction results in more base addition to maintain pH, a cycle could be formed between lactate production and base addition. As a remediation, we showed that lactate reproduction could be eliminated by improving CO_2 removal at 2,000 L scale.

MEDIUM DEVELOPMENT STRATEGIES AND SCALE DOWN MODELS FOR A HIGH DENSITY HIGH PRODUCTIVITY CELL LINE

Amy S. Johnson, Regeneron Pharmaceuticals, Inc.
amy.johnson@regeneron.com
Meghan E. Casey, Regeneron Pharmaceuticals, Inc.
Nancy Guillen, Regeneron Pharmaceuticals, Inc.
Shawn M. Lawrence, Regeneron Pharmaceuticals, Inc.

Key Words: Medium Development, Osmolality, Shake Flask, ambr® 250, DOE, scale-down model

Medium Development at Regeneron continues to enhance fed batch culture productivity. These efforts have been enabled through the development of high throughput scale down models in shake flasks and the ambr® 250. Design of Experiment (DOE) approaches have been applied to optimize the operating conditions in the small scale models leading to performance for growth and titer that match benchtop bioreactor with no off-set. The development of these representative scale down models and our approach to medium development will be described.

A medium development case study will be presented from a recent Regeneron fed batch process with a cell line achieving high cell densities and depleting the culture of key amino acids. The traditional medium development approach of supplementing the culture with the depleted nutrients was unsuccessful: high amino acid consumption rates required large amounts of amino acids resulting in significantly increased culture osmolality and reduced productivity. Leveraging high throughput culture systems and multifactor DOEs, multiple medium composition factors in combination were rapidly evaluated. Mathematical models relating medium input factors to process outputs are generated that allow for process optimization. Using this approach, a new feeding strategy was developed that limits increases in osmolality and yields titers approaching 10g/L in both the scale down systems and a process that has been implemented for clinical scale manufacturing of a monoclonal antibody.

PROCESS SCALE UP AND CHARACTERIZATION OF AN INTENSIFIED PERFUSION PROCESS

Jiuyi Lu, Sanofi
Jiuyi.lu@sanofi.com
Jonathan Wang, Sanofi
Myles Hollenbach, Sanofi
Jason Walther, Sanofi
Jean McLarty, Sanofi
Ken Karey, Sanofi

Key Words: Intensification, Perfusion, Process Characterization, Scale up.

Continuous biomanufacturing provides many advantages for the production of therapeutic proteins through process integration, automation and intensification. Sanofi currently has developed a robust and integrated continuous biomanufacturing platform to achieve improved volumetric productivity and consistent product quality. Process intensification reduces the physical footprint as well as capital and operating expenses of manufacturing facilities. This presentation is a case study on the implementation of the intensified process for commercialization of a biotherapeutic product.

Using a QbD approach, we successfully implemented an intensified perfusion process coupled with continuous product capture for a commercial product. High cell densities have resulted in a significant increase in volumetric productivity, which allows a substantial footprint reduction and increases flexibility in the commercial facility. To understand the impact of process parameters on critical quality attributes (CQAs), univariate and multivariate studies were conducted in small scale bioreactors. Mix model repeated measurement was applied in the data analysis to incorporate time-dependent information into the predictive model. This was followed by Monte Carlo simulation to determine proven acceptable ranges (PARs) for critical process parameters in support of process control strategy (PCS). Facilitated by computational fluid dynamics (CFD) simulation, we successfully scaled up the process to commercial scale. In this presentation, challenges associated with application of QbD approach for a perfusion process and the advantages of an intensified perfusion process will be discussed.

LEVERAGING A TECHNICAL PARTNERSHIP TO DELIVER HIGH TITER BIOLOGICS MANUFACTURING

Haofan (Eric) Peng, Biogen, USA
haofan.peng@biogen.com

Biogen has two of the best legacy manufacture facilities in Research Triangle Park, North Carolina and Hillerod, Denmark, and are able to maintain many well-established partnerships with different companies, who utilize these facilities and work collaboratively to bring many products to market. Biogen not only delivers extraordinary performance in technology transfer, engineering instrumentation, and manufacturing operation, but also has a very strong technical development platform that can generate high biologics titer in the internal and biosimilar programs.

Among partnership-based manufacturing experiences, some molecules have high clinical demand but relatively low titer output, which can require high volume and frequency in manufacture capacity; this low output might also require partners to engage more than one CMO and complicate the technology transfer and supply chain. Biogen has thus invested technical development resources in upstream, downstream and analytical development, all working together to develop high titer processes in numerous programs. Regardless of the challenge of multiple different cell lines which originally required different medium and processes, Biogen has shown the ability to successfully triple the titer and maintain comparable product quality in the new development.

This collaboration not only utilizes the strength from each side of the partnership, but also exchanges the expertise from technical teams, facilitates technology transfer, enhances the manufacturing support and opens more capacity in the manufacturing facilities for the other programs in need.

EFFICIENT TECHNOLOGY TRANSFERS TO INCREASE AGILITY, FLEXIBILITY, AND PRODUCTIVITY

Kara Calhoun, PhD, Genentech, a Member of the Roche Group
calhoun.kara@gene.com

Key Words: Technology transfer, scale-up, scale-down, CMOs

The current pharmaceutical business environment requires increasing agility, flexibility, and efficiency in bioprocess manufacturing to ensure competitiveness. Excellence in technology transfer execution is one way to become more agile and to develop a competitive advantage. Roche's large manufacturing network, using both internal and external (CMO) manufacturing capabilities, provides many examples of various bioprocess technology transfers. A consistent framework for technology transfer is used that incorporates elements of quality, speed, and risk management. The amount of complexity is dictated by the level of changes in process, scale, or analytical methods as well as by the experience level of donor and receiving parties. When external manufacturing (CMO) is included, additional complexities can arise due to differences in transfer methodologies, cultural norms, and communication styles. Despite the complexities, a unified framework for transfers has allowed for significant reductions in transfer time. A series of recent transfers for Roche commercial legacy processes will be described. Facility fit considerations for upstream unit operations, scale-up/scale-down approaches, and obstacles encountered during manufacturing campaigns illustrate challenges encountered and overcome. Specific examples to be discussed include approaches to address comparability challenges, cell culture scale-down model troubleshooting, and design modifications to media preparation equipment including high temperature short time (HTST) and bioburden control topics.

TOWARDS ADVANCED UNDERSTANDING OF SCALE-UP:
FROM COMPUTATIONAL FLUID DYNAMICS TO SYSTEMS BIOTECHNOLOGY APPROACHES

Jochen Schaub, Late Stage USP Development, Bioprocess Development Biologicals, Boehringer Ingelheim Pharma GmbH & Co.KG, Germany
jochen.schaub@boehringer-ingelheim.com
Anna Wippermann (same affiliation)
Carina Guelch (same affiliation)
Christoph Hold (same affiliation)
Jan Bechmann (same affiliation
Martina Berger (same affiliation
Thomas Wucherpfennig (same affiliation)

Key Words: Scale-up, systems biotechnology, CFD

Scale-up of mammalian cell culture processes from development scale to commercial manufacturing scale is routinely performed in biopharmaceutical process development. For this purpose, well established biochemical engineering principles, empirical formula and scale-up criteria were developed. Considering well characterized equipment as well as company specific process and platform knowledge, scale-up typically is successfully achieved. Yet, improved understanding of scale-up phenomena is desirable for various reasons.
Since miniaturized systems are increasingly used in biopharmaceutical process development and, at the same time, efforts with respect to resources and timelines to achieve final manufacturing scale are to be minimized, scale-up steps need to cope with larger bioreactor volume changes in the future. From a process science perspective, an integrated analysis of scale-up phenomena considering both the biochemical engineering aspects (e.g. power input, k_La) as well as cell-level data is needed.

In order to gain more profound understanding of scale-up, comprehensive characterization of our cultivation systems using computational fluid dynamics (CFD) was achieved (Figure 1). To further improve and integrate the understanding of an antibody producing CHO cell in a bioreactor environment across scales, we performed thorough analysis of metabolic rates and fluxes in different cultivation scales. In addition, gene expression data using NGS were obtained (Figure 2).

These tools were applied for an in-depth analysis of a production process where differences in cell culture performance (e.g. product titer) were observed between different scales (from 250mL to 12.000L). The results of this case study will be presented and the individual contributions of these tools towards a more comprehensive understanding of scale-up will be discussed.

