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THE BIOPHARMA MANUFACTURER

3. Laupheimer Zelltage 2014

Processes for Virus-based Biologics

ABSTRACTS

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CHO GENOME AND CHO QUASI-SPECIES: IMPACTS ON MANUFACTURING AND FUTURE

Chinese hamster ovary (CHO) cells are the source of multi-ton quantities of protein pharmaceuticals. They are derived from an immortalized population of cells (cell line) that apparently emerged spontaneously during culture in the late 1950s. As is true for all cell lines, cells of CHO populations are characterized by a high diversity in genetics and phenotypes. While modest fluctuations around an “averaged” phenotype of a population may occur at any time during culture such drifts stay typically small if culture conditions are kept within a narrow range of parameters. However, according to Darwin and evolution theory, any pre-existing diversity within a population of organisms (viruses, cells, bacteria, animals or plants) can and usually will be enhanced by selective forces when such populations become separated from each other so that the sharing and mixing of a given gene pool is prevented. In nature, such phenomena can result in in speciation. The culture of CHO cells under (very diverse, but practical) conditions in hundreds of laboratories resembles processes under Darwinian selection.

CHO cells have been used in cell culture for more than 50 years and a bewildering number of cell lines can be found in the literature. In many instances, the same name is used for many CHO cell lines, while, due to many factors, the culture conditions may vary dramatically from lab to lab and this on a long-term basis. This seminar tries to trace back the history of CHO cells, particularly the origin and subsequent fate of key industrially used cell lines. It is, in the opinion of the author, not a radical proposal to suggest that the name CHO represents many truly different cell “species”, based on their inherent genetic diversity and their highly dynamic rate of genetic change. The remodeling of the many genomic structures in clonal or non-clonal cell populations, further enhanced by the non-standardized culture conditions in hundreds of different labs, renders CHO cells a typical case for “groups of quasispecies”. This term was coined in the 1970s for families of related (genomic) sequences exposed to high mutation rate environments where a large fraction of offspring are expected to carry one or more mutations. The implications of the CHO-quasispecies concept for protein manufacturing are significant. Strictly speaking, any individual cell in a culture appears to carry a peculiar genomic structure. Only screening of many clonal cell lines derived directly and under controlled culture conditions from one relatively well-defined starting material may reveal a narrow diversity of geno/phenotypes that may allow relatively reliable predictions cell lines for high-yielding manufacturing processes.

Prof. Dr. Dorothee von Laer
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RE-ENGINEERING VESICULAR STOMATITIS VIRUS TO ABROGATE NEUROTOXICITY, CIRCUMVENT HUMORAL IMMUNITY AND ENHANCE ONCOLYTIC POTENCY

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Background: Oncolytic viruses destroy cancer cells specifically without harming normal tissues and are a highly promising new class of anti-cancer therapeutics. The Vesicular Stomatitis Virus (VSV) is an extremely potent oncolytic agent; however, clinical application has been limited by its devastating neurotoxicity and the rapid induction of a neutralizing antibody response. Here, we sought to exploit the many virtues of replication competent VSV as an anticancer therapeutic, while at the same time mitigating its propensity to infect and destroy normal brain cells.

Material and methods: We removed the VSV glycoprotein G as key neurovirulence deteminant and replaced it with the arenavirus glycoprotein LCMV-GP thereby generating a replicating therapeutic, rVSV(GP). Here, we analyzed safety and efficacy of this novel virus *in vitro* and *in vivo*.

Results: While there are no doses at which wildtype VSV (wtVSV) can be safely introduced into rodent brains, we found that our chimeric strain rVSV(GP) caused no significant neurotoxicity even at doses of 10^8 plaque forming units injected directly into the brains of rodents. In addition, rVSV(GP) was a much safer virus when delivered systemically compared to its parental rVSV strain. rVSV(GP), however, was significantly more potent against a spectrum of human cancer cell lines than current oncolytic virus candidates. Furthermore, it retained rVSVs potent oncolytic activity in both syngeneic and xenogeneic orthotopic human glioblastoma models as well as in a syngeneic CT26 colon carcinoma brain metastasis model. Most importantly, in contrast to wtVSV, rVSV(GP) was not inactivated by human serum complement and did not induce a neutralizing antibody response in mice. The lack of neutralizing antibody induction allowed rVSV(GP) to access and replicate within tumour tissue of pre-immunized animals. Thus, rVSV(GP) is the first oncolytic virus that has the potential to fully retain therapeutic efficacy upon repeated therapeutic application.

Conclusions: Taken together, rVSV(GP) is an extremely promising new oncolytic virus platform, which does not show any of the major drawbacks that have limited clinical efficacy of oncolytic viruses so far.

