

Modern Challenges in Therapeutic Protein Production

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: Friday, 11 June 2010 09:00 - 17:00

The purpose of this meeting is to look at the challenges facing therapeutic protein production and demystify some of the novel approaches and new technologies currently being developed. *Meeting chair - Dr Brendan Fish*, NPI-PT Director at GSK Barnard Castle

This event has CPD accreditation and will have a [discussion panel session](#).

On registration you will be able to submit your questions to the panel that will be asked by the chair on the day of the event

- 9:00 – 9:45 **Registration**
- 9:45 – 9:55 **Introduction by the Chair:** *Dr Brendan Fish*, NPI-PT Director at GSK Barnard Castle
- 9:55 – 10:20 **Faster, cheaper, better: How novel approaches are helping develop biotherapeutics for tomorrow.**
Mrs Allison Mason, MedImmune, Cambridge, UK
Developing successful biotherapeutics takes many years and is a costly process. Time to the clinic can be reduced by improving efficiency of the processes used at each stage of the drug development cycle and cost of goods can be decreased by optimising manufacturing processes. This talk focuses on some strategies and new technologies which have been adopted at MedImmune to streamline and optimise our cell line development and upstream production processes.
- 10:20 – 10:45 **Slonomics Technology - Generation of Multiple Length Variants in Synthetic Antibody Libraries**
Dr. Thomas Waldmann, Sloning BioTechnology GmbH, Germany
Traditional research has focussed on process development to improve therapeutic protein yield and quality. Another more recent approach is to engineer the genes themselves to obtain the desired protein - a process known as directed evolution. The Slonomics® technology platform generates highly diverse and precise combinatorial libraries for such protein engineering. Unlike traditional mutagenesis methods that rely on single stranded oligo nucleotides, the process uses double stranded DNA triplets as universal building blocks for the synthesis of any gene sequence - 'one amino acid at a time'. For library production, a mixture of codons can be introduced at any desired sequence position, in any combination and at any ratio. The absence of functional bias and the sequence independent synthesis process result in exceptionally high quality libraries containing the complete set of desired mutants. Any sequence position can be mutated individually, in a stretch, or in multiple sequence combinations. In addition, individual mutation patterns can be uniquely combined with length diversifications or randomly mutated sequence regions.
- 10:45 – 10:55 **Speakers photo**
- 10:55 – 11:20 **Mid-morning break and poster viewing**
- 11:20 – 11:45 **Strategies to decrease cell line development - a review of the current technologies**
Dr Jenny Thirlway, Eden Biodesign, Liverpool, UK
One of the largest lead times in moving a biological drug candidate from the bench to a 'first in man' clinical trial is the development of a stable, high expressing production cell line. Here we present a strategic review of the key activities required to develop a cell line and discuss the pros and cons of the current proprietary technologies (including generation of cDNA, expression technologies and clone screening & selection) that are used to compress the timelines for cell line development.
- 11:45 – 12:10 **Scaling up Protein Production in Animal Cells - Challenges and Solutions to the Bioreactor process**
Mr Gary Pettman, GSK, Harlow, UK
The challenges for scaling up protein production from animal cells comprise both economic and technical issues. Reducing costs and shortening timelines whilst at the same time maintaining high viability, highly productive cultures are vital for success, whether the protein is required as a drug target for screening or as a biopharmaceutical. The complete process, from cell line generation / selection, through to the downstream processing contributes to the final product but a key component from both an economic and technical point of view, is the choice of bioreactor

used. This talk will discuss developments in this area, namely the choice of bioreactor and the increased use of disposable technology and bioreactor miniaturisation for process development and optimisation.

12:10 – 12:35

The use and validation of disposable technologies in therapeutic protein manufacture

Mr John Moys, Sartorius Stedim, UK

The growth in the use of disposable technologies in the pharmaceutical and bio-pharmaceutical industries has been exponential over the last decade and more and more products are now nearing regulatory approval and launch. The adoption of these technologies has been discussed many times, however one area that is rarely discussed is the validation of these technologies and how this varies from the validation of traditional manufacturing technologies. This talk will therefore focus not just on the applications for disposable technologies but also the increasing validation burden as your therapeutic candidate moves through the clinical phases towards approval and launch.