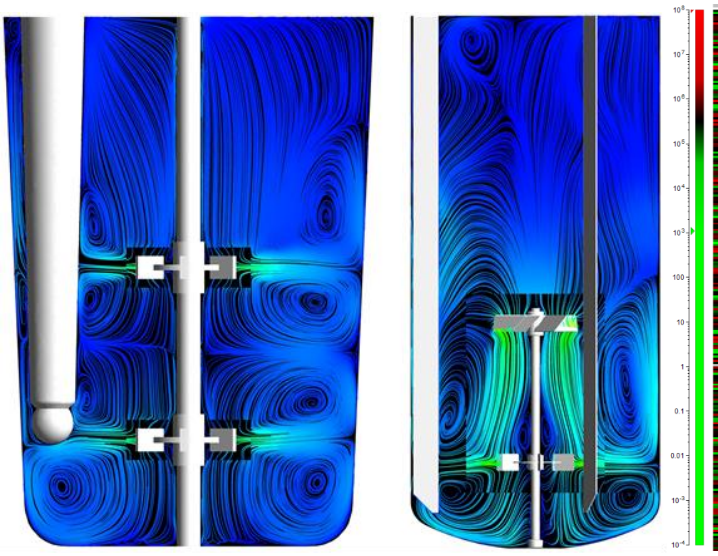

Figure 1 – Velocity profiles in 250mL and 12.000L scale

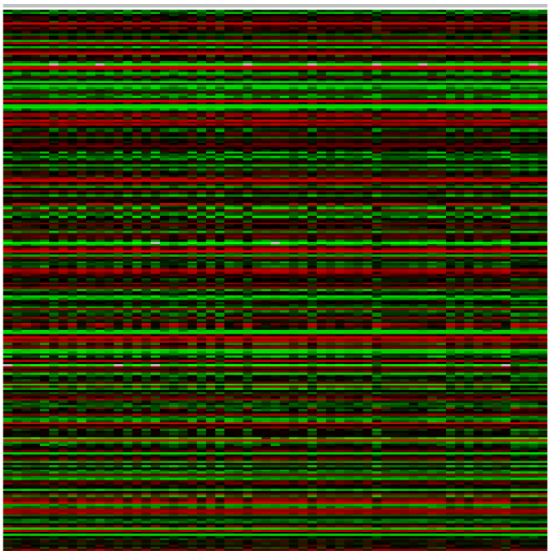

Figure 2 – NGS gene expression profiling

UNDERSTANDING AND IMPROVING CELL CULTURE PROCESSES THROUGH OMICS TECHNOLOGIES

Amanda M. Lewis
amanda.lewis@bms.com
Global Product Development & Supply
Bristol-Myers Squibb, Devens, MA

Biologics remain the fastest growing class of pharmaceutics, with global market growth projected to exceed 400 billion dollars by 2025 [1]. With this growth comes a need to manufacture diverse biological molecules to treat many serious diseases more cost effectively, and to deliver these medicines to the market quickly. Over the past 30 years, advances in life sciences and process engineering have directly contributed to advances in biologics manufacturing resulting in efficient processes with unprecedented protein titers. Nonetheless, common challenges still occur for many bioprocesses as they advance from early development through the process life cycle. This presentation discusses how omics technologies in conjunction with traditional monitoring techniques can be used to understand and improve cell culture processes [2]. Here we present three case studies where Omics technologies were successfully applied to common bioprocessing challenges, including scale-up/down, product quality attribute control, and raw material variability. First, we discuss a legacy process which does not scale effectively between lab and manufacturing scales. In this case, metabolomics and proteomics approaches were used to identify a difference in oxidative stress levels at different scales, resulting in reduced viability, viable cell density and titer [3]. This learning was then applied to the next generation process to successfully develop an optimized scale down model representative of manufacturing scale [4]. Next, we show how Omics technologies can be used to increase both understanding and control of product quality attributes. We provide examples where metabolomics and transcriptomics were applied to improve cellular and process understanding around protein sialylation, a desirable glycosylation attribute [4], as well as α-Gal, an undesirable glycosylation attribute. In our third case study, we present a metabolomics approach to monitor raw material variability in a legacy process. Manufacturing lots for chemically defined and complex media components were analyzed using NMR and LC/MS, and age and storage related degradation metabolites were identified. The impact on process performance was confirmed through spiking studies. Finally, we propose strategies for incorporating Omics technologies into bioprocess development and manufacturing monitoring to enable long-term, sustainable improvements.

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APPLICATION OF -OMICS KNOWLEDGE YIELDS ENHANCED BIOPROCESS PERFORMANCE

Alan Gilbert, Biogen
alan.gilbert@biogen.com
Ravali Raju, Biogen
Amr Ali, Biogen
Chris Kwiatkowski, Biogen
Kyle McElearney, Biogen
Rashmi Kshirsagar, Biogen

Key Words: metabolomics, transcriptomics, proteomics, platform process

The classic phrase to describe cell culture is “every cell line is different.” The unfortunate part of this idiom is the actual concealment of a crucial lack of fundamental understanding. Furthermore the phrase ignores the substantial success achieved to date in developing robust industrial cell culture platforms that are applied to all cell lines regardless of their intrinsic variation. At Biogen, our cell culture medium platform is agnostic to CHO host cell line, and the platform can accommodate this inherent genomic variation as cell lines come from different host backgrounds. This is also an opportunity for -omics work then as the differences in cell line performance can be linked back to fundamental differences within those host cell lines. However, the power of -omics technologies to influence process optimization is limited by the difficulty and time scale for execution and interpreting such studies.

Our approach to -omics implementation has been to utilize multiple targeted investigations and combine the learnings into an implementation strategy focused on enhancing the efficiency of manufacturing. Metabolic flux analysis was used to establish a baseline knowledge of central metabolism in the Biogen platform. The next step was to incorporate transcriptomics and proteomics with our metabolomics knowledge. With Biogen’s toolbox of CHO host cell lines, this approach identified intrinsic host cell line differences as well as unique limitations in cell culture. Specifically, we have determined sources of novel metabolic inhibitors that suppress cell growth as well as differences in lactate and ammonium metabolism that split according to host cell source. These conclusions ultimately lead to the optimized platform process yielding the desired product quality. Determining these differences led to an increased growth rate in scale up for cell lines from a more sensitive host as well as maintaining robust cell growth and productivity in production bioreactors. Ultimately still “every cell line is different.” Yet the more we know, the more opportunities there are to exploit both the similarities and the differences.

MULTIVARIATE DATA ANALYSIS ENABLING IMPROVED CLONE SELECTION

Stephen Goldrick, MedImmune Ltd, Cambridge, UK/ Advanced Centre for Biochemical Engineering, University College London, UK
s.goldrick@ucl.ac.uk
Nicholas J. Bond, MedImmune Ltd., Cambridge, UK
Clare Lovelady, MedImmune Ltd., Cambridge, UK
Rahul Pradhan, MedImmune Ltd., Cambridge, UK
Richard Turner, MedImmune Ltd., Cambridge, UK
Suzanne S. Farid, Advanced Centre for Biochemical Engineering, University College London, UK

Key Words: Cell line development, multivariate data analysis, critical quality attributes, critical process parameters, scale-up.

Selecting a single cell from a heterogeneous transfection pool that will scale-up appropriately from a micro-scale system to a commercial facility is a challenging and hugely important task. This clonal cell line needs to demonstrate the desired product quality attributes and ensure manufacturability throughout the entire drug manufacturing lifecycle. This process typically requires 6 to 12 months and is a time, capital and labour intensive process. High throughput (HT) methodologies are increasingly being adopted to speed up this cell line selection protocol. However, often the large quantities of data generated in combination with the increase in availability of analytics results in a daunting multivariate data analysis problem. Typically, the cell line selection strategy focuses on quality attributes recorded at point of harvest such as final concentrations of process parameters including titre and viable cell density, level of aggregates or addition product quality attributes. Time-series data such as dissolved oxygen, pH or gas flow rates are often overlooked due to challenges with visualization and interpretation of the large number of process variables recorded. This work describes a novel method that implements advanced multivariate tools including principal component analysis (PCA) to better leverage the available data to help guide this challenging decision making process. The inclusion of additional process variables was demonstrated to enhance the selection of a high-yielding mammalian cell line through inclusion of scale-up dependent process parameters related to high oxygen demands and varying nutrient uptake rates. Furthermore, this technique was demonstrated to highlight problematic product heterogeneities of parent clones that were not identified through univariate analysis of the multiple cell lines. The inclusion of this MVDA methodology demonstrated a more efficient and better decision-making protocol compared to conventional cell line selection processes.