Dr. Per Sonne Holm
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YB-1 BASED VIROTHERAPY: BASICS AND ACHIEVEMENTS

Glioblastoma have a dismal prognosis, and are typically dying within 3 months if untreated. Standard treatment increases the median survival to 12-15 months, although the disease tends to progress within 6–9 months and the 2-year survival rate is 25%. Patients with recurrent tumors usually survive < 1 year. This has remained essentially unchanged for 25 years despite impressive technological advances in imaging, surgery, and therapy, including targeted therapies, but which have thus far shown limited clinical improvement. The current standard of care for newly diagnosed GBM is surgical resection with concomitant daily temozolomide and radiotherapy. Overall, recurrent GBM is difficult to treat and available treatment options are limited.

Conditionally-replicating adenoviruses (CRAd) specifically replicate in and thus kill cancer cells, while leaving normal non-transformed cells intact. This new strategy, called virotherapy, is an exciting, increasing recognized strategy to treat cancer. XVir-N-31, a recombinant human adenovirus C serotype 5, was proven to replicate efficiently in cancerous cells containing the human oncogenic transcription factor YB-1, and kill these cells as a consequence.

Data will be presented regarding (1) general mode of action of XVir-N-31, (2) tumor growth inhibition in animal glioma models, (3) safety and biodistribution studies of XVir-N-31 in Syrian hamster, (4) GMP production and (5) a novel method to determine virus concentration in cell lysates during downstream process using light scattering

Based on the pre-clinical results as single center, open-label, phase I, dose finding and safety study on the oncolytic adenovirus XVir-N-31 in patients with recurrent glioblastoma is planned to be initiated end of 2014.

Dr. Marc Federspiel
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THE PRODUCTION AND PURIFICATION OF TWO ONCOLYTIC VIRUSES FOR HUMAN CLINICAL TRIALS TREATING CANCER

A wide variety of viruses have shown potent oncolytic activity as therapeutics against a variety of human cancers in animal models and are currently moving toward or already being tested in clinical trials in patients. The large-scale production and purification of larger enveloped viruses are particularly challenging requiring aseptic processing which limits some downstream purification techniques using Good Manufacturing Practices (GMP).

The VVPL has developed enveloped virus GMP production processes using suspension cells in combination with gentle but effective purification using hollow fiber tangential flow filtration that result in greater than 99.5% removal of contaminants and greater than 100-fold increases in final infectious virus titers. Virus production and purification processes developed for two oncolytic viruses, measles virus and vesicular stomatitis virus, will be described. Recent data from ongoing clinical trials will also be presented.

Dr. Kilian Guse
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HELPER-DEPENDENT ADENOVIRAL VECTORS FOR THE TREATMENT OF OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common form of arthritis and the most common musculoskeletal disease in man and other species such as horses. Neither long-term efficient symptomatic nor disease-modifying treatments exist to date. Blocking interleukin-1 (Il-1), which is a key mediator of inflammation, pain and cartilage degradation in osteoarthritis, has shown potential as a treatment strategy for OA. However, stability issues of protein-based Il-1 inhibitors prevent sustained efficacy. Gene therapy may be ideally suited for long-term expression of Il-1 inhibitors to achieve sustainable symptomatic and disease-modifying treatment. In particular, helper-dependent adenoviral vectors (a.k.a. gutless or high-capacity adenoviral vectors) may be ideal because of their high transduction efficiency in joints and their capacity to mediate long-term gene expression. Here we evaluated helper-dependent adenoviral (HDAd) vectors expressing Il-1 receptor antagonist (Il-1Ra) in mouse and horse models of osteoarthritis.

Significant improvement of OA parameters were observed in histologic evaluation of mouse joints treated with HDAd-Il-1Ra compared with mock and control vectors. Micro-CT imaging of mouse joints revealed significantly higher cartilage volume in the HDAd-Il-1Ra treated joints. In horses, no adverse effects were observed after Intraarticular application. Lameness was markedly reduced and range of motion and pain on flexion were significantly improved in HDAd-Il-1Ra treated joints compared to controls. Moreover, macroscopic evaluation of the cartilage surface suggests disease-modifying activity of HDAd-Il-1Ra. Further analyses including histology are ongoing.

We are currently developing a scalable GMP manufacturing process for clinical and commercial grade HDAds. The manufacturing process will serve as a versatile platform for GMP production of any HDAd-based gene therapy vectors.

In conclusion, we have shown that HDAds are well suited for joint gene therapy. HDAd mediated Il-1Ra expression resulted in significant improvement of OA symptoms in mice and horses with signs of disease-modifying activity.