12:35–13:30

Lunch and Poster Viewing

13:30 - 14:20

Question and Answer Session

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

14:20 – 14:45 -

xCELLigence, Real Time, Label Free Cell Analyser for True and Physiologically Relevant Data in Living Cells

Dr Pete Hughes, Roche Diagnostics Limited, UK

The xCELLigence Systems allow for label-free and real-time monitoring of cellular processes such as cell proliferation, cytotoxicity, adhesion, viability, invasion, and migration, using electronic cell sensor array technology. Real-time monitoring of cellular processes by the xCELLigence Systems offers distinct and important advantages over traditional end-point assays. First, the avoidance of labels allows for more physiologically relevant assays which save on time, labor, and resources. Second, a comprehensive representation of entire length of the assay is possible allowing the user to make informed decisions regarding the timing of certain manipulations or treatments. Finally, the actual kinetic response of the cells within an assay prior or subsequent to certain manipulations provides important information regarding the biological status of the cell such as cell growth, arrest, morphological changes and apoptosis. The xCELLigence System serves the increasing needs of the life science research and drug discovery markets. The benefits offered by this exciting technology include broad applications (including GPCR, Tyrosine Kinase activation, Natural Killer Cells and Antibody Dependant Cell Mediated Cytotoxicity as well as Cell invasion and migration), high data quality, complete data record, convenience, physiological relevance and a versatile software.

14:45 – 15:10

Forced degradation studies of proteins formulated by high-throughput techniques

Yitzchak Grant, University College, London

Microplate-based, and increasingly microfluidic platforms, enable very small quantities of proteins to be analysed under a wide range of formulation, stress and bioprocess conditions. A platform of automated high-throughput methods for pre-formulating protein conformational stability, solubility and tolerance to freeze-drying will first be introduced. Their application to pre-formulation, the design of bioprocesses and the early identification of problematic protein leads will be discussed. The benefits and current limitations of high-throughput approaches for gaining increased understanding of protein behaviour will also be discussed by comparison to forced degradation results and further detailed biophysical analyses of proteins in solution.

15:10 – 15:40

Afternoon Tea/Coffee and Last Poster Viewing

15:40 – 16:05

Quality Issues, Guidelines and Requirements for Biological Products

Charles Christy, Covance Laboratories Ltd, Harrogate, UK

The talk will cover the range and specific details of analytical testing that is currently required for biological products with specific reference to cell line release, process validation for contaminant clearance (virus, HCP, rDNA), and to characterise, ensure batch-to-batch consistency and appropriate product stability. This talk will cover the regulatory requirements and the relevant guidelines for both the EMEA and the FDA for biological therapies. Finally the talk will give guidance on the most appropriate analytical techniques required to demonstrate product consistency, purity, safety and potency.

16:05 – 16:30

Regulatory aspects of therapeutic protein production

Dr Stephen Thompson, S-cubed Ltd, Oxfordshire, UK

The evolution of regulation of biological products has resulted in the key concept of 'the process is the product, and the product is the process'. Biotech products are complex and diverse, resulting in a 'case by case' approach to

assessment. While there is an absence of useful precedent in the regulatory requirements for biologicals, there is more scope for devising development programmes based on knowledge of the product and process. This presentation will cover the regulatory requirements and relevant guidelines for biological therapies, and the evidence require to meet regulator's expectations for a well-characterised product and well-characterised process.

16:30 - 17:00 **Chairman's summing up.**

*This meeting was **organised by Euroscicon** (www.euroscicon.com), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry. The event was hosted by **'BioPark** (www.biopark.co.uk), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies*

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About the chair

Brendan Fish is Director of New Product Introduction and Process Technology for GSK at Barnard Castle. With a career spanning over 20 years, he was Director of Bioprocess Sciences at MedImmune Cambridge where he was responsible for all aspects of the development of purification methodologies, product characterisation, QC, formulation and delivery for MedImmune products in relation to their use in commercial pharmaceutical processes. This included initial design and optimisation, scale-up, process cost modelling, process integration and technology transfer to GMP Production for clinical trial supply. Brendan was also at Delta Biotechnology Limited as a Consulting Scientist. He played a key role in the development of their biotechnology-based products providing expert opinion and strategies for QA, QC, Production, Marketing, Operations, Regulatory Affairs and Engineering on all aspects of Process Development. Early in his career, he was a Post-doctoral fellowship at University of Toronto in Canada working in the School of Nutritional Sciences, studying the anti-nutritional effects of lectins in the diet.