QUANTIFYING THE PARTITION OF METABOLIC RESOURCES BETWEEN CELLULAR AND RECOMBINANT PROTEIN GLYCOSYLATION IN GS-CHO CELLS

Ioscani Jiménez del Val, School of Chemical & Bioprocess Engineering, University College Dublin, Dublin, Ireland, D04 V1WD
ioscani.jimenezdelval@ucd.ie
Sarantos Kyriakopoulos, MS&T BioMarin Manufacturing Ireland, Shanbally, Ireland, P43 R298
Simone Albrecht, National Institute for Bioprocessing Research & Training, Dublin, Ireland, A94 X099
Henning Stockmann, National Institute for Bioprocessing Research & Training, Dublin, Ireland, A94 X099
Pauline M Rudd, National Institute for Bioprocessing Research & Training, Dublin, Ireland, A94 X099
Karen M Polizzi, Department of Life Sciences, Imperial College London, London, UK SW7 2AZ
Cleo Kontoravdi, Department of Chemical Engineering, Imperial College London, London, UK SW7 2AZ

Key Words: CHO cells, Glycosylation, mAbs, Metabolic Flux Analysis

All commercially-available therapeutic monoclonal antibodies (mAbs) contain a consensus N-linked glycosylation site on the constant fragment (Fc) of their heavy chains. The composition of the carbohydrates (glycans) bound to these products determines their safety and therapeutic efficacy [1]. While production cell lines synthesize, glycosylate, and secrete the mAb, they also glycosylate their own components. Therefore, there is a partition of glycan biosynthetic precursors – nucleotide sugars (NSs) – that directly couples mAb glycosylation with cellular growth and metabolism. With this work, we present a novel metabolic flux analysis (MFA) model that establishes a mechanistic and quantitative representation of the partition of metabolic resources between cellular and mAb glycosylation.

Experimentally, IgG-producing GS-CHO cells were cultured with three amino acid feeding strategies. Data for cell density, nutrient availability, metabolite accumulation, product titer, intracellular NS concentration [2], and mAb glycoprofiles [3] were collected. Computationally, a metabolic flux analysis (MFA) model that represents 101 metabolites connected by 143 reactions has been developed. In addition to central carbon, amino acid, nucleic acid and lipid metabolism, the MFA also includes the aspartate-malate shuttle, the urea cycle and detailed balances for ATP as well as the $NAD(P)^+$/NAD(P)H redox pair. Demand of cellular resources towards cellular glycosylation has been represented by including stoichiometric coefficients for O-GalNAc, N-linked, glycosphingolipid and GPI anchor glycans in the biomass equation [4]. Product glycosylation has been included in the equation describing mAb composition. The underdetermined MFA (42 degrees of freedom) was constrained to account for reaction reversibility and was solved through multi-objective optimization, where the squared error between experimentally-determined and MFA-calculated fluxes is minimized and ATP synthesis per flux unit was simultaneously maximized [5].

The proposed MFA framework allows us to analyze how metabolic resources are partitioned between cellular and mAb glycosylation. Our results indicate that during exponential growth, cellular glycosylation consumes considerably higher amounts of NS biosynthetic precursors (ATP, glucose, and glutamine). As growth ceases, a larger fraction of metabolic resources is allocated to mAb glycosylation, but total NS consumption decreases. This suggests that cellular glycosylation is the larger metabolic ‘sink’ within our cell line, a result that is consistent with the intracellular accumulation of NSs observed towards latter stages of culture. With further refinement – in particular, with data for dynamic variations in cellular glycosylation – our MFA framework can serve as a computational tool to design optimal NS precursor feeding strategies that control mAb glycosylation and minimize negative impacts on cell growth and productivity.

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MORE ACCURATE PROCESS UNDERSTANDING FROM PROCESS CHARACTERIZATION STUDIES USING MONTE CARLO SIMULATION, REGULARIZED REGRESSION, AND CLASSIFICATION MODELS

Cary F. Opel, Gilead Sciences
cary.opel@gilead.com
Cerintha J. Hui, Gilead Sciences
Patrick Y. Yang, Gilead Sciences
Daniel J. Tien, Gilead Sciences
Gayle E. Derfus, Gilead Sciences
Rajesh Krishnan, Gilead Sciences

Key Words: DOE, Monte Carlo Simulation, Process Characterization, Machine Learning, QbD

Establishment of an appropriate control strategy with defined operating ranges (OR) predicted to meet a target product profile is a critical component of commercializing new biologics under the Quality by Design (QbD) approach. Process characterization (PC) studies are performed to expand process understanding by achieving two main goals: 1) determining which process parameters have significant effects on quality attributes and 2) establishing models describing the relationships between these critical process parameters (CPP) and critical quality attributes (CQA). Risk assessment and design of experiments (DOE) techniques are effectively deployed in the industry to identify parameters to study and build process understanding. However, the true value of the data produced by these studies can be compromised by the inherent flaws with traditional data analysis techniques. In particular, p-value based methods such as stepwise regression are prone to generate false positives and overestimated parameter coefficients.

Many of the deficiencies of traditional stepwise regression can be alleviated by applying Monte Carlo cross validation (MCCV) and simulations to stepwise algorithms. These methods can greatly enhance process understanding and assist in the selection of CPPs. Regularized regression methods such as LASSO, ridge, and elastic net are also designed to overcome many of the issues inherent in techniques based on ordinary least squares. However, a superior strategy is to build multiple models using a variety of techniques and use the insights gained from each to establish the relationships between CPPs and CQAs. Use of complementary methods during data analysis allows more informed decisions to be made during model construction.

A series of PC studies were performed to evaluate a process for the production of a recombinant monoclonal antibody. Four steps of the manufacturing process were analyzed consisting of production in a fed-batch bioreactor followed by three chromatography steps. All four steps had separate designs and were analyzed independently to determine CPPs and ORs. The bioreactor stage examined dissolved oxygen, pH, temperature, inoculation density, and feeding strategy. The parameters for the chromatography operations included conductivity, pH, flowrate, temperature, and load density. The resulting data were analyzed using the methods described above (Figure 1) and used to identify CPPs. Several examples of false positives and overfitting were identified and avoided using MCCV Stepwise Regression. After parameter selection, MCCV was used to assess the accuracy of the models. Monte Carlo simulations were then used to establish simulated target product profiles. MCCV Stepwise Regression outperformed the more advanced regularization methods in several situations. Finally, classification analysis was performed. Analysis of PC data by building and comparing multiple models, as well as applying Monte Carlo simulations enables deeper process understanding. This understanding provides higher statistical confidence in both CPP selection and establishment of ORs.

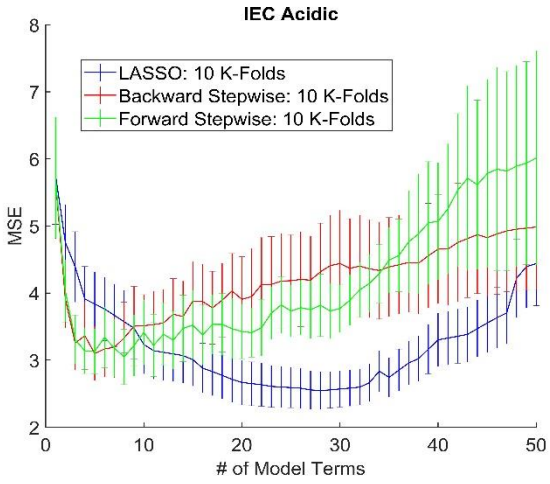

Figure 1 – Mean Squared Error as a function of Model Size for Several Methods

ENHANCING CHO PROCESS UNDERSTANDING FROM CHO MANUFACTURING PROCESS DATA

Thomas Mistretta, Amgen, Cambridge, MA
tmistret@amgen.com
Patrick Gammell, Amgen, Cambridge, MA
Cenk Undey, Amgen, Thousand Oaks, CA
Greg Naugle, Amgen, West Greenwich, RI

Key Words: Big Data, Data Lake, Predictive Models, Process Monitoring, CPV

It has been said that the biggest data can come from the smallest packages and this is certainly true in the case of CHO cells, CHO-based commercial bioprocesses can generate up to 500,000,000 data points per batch. When aggregated, organized, and analyzed, these data represent a significant, but generally underutilized, opportunity to advance product understanding and process control opportunities.

Traditional approaches to CHO process analytics have typically leveraged discrete in-process control and analytical release test data for batch over batch trending to ensure that processes remain in a state of statistical control. While this methodology can be effective to ensure that a control strategy is operating as intended, it is a reactive, lagging approach to process understanding. Advances such as deployment of real time multivariate statistical process monitoring have helped to drive proactive approaches to detect weak multivariate signals within complex datasets and have been successfully utilized at Amgen to enhance monitoring controls to ensure robust performance, early detection of issues and to rapid root cause determination when process deviations occur.