Dr. Harald Petry
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THE DEVELOPMENT OF A COMMERCIAL PRODUCTION PROCESS WAS KEY IN ACHIEVING REGULATORY APPROVAL FOR GLYBERA®

uniQure has developed the first and currently the only gene therapy product, Glybera®, to receive regulatory approval in the European Union. Gene therapy offers the prospect of long-term and potentially curative benefit to patients with genetic or acquired diseases by directing the expression of a therapeutic protein or restoring the expression of a missing protein through a single administration. The first product, Glybera, was approved by the European Commission in October 2012 under exceptional circumstances for the treatment of a subset of patients with lipoprotein lipase deficiency, or LPLD, a potentially life-threatening, orphan metabolic disease. It is expected that Glybera is launched commercially in selected European countries in the first half of 2014 through our collaboration with Chiesi Farmaceutici S.p.A..

A main contributor to the success of Glybera is the robust production process that was developed at uniQure and which is required to provide such a complex product to the market. The manufacturing process, which uses insect cells, is designed to produce higher yields of vectors more cost-effectively and efficiently than other production processes used in the field of AAV-based gene therapy. So far AAV-based vectors could not be manufactured at commercial scale on a cost-effective, reliable and reproducible basis. Difficulties in increasing production levels to commercial scale were particularly challenging in manufacturing processes that utilized mammalian cells. These mammalian cell processes typically use adherent cells, which are cells that only grow on a surface. As a result, production of larger quantities of vector required large surfaces, which is more challenging and less economical than manufacturing processes that use cells that can be grown in a suspension culture.

The production process of AAV products we have developed in insect cells, including Glybera®, starts with a gene cassette containing the therapeutic transgene together with the appropriate promoter and other DNA components required for replication and packaging by the AAV vector. This gene cassette is inserted into the genome of a baculovirus, which we use as an engineering tool in our manufacturing process to generate the AAV particles that are ultimately used for the therapeutic intervention. Those recombinant baculoviruses are then used to infect insect cells derived from Spodoptera frugiperda, the caterpillar stage of the fall army worm moth, with the baculovirus containing the gene cassette. The insect cells are infected at the same time with three different baculoviruses that contain either the therapeutic gene or containing the elements needed for the proteins of the shell, or capsid, of the AAV vector, and the replication proteins required to create multiple copies of the gene cassette that are subsequently packaged into the AAV capsids.

The presentation will cover a description of our insect cell based production process that we use for the production of Glybera. In addition the insect cell production process will be put into perspective of other manufacturing processes used in the field of AAV gene therapy.

Prof. Dr. Stefan Kochanek
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IN VIVO BARRIERS FOR GENE- AND VIROTHERAPY

Viruses are perceived as very efficient gene delivery vehicles, not only for *ex vivo* modification of mammalian cells but in particular also for *in vivo* gene delivery and therapy. However, due to the large amount of vector/virus particles required for many of the *in vivo* applications, the production of different replication deficient viral vectors or oncolytic viruses that are suitable for *in vivo* administration has frequently been found to be very challenging, requiring a significant investment in the development of improved processes and production technologies. Examples are the production of adeno-associated virus (AAV) vectors being under clinical development for the treatment of coagulation disorders and for diseases of lipid metabolism, respectively, or oncolytic viruses such as measles virus (MV) currently used in clinical studies for virotherapy of cancers.

When having a closer look at the vector doses required to achieve a therapeutic effect it frequently becomes clear that only a very small fraction of vector particles is delivered to the target cell or the target tissue. This inefficiency can be explained, at least in part, by *in vivo* barriers, which the different viral vectors encounter when injected either systemically or locally.

Using adenovirus as an example for a frequently used vector type with significant clinical potential and solid tumours as a therapeutic target recognized barriers will be discussed and their impact on vector delivery and vector activity. An improved molecular understanding of these barriers may lead to the development of improved technologies with increased potency at lower doses directly facilitating market supply in the future.

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COMMERCIAL RAAV MANUFACTURING USING THE INTEGRITY ICELLISTM SINGLE USE FIXED-BED BIOREACTOR AND A TRANSFECTION-BASED PRODUCTION PROCESS WITH NOVEL TOOLS TOWARDS VERSATILE STABLE PRODUCER CELL LINES

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In July 2013, the Journal of Gene Medicine counted a total of 1902 gene therapy clinical trials, of which 5.1 % are using AAV as vectors to deliver the therapeutic gene. In fact, AAV technologies show a strong upward trend in view of current preclinical work. Despite the relatively small genome (4.7 kb) that limits its packaging capacity, AAV is getting increasingly popular for the advantages it has to offer: lack of pathogenicity, low immunogenicity, ability to infect quiescent cells, long-term transgene expression without risk of insertional mutagenesis, etc. The boost of the gene therapy field among others driven by approval of Glybera® in the European Union, raises the demand for robust and cost-effective manufacturing of gene transfer vectors for market supply. In this respect, technology platforms are desirable to manufacture high-quality vectors based on rAAV allowing for fast and easy switch of capsid and vector appropriate for a variety of applications.