About the speakers

Gary Pettman has 29 years industrial experience within GSK and its legacy companies in the area of Animal Cell Technology. He has extensive experience in all areas relating to the culture of animal cells, including, cell banking, serum free media development, use of different bioreactor systems and process optimisation, mammalian and insect cell expression systems and large scale transient expression. He has previously worked in Departments responsible for therapeutic protein development but for the last 10 years has headed a group responsible for the supply of a variety of protein reagents for use as targets and reagents in Drug Discovery at GSK. Recently, he has brought his experience to the area of Stem Cell culture, developing methods for the scale up of Stem Cells for Drug Screening.

Yitzchak Grant, is in his final year of an EngD (PhD in Engineering) in biochemical engineering at University College London.

Jenny Thirlway is responsible for Eden Biodesign's in house Cell and Strain Development capabilities and management of its outsourcing requirements. Jenny's team's capabilities include molecular biology (including viral engineering), mammalian and microbial cell/strain development and small scale fermentation. Prior to joining Eden, Jenny has worked extensively in academia and held a prestigious BBSRC Post Doctoral Research Associate position at the University of Manchester focusing on the engineered biosynthesis of calcium dependent antibiotics from *Streptomyces coelicolor*. Jenny graduated with a Biochemistry and Biological Chemistry degree from the University of Nottingham in 2001 and subsequently obtained a PhD from the School of Chemistry, University of Nottingham studying the interaction between the helicase and primase proteins of *B. stearothermophilus*.

Alison Mason has over 11 years of experience of cell culture and bioreactor process development within the field of therapeutic protein production. After working at Lonza Biologics as a fermentation scientist, Alison moved to MedImmune Ltd (formerly Cambridge Antibody Technology) in 2000. At MedImmune, Alison has been responsible for developing cell culture media and feeds, optimising bioreactor processes and tech transfer of these processes for cGMP production at both an external CMO and at MedImmune's cGMP facility in Gaithersburg, Maryland. Alison holds a degree in industrial microbiology from the University of Manchester.

Charles Christy is the Associate Director of Biotechnology at Covance Laboratories. Covance is a global supplier of drug development services. The Biotechnology Division provides specialised support to biologics developers and manufacturers in the key areas of cell banking, cell line qualification, biosafety testing, process validation, GMP batch release, GMP protein characterisation, product stability and potency studies, and immunochemistry. Charles has a degree in Biochemical engineering from University College London, where he also followed research into the large-scale fermentation and isolation of a chloroperoxidase from a filamentous fungus. He then joined the engineering contracting company APV as a process engineer, and was mainly involved in the design of a state-of-the-art large-scale plasma fractionation facility for the Australian government. Charles then joined the leading specialist separations company Millipore Corporation and served in a variety of roles over 13 years, including system specialist, tangential flow product manager and finally as biotechnology market manager. In these roles Charles developed expertise in chromatography, filtration and process system scale-up, design and optimisation. During his time at Millipore Charles developed multiple training courses for the industry on topics such as separation technology, scale-up, and process design and optimisation. Charles has been widely published on downstream processing topics and holds a US patent regarding the design of a novel heat exchanger.

Thomas Waldmann is Director Science & Technology Support at Sloning BioTechnology. Since 2006 he is responsible for technical customer liaison and support in Europe, USA and Japan. He joined Sloning BioTechnology as a scientific group leader in 2002 and contributed significantly to the development of the Slonomics[®] technology. Before, he has worked successfully in the field of automated screening technologies for several Munich-based biotech companies. Dr. Waldmann gained extensive scientific experience from his postdoctoral fellowships at the Memorial Sloan-Kettering Cancer Center in New York, USA. He holds a degree in biology from the Technical University in Munich, Germany and graduated at the Max Planck Institute of Biochemistry, Martinsried, Germany.

Stephen Thompson has over twelve years' regulatory affairs experience in the pharmaceutical industry as a consultant, joining Origin Pharmaceutical Services (which then became Constella Group), before moving to S-cubed Ltd as Director of Regulatory Affairs in March 2009. Stephen has provided regulatory support to many pharmaceutical and biotechnology companies in both Europe and the USA, and has worked with European regulatory agencies and the US FDA. His experience includes the preparation, submission and approval of clinical trial applications for clinical studies in the EU and USA, as well as marketing authorisation applications and subsequent maintenance, for both small molecules and biologicals.

John Moys is the head of applications support, North Europe for Sartorius Stedim Biotech. He has over 15 years experience with Sartorius in both technical and commercial roles and has carried out projects in the UK, Europe and Asia. Prior to joining Sartorius he had over 8 years working in industry in manufacturing, quality control and R&D roles in the in-vitro diagnostics and antibody manufacturing sectors.