The ideal future state however is to truly implement an automated data infrastructure to enable the capability to allow developers, data scientists, and users within manufacturing and PD to access a variety of structured and unstructured data sources from all stages of the process lifecycle to enhance monitoring, analytics and development of predictive process models. This presentation will describe Amgen's efforts to drive towards that ideal state through implementation of a data infrastructure that enables network wide data aggregation, predictive modeling, advanced process monitoring, and data science driven approaches to extract the knowledge within our big data.

GAUSSIAN MIXTURE MODELS AND MACHINE LEARNING PREDICT MEGAKARYOCYTE GROWTH AND DIFFERENTIATION POTENTIAL EX VIVO

William M. Miller, Chemical and Biological Engineering, Northwestern University, Evanston, IL
wmmiller@northwestern.edu
Jia J. Wu, Darryl A. Abbott, Neda Bagheri; Chemical and Biological Engineering, Northwestern University
Meryem K. Terzioglu, Dolores Mahmud, Nadim Mahmud; Hematology/Oncology, University of IL at Chicago

Key Words: culture-derived platelets, flow cytometry, cell therapies, time-course data, cell-surface markers

The ability to analyze single cells via flow cytometry has resulted in a wide range of biological and medical applications. Currently, there is no established framework to compare and interpret time-series flow cytometry data for cell engineering applications. Manual analysis of temporal trends is time-consuming and subjective for large-scale datasets. We resolved this bottleneck by developing TEmporal Gaussian Mixture models (TEGM), an unbiased computational strategy to quantify and predict temporal trends of developing cell subpopulations indicative of cellular phenotype.

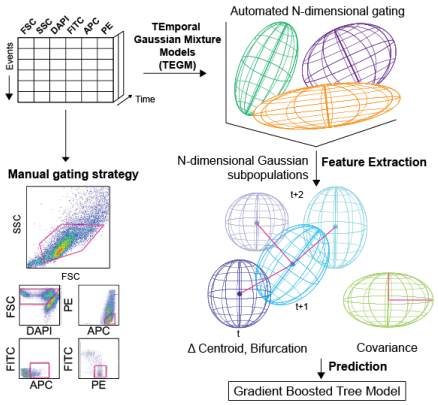

Figure 1 – Gaussian mixture models characterize each population subset in MK culture time-course data.

TEGM applies Gaussian mixture models and gradient boosted trees for cell engineering applications. TEGM enables the extraction of subtle features, such as the dispersion and rate of change of surface marker expression for each subpopulation over time. These critical, yet hard-to-discern, features are fed into machine-learning algorithms that predict underlying cell classes. Our framework can be flexibly applied to conventional flow cytometry sampling schemes, and allows for faster and more consistent processing of time-series flow cytometry data.

As a proof-of-concept, we applied our method to the analysis of *ex vivo* megakaryocytic (MK) differentiation and maturation of hematopoietic cells from donors with varying potential to generate $CD41^+/CD42^+$ mature MK cells. We illustrate the major steps of the computational approach by predicting peak $\%CD41^+/CD42^+$ MK maturation of $CD34^+$-selected umbilical cord blood (CB) cells from 16 independent donors (Figure 1). Cells were cultured over a 19-day multi-phase differentiation culture, consisting of a pre-expansion phase and a differentiation phase. The novel dataset comprised 720 measurements from 80 perturbations of 16 individual donors, with 9 time-point measurements sampled every 2-5 days for each donor. We constructed an automated gating strategy to extract surface marker expression of various clusters of $DAPI^{low}/CD41^+$ MK cells. Notably, we demonstrate that estimation of the $\%CD34^+$ and $\%CD42^+$ cells was within 1% of manual gating estimates, thus illustrating the consistency and accuracy of the technique. A gradient boosted tree model was trained using an explanatory matrix describing early characteristics and tested to predict peak $CD41^+/CD42^+$ marker expression. We then performed feature extraction for each flow cytometry time-course dataset on several descriptors, such as growth rate, viability, production, percentage positivity of each surface marker, covariance of mean fluorescence intensity, rate of change, and bifurcation of each subpopulation. A gradient boosted tree model was trained using an explanatory matrix describing early characteristics (Day 0 to Day 9) and tested to predict peak $CD41^+/CD42^+$ marker expression, which typically occurs on Day 14 to Day 17.

Overall, we identify several influential early culture factors that are predictive of peak $\%CD42^+$ expression. We show that $\%CD41^+$ on Day 5 and Day 7 is highly predictive, while cell viability and $\%CD34^+$ are comparatively less predictive of peak $\%CD42^+$. We are able to identify the best performing cultures with high sensitivity and specificity (AUROC = 0.92, where 1 denotes perfect accuracy). Predicted and actual $CD41^+/CD42^+$ responses are highly correlated using three independently selected partitions of test/training sets of our data (Figure 2; p = 7.4e-09, R = 0.87). Identifying CB units with high and low MK potential early in the 19-day culture process can save expensive resources and time, and provides the potential to intervene during the culture process.

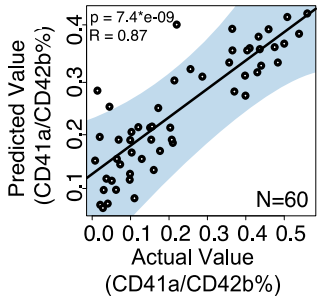

Figure 2 – TEGM with gradient boosted tree model predicts peak $\%CD41a^+/CD42b^+$ of MK cultures.

AUTOMATED AND ENHANCED CLONE SCREENING USING A FULLY AUTOMATED MICROTITER PLATE-BASED SYSTEM FOR SUSPENSION CELL CULTURE

Sven Markert, Roche Diagnostics GmbH, Pharmaceutical Biotech Production and Development, Penzberg
sven.markert@roche.com
Carsten Musmann, Roche Diagnostics GmbH, Pharmaceutical Biotech Production and Development, Penzberg
Klaus Joeris, Roche Diagnostics GmbH, Pharmaceutical Biotech Production and Development, Penzberg
Peter Hülsmann, Roche Diagnostics GmbH, pRED, Large Molecule Research, Penzberg

Key Words: early stage clone characterization; glycosylation analysis; RT-qPCR analysis; automated clone screening; clone evaluation parameters

In this talk we will present how we accelerated a clone selection process by 4 weeks while increasing the information density obtained for each clone. This was achieved by increasing the throughput and integrating new powerful analytical technologies as gene expressions and glycosylation analysis at a very early stage. Additionally, we could identify the overall top clone that was lost during the pre-selection phase of the reference process which is based on a considerably lower automation degree.

Using our in-house developed MTP-based cell culture system for clone screening and selection offers substantial potential for improvement and acceleration: The system provides a fully automated process and enables fed-batch cultivation at an early stage which is of high importance for successful clone selection. Due the high throughput of more than 600 cultivations at the same time the MTP-based system considerably increases the number of clones that can be evaluated and speeds up the processing. The potential to integrate real-time monitoring of metabolites and other secondary selection criteria crucial for productivity and product quality further supports reliable clone selection. Moreover, the MTP-based system establishes an interface for advanced HT analytics to identify additional parameters for clone evaluation. Thus, we demonstrated the technical feasibility to couple the MTP-based cell culture system with glycosylation analysis as well as the LightCycler® technology to perform automated RT-qPCR gene expression analysis for a large number of cell culture samples. The results of the RT-qPCR analysis showed that the identified top clone displayed the highest mRNA expression level for the HC of the examined mAb which correlated with the highest specific productivity recorded at the protein level. Furthermore, RT-qPCR analysis may also be beneficial to monitor the expression of stress markers like chaperones and factors related to endoplasmic reticulum stress potentially correlating with product concentration or it can be applied to improve prediction of clone stability attributes related to promoter methylation or transgene copy number.

This particular advancement combined with the high flexibility of the system opens up future perspectives for optimizing the selection process at an early stage, e.g. by using multiple selection criteria that are especially tailored for each product. The early availability of product quality data will also improve the chances to select the most suitable clone and to reduce risks and required effort during later development stages. This is of particular importance when expressing complex molecule formats such as bispecific antibodies, glycoengineered antibodies or antibody cytokine fusion proteins. Our high-throughput MTP-based cell culture system appears most suitable to enhance efficiency and robustness of the clone screening procedure as well as the quality of the selected clones. Product quality control can be reached by selecting the clone with the desired product quality pattern at a very early stage using our cell culture system that is proven to be predictive for large scale bioreactors.