Many virus-based biologics (viral vectors, virus-based vaccines and oncolytic viruses) are still produced in anchorage-dependent cells, requiring production processes of up to 1000 m^2. Conventional cell culture technologies, such as roller bottles and multitray systems will, therefore, hardly be able to meet future demands of the growing market as they imply some major drawbacks: bulkiness, substantial handling operations and limited process control. Novel cell cultivation technologies such as ATMI's Integrity iCellis^{TM} single use fixed-bed bioreactors, offering surface areas of 0.5-500 m^2, provide a very auspicious alternative. Cell growth, metabolic data and virus yields obtained by transfection-based production of rAAV in this bioreactor format will be presented.

Stable producer cell lines rendering transfection at production scale dispensable, are a promising tool for the establishment of more classical bioreactor processes. Based on a HeLa cell, a suspension cell line carrying stably integrated AAV *rep*, *cap* and vector sequences and allowing for RMCE mediated *cap* serotype and vector exchange has been generated. The final goal is to additionally integrate the Adenovirus helper genes into this cell line and to render the rAAV production cascade inducible by temperature switch. Making use of a temperature downshift as primary switch for rAAV production, we would combine the inevitable induction event due to the formation of toxic proteins with conditions presumably enhancing rAAV production. Tools towards such a highly versatile rAAV producer cell line and first data will be presented.

Prof. Dr. Amine Kamen
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EFFICIENT PRODUCTION OF VIRAL VECTORS FOR GENE THERAPY AND VACCINATION

HEK-293 cell lines have been widely used for over 30 years by the scientific community. Currently HEK-293 cells are the most efficient system for improved production of r-proteins and viral vector by large-scale transfection of suspension-growing cells in serum-free medium. Also, it is the most established cell line for the production of adeno- and adeno-associated viruses, retro-and lentiviruses for gene therapy application. Additionally HEK 293 cells sustain replication of many viruses that are evaluated as vaccines or viro-therapeutic agents. Consequently numerous viral vectors produced in HEK-293 cells have been approved for phase II and phase III clinical trials.
In this presentation, we will discuss the major achievements in process developments completed at NRC to support the large scale manufacturing of viral vectors and vaccines. On-going efforts include characterizing the metabolism of HEK-293 cells in culture, design of efficient operating and feeding strategies, and the development of advanced monitoring tools. Beside viral vectors for gene delivery, recent data demonstrating the suitability of the HEK-293 cells for manufacturing vectored vaccines and influenza vaccine will be presented. Through different examples, the presentation will also discuss practises and experiences in supporting translational research.

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Pierre Heimendinger
Transgene
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Novel Virus-based biologics are edging into the vaccine markets

APPROACH OF PAT IN DEVELOPMENT: NEW TOOLS FOR ONCOLYTIC VIRUS PRODUCTION

The concepts described in the ICH Q8-Q11, commonly referred to as Quality by Design (QbD) have lead Pharmaceutical Development to a better understanding of the interactions between product and the associated process. Process Analytical Technology (PAT) is needed in the implementation of the QbD in Pharmaceutical Development. The PAT is a continuous follow up of the Critical Process Parameters (CPP) and Critical Quality Attributes (CQA). The presentation is about the on-going development of an in-line measurement for process monitoring by using Raman spectroscopy.

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DEVELOPMENT AND CONTROL OF A PRODUCTION PROCESS OF VIRUS PARTICLES

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Tissue culture–adapted Edmonston strains of measles virus (MV) have altered its receptor specificity and became selectively oncolytic with attenuated pathogenicity. This property of the MV eventually leads to selective tumor cell killing. According to the dose needed for measles vaccination in cancer therapy a much larger amount of the MV is needed. So the subject of the presented project is to scale up the production of MV in Vero cells in order to achieve an optimized and standardized fermentation process by providing more MV particles for the use in cancer therapy.

The cells have been adapted to a commercially available serum-free medium. The growth surface was provided by micro carriers for the cultivation of adherent cells in stirred systems.

Similar maximum titers were reached for cell-associated and extracellular MV. Repeated harvesting of the extracellular MV showed no negative influence on MV yields, but raised cumulative virus titers. In addition research on the temperature stability of the MV was carried out and it has been shown that the MV in the supernatant under culture conditions was very unstable due to temperature inactivation.

To meet commercial and regulatory requirements, this process must be high yielding, scalable and reproducible. These requirements are met by establishing a cell culture process employing stirred system and serum-free cell culture medium. For the control of the several online methods are used. The description of MV production kinetics is used for a successful optimization of the process.

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