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POSTERS

A FULLY AUTOMATED STIRRED, SPARGED BIOREACTOR MIMIC

Dr Ralph von Strandmann, The Automation Partnershi, UK

The infrastructure requirements, overheads and labor associated with the use of traditional bench-scale bioreactors limits their application in cell line selection and early process development. The Automation Partnership has developed a stirred, sparged bioreactor providing closed-loop control of pH and DO. The automation package provides for automated feed addition, sampling and maintenance of 24 individual bioreactors. The poster describes the system function and capability and shows data from cell culture trials to provide an understanding of the potential benefits of the system

ENGINEERING QUALITY IN BIOPHARMACEUTICALS: RE-DESIGNING ANTIBODIES TO REDUCE AGGREGATION & INCREASE PRODUCTIVITY

Y Stallwood, R Michael, J.L. Jiménez, M Demir, A Arnell & J Zurdo
*Advanced Protein Technologies, LONZA Biologics plc, Babraham Research Campus,
Cambridge, CB22 3AT, UK*

Protein stability and aggregation are major, and still largely unsolved, issues affecting the development and production of biopharmaceuticals. Besides their impact on development costs, the safety of biopharmaceuticals can also be significantly affected.

Traditionally, protein misfolding and aggregation problems have been approached by implementing costly DSP and formulation strategies. However, the growing concern of regulators with the safety of biopharmaceuticals is increasing the pressure to optimise current procedures used in drug development and to implement new strategies aimed to increase the quality and safety of biologics.

Lonza APTs aggregation prediction platform (AggreSolve™) can be applied to overcome protein stability and aggregation issues, thereby improving the manufacturability of polypeptide drugs. The calculation of solvent accessibility, structural preferences, aggregation propensity, and potential inter-molecular interactions can be combined to propose potential modifications that could contribute to the stabilisation of a polypeptide.

We have previously shown that the AggreSolve™ platform can be successfully applied to reduce aggregation in full antibodies (PEPTALK, San Diego, 2009; IBC Antibody Development and production, Carlsbad, 2009). Here we show how AggreSolve™ can be applied to a specific region of a full antibody with known stability and aggregation problems to generate variants with reduced aggregation and enhanced thermal stability, and how, by doing that, the productivity of the molecule can also be improved.

P200 SCREENTAPE – AUTOMATED SDS-PAGE AND ANALYSIS

Andrew Hayward, Broadley Technologies Ltd, UK

P200 ScreenTape is a fully automated electrophoresis platform that fully supports the QA/QC process during research, development and manufacture of antibody products. We present here the at-line monitoring of mAb expression with side-by-side reducing and non-reducing analysis directly from the bioreactor. We also present the use of P200 as a tool for the analysis of mAb purification as well as a final quality check of the purified product.

NOVEL STRATEGIES FOR THE USE OF ENGINEERED AMINOPEPTIDASES AND ON-COLUMN TAG REMOVAL FOR THE PURIFICATION OF RECOMBINANT PROTEINS

Kuo, W.H.K. and Chase, H.A.

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The adoption of histidine-rich tags, used in conjunction with Immobilized Metal Affinity Chromatography (IMAC), is a cost-effective technology that has a much higher potential for use at large scale protein purification than currently documented. Difficulties in removing the tag after purification pose a barrier to the wider use of this technology. Previous studies have revealed DAPase, a recombinant hexa-histidine tagged exopeptidase, to be a highly efficient protease for poly-histidine tag removal¹. Consequently the optimisation of tag removal using DAPase has been the subject of this work. We have addressed some of the issues raised in the processing of histidine-rich tagged proteins, namely tag removal and its detection, clearance of process contaminants derived from enzymatic tag cleavage, and process intensification. "On-column" exopeptidase cleavage, i.e. the removal of the tag by DAPase while the target protein remains adsorbed to IMAC adsorbent used to purify it, has been shown for the first time to prove the processing of tagged proteins. Results will be presented that identify two strategies for the on-column tag cleavage by DAPase. Overall, on-column exopeptidase cleavage is shown to provide significant benefits over the supplier's currently recommended batch process for tag removal².

[1] Pedersen, J., C. Lauritzen, et al. (1999). "Removal of N-terminal poly-histidine tags from recombinant proteins using engineered aminopeptidases." *Protein Expr Purif* 15(3): 389-400.
[2] Arnau, J., C. Lauritzen, et al. (2006). "Current strategies for the use of affinity tags and tag removal for the purification of recombinant proteins." *Protein Expr Purif* 48(1): 1-13.