SYSTEMS BIOLOGY APPROACH IN THE DEVELOPMENT OF CHEMICALLY-DEFINED MEDIA FOR PRODUCTION OF PROTEIN THERAPEUTICS IN CHINESE HAMSTER OVARY CELLS

Wai Lam W. Ling, Biologics & Vaccines, Merck Sharp & Dohme Corp.
wai_lam.ling@merck.com
Taha Salim, Biologics & Vaccines, Merck Sharp & Dohme Corp.
Gaurav Chauhan, Biologics & Vaccines, Merck Sharp & Dohme Corp.
Neil Templeton, Biologics & Vaccines, Merck Sharp & Dohme Corp.
Brian Kwan, Biologics & Vaccines, Merck Sharp & Dohme Corp.
Nicholas Murgolo, Pharmacogenetics & Bioinformatics, Merck Sharp & Dohme Corp.
Jennifer Cho, Pharmacogenetics & Bioinformatics, Merck Sharp & Dohme Corp.
Qiuwei Xu, Analytical & Biochemical Toxicology, Merck Sharp & Dohme Corp.
Heather Vu, Analytical & Biochemical Toxicology, Merck Sharp & Dohme Corp.
Vaclav Belak, Advanced Analytics Data Science, Merck Sharp & Dohme Corp.
David Dufour Rausell, Advanced Analytics Data Science, Merck Sharp & Dohme Corp.
Pavlo Minayev, Advanced Analytics Data Science, Merck Sharp & Dohme Corp.

Key Words: CHO cells, Chemically-defined media, ‘omics tools

Cell culture medium plays a critical role on mammalian cell growth, protein expression and quality. Typical cell culture medium formulations consist of >50 components which include amino acids, vitamins, trace metals, lipids and proteins. Chinese Hamster Ovary (CHO) cells that produce biotherapeutics are propagated in specific cell culture media to ensure robust productivity and product quality.

Systems biology has been applied to multiple areas of biological research to gain a better understanding of disease origins and to identify potential new drug targets. Although CHO cells are simpler systems, they share similar biochemistry and cellular pathways. Therefore, leveraging the systems biology knowledge from animal systems and applying these strategic systems biological tools to bioprocess development can be valuable in gaining better understanding of CHO cell culture performance, optimizing cell culture media, and subsequently resulting in better control of the overall production processes.

In this presentation, we will present several case studies of various ‘omics tools applied to (1) optimize cell culture medium formulation for improve cell growth and productivity via metabolomics, (2) understand effects of medium components on cellular gene expression via transcriptomics, and on product quality via glycomics, and (3) identify potential cellular protein targets that are affected by stress imposed during production process via proteomics. The development of a statistical model that aims to highlight key metabolites and a machine learning model that identifies significantly important genes which are involved in monoclonal antibody production will also be discussed.

FEEDBACK CONTROL OF LACTATE USING RAMAN SPECTROSCOPY

Derrick Ruble, Pfizer, USA
derrick.l.ruble@pfizer.com
Peter Slade, Pfizer, USA
Hamid Mehdizadeh, Pfizer, USA
Jun Huang, Pfizer, USA
Dunie Navarro, Pfizer, USA
Erwin Yu, Pfizer, USA

In fed batch cell culture lactate is produced during the growth phase; its consumption is observed when cells enter the stationary phase. This metabolic shift is desirable and tends to favor optimal process performance and product quality. A CHO cell line known not to demonstrate this metabolic shift and produce high lactate was used as an experimental system. Multifactor DOE experiments were run on process conditions known to influence lactate which included glucose, pH, cell generational age, base control, cell bank and scale-up. Conditions were established for high and low lactate results at bench-top and pilot scale. Multivariate models of lactate production were established. Although multiple factors influenced lactate production it was thought that a flexible manufacturing system could convert a worst-case high-lactate profile into desirable low-lactate profile. Previous work has demonstrated the feasibility of controlling lactate and glucose on-line with Raman spectroscopy. Models were established to quantitate in real-time both glucose and lactate concentrations during the bioreactor process. A feedback loop was developed, in which glucose and lactate concentrations were both monitored and used to control a glucose feed pump. The high-lactate process conditions were run with this two factor feedback control in both bench-top scale and pilot scale reactors. The results demonstrated that a culture could be shifted to lactate consumption converting a high-lactate profile to a low-lactate profile. The system also demonstrated an equivalent or potential increase in over-all titer. Work is continuing to establish this system as a back-up control to prevent high-lactate in future manufacturing.

AN ENERGY-BASED MODELLING TOOL FOR CULTURE MEDIUM DESIGN AND BIOMANUFACTURING OPTIMIZATION

Athanasios Mantalaris, Imperial College London
a.mantalaris@imperial.ac.uk
Ana L. Quiroga-Campano, Imperial College London
Nicki Panoskaltsis, Imperial College London

Key Words: Energy metabolism, model-based optimisation, medium design, monoclonal antibodies, mammalian cell culture.

Demand for high-value biologics, a rapidly growing pipeline, and pressure from competition, time-to-market and regulators, necessitate novel biomanufacturing approaches, including Quality by Design (QbD) principles and Process Analytical Technologies (PAT), to facilitate accelerated, efficient and effective process development platforms that ensure consistent product quality and reduced lot-to-lot variability. Herein, QbD and PAT principles were incorporated within an innovative *in vitro-in silico* integrated framework for upstream process development (UPD). The central component of the UPD framework is a mathematical model that predicts dynamic nutrient uptake and average intracellular ATP content, based on biochemical reaction networks, to quantify and characterize energy metabolism and its adaptive response, metabolic shifts, to maintain ATP homeostasis. The accuracy and flexibility of the model depends on critical cell type/product/clone-specific parameters, which are experimentally estimated. The integrated *in vitro-in silico* platform and the model's predictive capacity reduced burden, time and expense of experimentation resulting in optimal medium design compared to commercially available culture media (80% amino acid reduction) and a fed-batch feeding strategy that increased productivity by 129%. The framework represents a flexible and efficient tool that transforms, improves and accelerates conventional process development in biomanufacturing with wide applications, including stem cell-based therapies.

NOVEL MODELING METHODOLOGY TO PREDICT PRODUCT QUALITY AND CELL CULTURE PERFORMANCE IN FED-BATCH AND PERFUSION CULTURES

Bassem Ben Yahia, Biochemical Engineering Institute, Saarland University, Germany, Upstream Process Sciences Biotech Sciences, UCB Pharma S.A., Avenue de l'Industrie, Belgium
bassem.benyahia@ucb.com
Boris Gourevitch, Institut de NeuroScience Paris-Saclay (NeuroPSI), France, Université Paris-Sud, France
Elmar Heinzle, Biochemical Engineering Institute, Saarland University, Saarbrücken, Germany
Laetitia Malphettes, Upstream Process Sciences Biotech Sciences, UCB Pharma S.A., Avenue de l'Industrie, Belgium

Key Words: Macroscopic model, CHO, Fed-Batch, Perfusion, Product Quality, Metabolism, mAb, Cell growth, Intracellular pool,

The acceleration of biopharmaceutical process development is difficult when traditional experience-based sequential approaches are used. As a result, fully optimized and well understood cell culture processes prior to scale-up are rare. Here we show that an accurate, scalable and simple model able to predict cell growth, cell metabolism, titer and some product quality attributes will significantly accelerate process development, improve process development outcomes and reduce development and production costs.

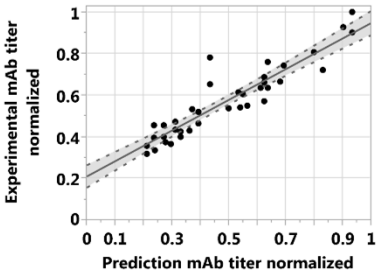

Figure 1 – Comparison of experimental with predicted mAb titers

In this paper we will present a simple systematic modeling methodology to study and predict fed-batch cell culture performance [**1**]. We will also show that this approach can be applied to the optimization of perfusion processes. Only a limited number of parameters need to be identified based on experimental results, which means that for each production process to be modeled, a minimal number of small scale bioreactor runs need to be performed before switching to *in silico* process development. We will show that this approach can accurately predict process performance both at small and large scale. Furthermore, various feeding strategies could be tested and optimized *in silico*. Moreover, the model was able to predict the impact of the depletion of essential metabolites on the specific productivity and also the impact of intracellular metabolite pools on cell growth.

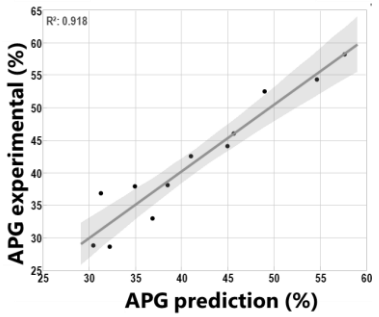

Figure 2 – Comparison of experimental with predicted acidic peak group (APG)

In a second step, the model was extended and applied to critical product quality attributes such as charge variants. This modeling approach shed further light on the impact of the feeding strategy on product quality. For instance, we will show that the total quantity of specific metabolites used throughout the bioreactor production process controls charge variants distribution, whereas within a given concentration range the daily concentration of these same metabolites is not predictive. To the best of our knowledge, this is the first study that shows that it is the total quantity of metabolites used that impacts mAb microheterogeneity.

Finally, the model was also applied to the development of continuous and hybrid/intensified production processes. The perfusion rate was controlled daily using the model calibrated with fed-batch production data. Moreover, a concentrated perfusion medium was developed and optimized *in silico*.

In summary, our modeling methodology provides a much better insight into the impact of process parameters on production yields and product quality, thus improving process understanding and control as well as accelerating process development.

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FROM BIOREACTORS FOR PROTEIN THERAPEUTIC PRODUCTION TO BIOREACTORS FOR TESTING EFFICACY AND SAFETY OF PROTEIN THERAPEUTICS

Linda G. Griffith, Massachusetts Institute of Technology
griff@mit.edu

Key Words: Tissue Engineering, Drug Development, Biologics, Preclinical Testing

The tremendous successes of mammalian cell culture engineering since the 1980's made our modern era of protein therapeutics a reality, bringing tremendous new possibilities for targeted intervention, but also additional challenges in pre-clinical development due to the species specificity of biologics. While the efficacy and safety of all drugs intended for humans is often difficult to extrapolate from assays in animal models and traditional cell cultures, the situation is significantly more difficult for biologics. Traditional cell culture modes, even with human cells, often fail to capture the complexity of pathways, which may involve multiple cell types and cross-talk between different organ systems. The species specificity of most biologics precludes testing in most common animal models. Thus, adverse events are observed in the clinic due to lack of adequate predictive models. For example, the anti IL-6 receptor Tocilizumab, developed to treat chronic inflammatory diseases like arthritis, earned a warning label from the FDA after clinical evidence that the metabolism of statins and other drugs was altered by Tocilizumab in ways that were not predicted by pre-clinical models. (Long, Cosgrove et al. 2016).

The modern field of "organs-on-chips" or "microphysiological systems (MPS)" is poised to address these gaps, and is coming full circle back to the wealth of knowledge about cell culture bioreactor performance produced by the therapeutic protein field. The field of "organs on chips" had its origins several decades ago with demonstrations by Michael Shuler and others that facets of human drug pharmacokinetics could be replicated by interconnected cultures of various cell lines (liver, fat, etc). These initial demonstrations were powerful but ultimately limited by the simplicity of the cell cultures – cell lines that mimicked only modest facets of the functions of the in vivo organ system. Over the past decades, tremendous advances in microfluidics, biomaterials, and technologies to process and make available primary human cells have dramatically increased the human-ness of in vitro cultures. Interestingly, cell lines are also still commonly used, and there has not yet been a thoughtful appreciation of how the basic metabolic functions of the tissue engineered constructs may affect the performance of these systems. Special challenges exist in formulating common media, for example, in interconnected organ systems where some cells are primary, some are tumor-derived lines, and yet others are from iPS-derived sources.

In this talk, I will highlight the past, present and future interplay between these two dynamic, vibrant fields, with illustrations in particular of how the organs-on-chips technologies are poised to aid the therapeutic protein production field.

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TAILORING ANTIBODY GLYCOSYLATION VIA INTEGRATING GENOME AND PROTEIN ENGINEERING TO GENERATE PREFERRED GLYCOFORMS ON THE FC REGION

Mike Betenbaugh, Johns Hopkins University, USA
beten@jhu.edu

One critical quality attribute of therapeutic antibodies is the glycosylation pattern at the Fc region.We combined genome editing of CHO cells and protein engineering of the IgG Fc region to allow antibodies presenting high level of galactosylation or exclusively α-2,6 sialylation. To generate IgG with high α-2,6 sialylation, we combined amino acid mutations in the Fc region of IgG and introduction of α-2,6 sialyltransferase in CHO to produce IgGs with significant levels of both α-2,6 and α-2,3 sialylation. Furthermore, to produce exclusively α-2,6 sialylation IgG in CHO, CRISPR/Cas9 was implemented to disrupt two dominant α-2,3 sialyltransferase genes (ST3GAL4 and ST3GAL6), then α-2,6 sialyltransferewas introduced in a α-2,3 sialylation knockout cell line. Notably, no α-2,3 linked sialic acids of IgG produced from the α-2,3 sialyltransferase knockout-α-2,6 sialyltransferase overexpression pools were detected by HPLC sialic acid quantification after the α-2,3 linkage specific sialidase cleavage. Finally, glycosylation analysis of IgG with four amino acid mutations generated by an α-2,3 sialyltransferase knockout-α-2,6 sialyltransferase overexpression stable CHO clone rendered >75% of sialylated glycans, among which 62.5 % was **biantennary disialylated** glycans.

Interestingly, the disruption of two α-2,3 sialyltransferases (ST3GAL4 and ST3GAL6) from CHO cells in conjunction with protein engineering of the Fc region produced IgGs with a great majority of bigalactosylated and fucosylated (G2F) glycoforms. Expression of the IgG with engineered Fc region (F241A) in triple gene knockout (FuT8-/-, ST3GAL4-/- and ST3GAL6-/-) CHO cells lowered the galactosylation content to 65% bigalactosylated glycoform (G2). However, overexpression of IgGs with four amino acid substitutions from the α-2,3 sialyltransferases knocked out CHO cells reconstituted the fraction of G2 glycoform back up to approximately 80%. Collectively, this study, to our knowledge, is the first attempt for generating highly galactosylated or solely α-2,6 sialylated N-glycans on antibodies *in vivo*, allowing researchers in both academia and industry to evaluate the significance of tailoring glycosylation on IgGs in biomedicine and biotechnology applications.

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PRODUCT QUALITY CONTROL STRATEGY DEVELOPMENT FOR NON-MAB COMPLEX MODALITIES BY USING COMBINATORIAL CELL ENGINEERING AND OMICS SCREENING TOOLS

Zhimei Du, Biologics Proc Dev & Clin Mfg, Merck Research Labs, Kenilworth, NJ
zhimei.du@merck.com
Ren Liu, Biologics Proc Dev & Clin Mfg, Merck Research Labs, Kenilworth, NJ
Bo Jiang, Biologics Proc Dev & Clin Mfg, Merck Research Labs, Kenilworth, NJ
Steve Huhn, Biologics Proc Dev & Clin Mfg, Merck Research Labs, Kenilworth, NJ
Yang Ou, Biologics Proc Dev & Clin Mfg, Merck Research Labs, Kenilworth, NJ
Gregg Nyberg, Biologics Proc Dev & Clin Mfg, Merck Research Labs, Kenilworth, NJ

Key Words: Product quality attributes, Cell engineering, OMICS, complex protein modality, High throughput screening.

Product quality control without compromising productivity has been a major goal in biotherapeutics process development. The challenge is further increased for new modalities using complex and hybrid protein structures, such as nanobodies and bispecific antibodies. New product-related impurities and unique product quality attribute (PQA) species have been found to accompany these new protein scaffolds, which usually don't exist in standard mAb production. Undesired attributes include unique patterns of glycosylation, conformation heterogeneity, mis-pairing, and partial molecules. Many of these PQAs are related to protein folding and assembly efficiency inside the cell, which impact post-translational modifications such as disulfide bond formation and glycosylation processes, directly or indirectly. We have identified multiple intracellular causal factors that link some PQAs directly to host cell lineage. To improve understanding and increase options in developing a successful production cell line with desired product quality profile, we have used this information to develop diversified CHO host lineages using both conditioned-culture adaptation and CRISPR genome editing approaches. The resulting CHO hosts showed significant differences in cell growth and recombinant protein production, including productivity and quality attribute profiles. Furthermore, the hosts respond differently to changes in medium components and process conditions. These differences were more significant for complex/ hybrid proteins such as nanobodies and bispecific antibodies. OMICS tools were systematically utilized to identify the evolutionarily significance of genetic and epigenetic variability of individual host cell lineages, which determine the specific PQA profile of the expressed recombinant protein. Overall, our presentation will illustrate the importance of selecting the appropriate host cell line through screening and/or engineering, as part of quality control strategy to obtain the desired recombinant protein PQA profile. .

CONTROLLING TRYPTOPHAN OXIDATION THROUGH MEDIUM/FEED MODIFICATIONS AND POTENTIAL MOA UNVEILED BY TRANSCRIPTOMICS ANALYSIS

Luhong He, Eli Lilly, USA
he_luhong@lilly.com,
Laurie Hazeltine, Jairav Desai, Jinxin Gao, John Calley, Christopher Frye, Eli Lilly, USA

Key Words: antibody, tryptophan oxidation, Chinese Hamster Ovary, cell culture, transcriptomics

Oxidation of monoclonal antibodies (mAbs) is one of the major product quality issues with potential impacts on product activity and immunogenicity. Tryptophan oxidation (Trp-ox) leads to addition of one or two oxygen atoms to the indole ring of its side chain and other subsequent degradation end products. It was reported that Trp-ox in the complementarity-determining region of a mAb led to a progressive loss of antigen binding and biological activity [1]. Trp-ox was also reported to cause color changes in near UV−visible light-irradiated and heat-stressed monoclonal antibody (mAb) drug product in liquid formulation [2]. In addition, Trp-ox was observed under real-time storage and elevated temperature conditions [3]. Recently, we observed that modifying the concentrations of copper, manganese, tryptophan, and cysteine in cell culture media/feed had a significant impact on Trp-ox levels of two mAbs in development produced in Chinese hamster ovary (CHO) cells [4]. We have demonstrated that Trp-ox level can be effectively controlled while maintaining productivity and overall suitable product quality profiles. In this presentation we will summarize those findings and the results from the systematic studies that enabled us to control the Trp-ox levels at both the shake flask and benchtop bioreactor scales. Moreover, we will describe new studies that aimed to understand the potential mechanism of action (MOA) of those components on controlling Trp-ox levels.

The advent of NGS technologies and the availability of CHO reference genomes have enabled the systematic analysis of CHO biology and its capacity for recombinant protein production [5, 6]. Here we applied transcriptomic analysis using RNA-Seq to explore the underlying mechanisms of cell culture's impact on Trp-ox. Cell samples from fed-batch bioreactors cultured with control or modified media/feed were harvested and subjected to RNA-Seq analysis. The results showed that cell culture conditions had little impact on the expression of the mAb transgenes (LC and HC), nor genes related to glycosylation, which is consistent with the previous findings on mAb productivity and glycosylation profile [4]. However, cell culture conditions did significantly alter the expression of multiple genes (fold change ≥1.5, p-value ≤0.05). Specific subsets of genes involved in control of oxidative stress and metabolism of copper, manganese, tryptophan cysteine will be discussed in detail. The analyses will focus on genes engaged in scavenging of free radicals because of their known roles in oxidation chemistry and production of reactive oxygen species (ROS). We postulate that these changes in gene expression may provide molecular means to balance the copper availability and glutathione pool, which in turn might result in the observed impact on mAb quality without changing the CHO cell growth and productivity. The work presented here provide another example of how gene expression analyses can shed additional light on potential mechanisms for observed cell culture performance and specifically in this case, changes in recombinant protein product quality attributes. Such understanding could eventually lead to a biomarker-based approach for process optimizations. To the best of our knowledge, this is the first example of using transcriptomic analysis to mechanistically understand the impact of cell culture on critical quality attributes other than glycosylation. Therefore, we believe this presentation is of great interest to general biopharmaceutical community and is relevant the themes of the Conference, especially to the section "Advances in cell culture control of product quality attributes".

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ONLINE CONTROL OF CELL CULTURE REDOX POTENTIAL PREVENTS ANTIBODY REDUCTION

Michael Handlogten, Cell Culture & Fermentation Sciences, MedImmune
handlogtenm@medimmune.com
Sanjeev Ahuja, Cell Culture & Fermentation Sciences, MedImmune

Key Words: Antibody Reduction, Redox Potential, Cell Culture

The phenomenon of monoclonal antibody (mAb) interchain disulfide bond reduction during manufacturing processes has been reported widely across the biotechnology industry. Reduction results in loss of product, requires more complex purification processes, and leads to reduced stability of the final drug product. Here, we show the development of a control system to prevent mAb reduction in the bioreactor based on the cell culture redox potential. Cell culture redox potential indicates the reducing/oxidizing potential of the extracellular environment. This work describes the process development for an IgG_2 mAb where a high level of mAb reduction was observed at harvest. Further analysis revealed reduced mAb in the bioreactor prior to harvest. To understand the impact of reducing/oxidizing environment on mAb reduction, the process was run with several different conditions including increased concentrations of metal ions, 2-mercaptoethanol, glutathione, cystine, and an increased DO set-point. Samples were taken from these bioreactors on days 12 and 14 and the amounts of intact mAb in the cell culture supernatants were quantified. Many of these bioreactors were run with redox probes that allowed monitoring of the cell culture redox potential. Analysis of the data revealed a clear correlation between the cell culture redox potential and mAb reduction. Based on this information, we identified a threshold redox potential above which the mAb remained intact and below which there was significant and highly variable amounts of reduced mAb. Using this knowledge, we developed three control schemes to mitigate mAb reduction. These control methodologies functioned by increasing the concentrations of dissolved oxygen (DO), Cu, or both DO and Cu to maintain the redox potential above the threshold value. The redox control strategies based on the addition of Cu or Cu and DO maintained the cell culture redox potential above the threshold value and prevented mAb reduction, whereas the control strategy based on DO control alone was insufficient to maintain the redox potential above the threshold value and had high levels of reduced mAb (Figure 1). Importantly, the redox control strategies did not significantly impact the cell growth, viability, mAb production, or product aggregates. This method of using on-line cell culture redox potential can be used to predict the likelihood of reduction occurring in the bioreactor and evaluate the effectiveness of new mitigation/control strategies; it can also be extended to prevent mAb reduction from occurring during or after the harvest. Finally, the methods described in this work to control mAb reduction would ensure simpler purification processes, improved product quality, and prolonged drug product stability compared to processes with uncontrolled reduction.

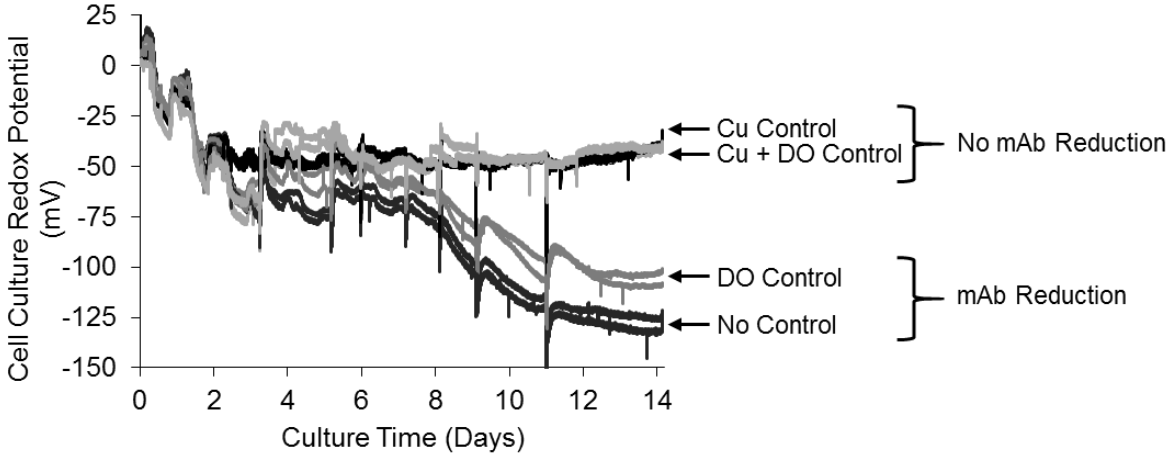

Figure 1 – Online Cell Culture Redox Potential

IDENTIFICATION OF COPPER AS A CELL CULTURE MEDIA COMPONENT CAUSING METABOLITE DEPLETION AND PRODUCT SEQUENCE VARIANTS

Brian E. Mickus, Gilead Sciences, Inc., Biologics Development
Brian.Mickus@gilead.com
Matt Schenauer, Gilead Sciences, Inc., Biologics Development
Winnie Yeung, Gilead Sciences, Inc., Biologics Development
Ambrose Wesel, Gilead Sciences, Inc., Biologics Development
Daniel Tien, Gilead Sciences, Inc., Biologics Development
Doug Rehder, Gilead Sciences, Inc., Biologics Development
Tim Kaschak, Gilead Sciences, Inc., Biologics Development
Gayle Derfus, Gilead Sciences, Inc., Biologics Development
Dell Farnan, Gilead Sciences, Inc., Biologics Development
Rajesh Krishnan, Gilead Sciences, Inc., Biologics Development

Key Words: Sequence variants, copper, amino acid depletion, lactate, cell culture media

The level of peptide sequence variants in a biologic drug substance batch is a critical product quality attribute that should be monitored and controlled. These sequence variants are typically caused by DNA single nucleotide variants that arise in cloning and amplification, mistranscription due to unstable vector DNA or cell age/production stresses, or mistranslation via tRNA wobble or mischarging. In this work, a low frequency of monoclonal antibody sequence variants was detected by mass spectrometry in a drug substance batch. The variants were distributed throughout the heavy and light chains at average levels of under 1% per site with no apparent codon bias. No product-coding DNA mutations were detected via deep sequencing data. This pattern of low level, widely-distributed variation strongly suggested a misincorporation mechanism via mischarging of aminoacyl-tRNA, presumably due to amino acid depletion during the process.

Copper is a critical cell culture media component that can be modulated in fed-batch processes to induce lactate consumption via its role as a cofactor for mitochondrial function and respiration. However, complete consumption of lactate can also trigger reduced levels of other metabolites required for recombinant protein assembly, which can lead to product sequence variants. To investigate the potential relationship between media copper supplementation and sequence variants, various levels of copper were supplemented into the basal media for fed-batch cultures at the 250 mL bioreactor scale. Mass spectrometry analysis of the partially purified antibody indicated a positive correlation between the amount of copper supplemented and the level of detected sequence variants as well as a mechanism for sequence variant reduction via targeted nutrient feeding.

This work has identified a potential mechanism of sequence variant generation related to cell culture media copper levels as well as process alterations to prevent such variation in future batches, highlighting the importance of carefully controlling trace metal levels. Additional studies may be required to validate the potential mechanism.

A SYNTHETIC BIOLOGY BASED CELL LINE ENGINEERING PIPELINE

Christopher S. Stach, University of Minnesota, Minneapolis, USA
Sofie O'Brien, Tung Le, Arpan Bandyopadhyay, Meghan McCann, Conor O'Brien, Zion Lee, Prodromos Daoutidis, Wei-Shou Hu, Nikunj Somia, Michael J. Smanski, University of Minnesota, Minneapolis, USA
Xinning Chen, East China University of Science and Technology, China
Liang Zhao, East China University of Science and Technology, China

Keywords: Synthetic biology, glyco-engineeering, combinatorial design, genome sequencing, ATAC-seq

An ideal host cell line for deriving cell lines of high recombinant protein production should be stable, predictable, and amenable to rapid cell engineering or other forms of phenotypical manipulation. In the past few years we have employed genomic information to identify "safe harbors" for exogenous gene integration in CHO cells, deployed systems modeling and optimization to design pathways and control strategies to modify important aspects of recombinant protein productivity, and established a synthetic biology approach to implement genetic changes, all with the goal of creating a pipeline to produce "designer" cell lines.

Chinese hamster ovary (CHO) cells are the preferred platform for protein production. However, the Chinese hamster genome is unstable in its ploidy, is subject to long and short deletions, duplications, and translocations. In addition, gene expression is subject to epigenetic changes including DNA methylation, histone modification and heterochromatin invasion, thus further complicating transgene expression for protein production in cell lines. With these issues in mind, we set out to engineer a CHO cell line highly amenable to stable protein production using a synthetic biology approach. We compiled karyotyping and chromosome number data of several CHO cell lines and sublines, identified genomic regions with high a frequency of gain and loss of copy number using comparative genome hybridization (CGH), and verified structural variants using sequencing data. We further used ATAC (Assay for Transposase-Accessible Chromatin) sequencing to study chromatin accessibility and epigenetic stability within the CHO genome. RNA-seq data from multiple cell lines were also used to identify regions with high transcriptional activity. Analysis of these data allowed the identification of several "safe harbor" loci that could be used for cell engineering.

Based on results of the data analysis and identification of "safe harbors", we engineered an IgG producing cell line with a single copy of the product transgene as a template cell line. This product gene site is flanked by sequences for recombinase mediated cassette exchange, therefore allowing easy substitution of the IgG producing gene for an alternative product gene. Furthermore, a "landing pad" for multi-gene cassette insertion was integrated into the genome at an additional site. Together, these sites allowed engineering of new cell lines producing a fusion protein and Erythropoietin to be generated from the template cell line. To enable rapid assembly of product transgenes and genetic elements for engineering cell attributes into multi-gene cassettes, we adopted a golden-gate based synthetic biology approach. The assembly of genetic parts into multi-gene cassettes in a LEGO-like fashion allowed different combinations of genes under the control of various promoters to be generated quickly for introduction into the template cell line.

Using this engineered CHO cell line, we set out to study metabolism and product protein glycosylation for cell engineering. To guide the selection of genetic elements for cell engineering, we developed a multi-compartment kinetic model, as well as a flux model of energy metabolism and glycosylation. The transcriptome meta-data was used extensively to identify genes and isoforms expressed in the cell line and to estimate the enzyme levels in the model. The flux model was used to identify and the LEGO-like platform was used to implement the genetic changes that can alter the glycosylation pattern of the IgG produced by the template cell line. Concurrently we employed a systems optimization approach to identify the genetic alterations in the metabolic pathway to guide cell metabolism toward a favorable state. The model prediction is being implemented experimentally using the synthetic biology approach.

In conclusion, we have illustrated a pipeline of rational cell line engineering that integrates genomic science, systems engineering and synthetic biology approaches. The promise, the technical challenges and possible limitations will be discussed in this presentation.

ENGINEERING STEM CELL FATE

Peter Zandstra, University of British Columbia, Canada
peter.zandstra@ubc.ca

Regenerative medicine (RM) offers an opportunity to address the root causes of chronic diseases, which typically result from the breakdown of tissues maintained by stem cells (SC). In Canada, chronic disease consumes 67% of direct health care costs, with approximately $68 billion attributable to treatment costs. Canada is a leader in SC-based RM and my group has contributed to discovery and innovation in this sector.
Early RM technologies focused on the transplantation of cells from the same individual. RM is now at a stage where cell products, such as blood SC, are manufactured for clinical testing. Our program is building the next generation of RM therapeutics – living cells and tissues designed to treat specific indications. Our vision is to understand, at a fundamental level, the mechanisms by which complex tissues develop from SC, and to use this understanding to advance new cell therapies and regenerative medicines.

Our approach is based on three complementary thrusts. First, we are developing computer simulations of normal and diseased human tissue development. These simulations allow us to connect the genetic coding inside SCs to the environment that influences SC growth. These simulations will one day dramatically shorten the time it takes to develop new therapies for degenerative diseases. Second, we are rewiring the genetic code in SC, and engineering the environment around SC, to understand the key requirements of SC-based tissue development, and to increase the quantity and quality of cells SC produced. Third, with our partners, we are moving promising discoveries towards the clinic using advanced models of disease, focusing on testing specialized blood cells as blood cancer and autoimmune disease therapeutics.

Our work is revealing new rules that govern tissue development, generating new technologies for RM applications, and yielding new SC-based therapies.

FUTURE CHALLENGES IN BIOLOGICS CELL CULTURE ENGINEERING

Lynne A. Krummen, Head Pharma Technical Development Biologics, Genentech Inc.
Krummen@gene.com

Michael Laird, Genentech, Inc., USA
laird.michael@gene.com

The biotechnology industry is at an important transition point. Over the last 30 years much has been learned about development and industrialization of Cell Culture processes largely from broad spectrum development of monoclonal antibodies in CHO cells. However, important learning has also been taken from expression and culture of complex proteins. Now we look to a future where designer molecules will replace the standard monoclonal modalities in new product development and competition and changing regulatory and economic paradigms will drive the need for unprecedented titers, product quality control and speed to market. Fortunately, we face these new challenges armed not only with historical knowledge, but with a new spectrum of molecular engineering, process modeling and analytical tools that promise unprecedented productivity combined with metabolic and product quality control. This talk will outline the opportunities of the future and highlight the technology developments that position the industry to meet these challenges.

IGNORANT EMPIRICISM IN CELL CULTURE ENGINEERING: 30 YEARS OF EXPENSIVE LESSONS

Matt Croughan, Keck Graduate Institute, USA
croughanmk@aol.com

Key Words: ignorant empiricism, performance barriers, operationally complex, impractical contraptions

Although cell culture engineering has generally been quite successful, the path forward was not steady, but instead has had many stalls and diversions. These have largely been due to approaches based upon ignorant empiricism --- i.e., "it just seems to work, but we don't know why". As it turns out, ignorant empiricism often goes hand-in-hand with performance barriers that are not identified and thus not overcome for years. Examples will be discussed such as early sparging, agitation, and cell line handling protocols that limited the impact of nutrient enrichment and modern medium development. Until such barriers were identified and overcome, industrial cell culture could not have met the cost requirements and market demands for monoclonal antibody therapeutics. Ignorant empiricism also led to the expensive development and testing of many creative but operationally complex and impractical bioreactor designs, as will be shown. It also led to both near and complete stock outs of life saving drugs as well as the failure and take-over of a major biopharmaceutical company. Lastly, it continues to lead to unexpected run failures, operational crises, and process performance variability, including unacceptable variability in product quality. This talk will cover 30 years of expensive lessons learned, including some being learned only now.