# Producing viral vectors on an industrial scale

This year, the annual conference of the European Society of Gene and Cell Therapy carried the subtitle 'changing the face of modern medicine.' This was a reference to not just new therapies, but also to the latest drug production technologies. While pharma and biotech companies aim to develop novel therapies eliminating the underlying cause of a disease and thereby healing the patient instead of just targeting the symptoms, technology companies explore innovative production systems to provide enough material to bring these modern medicines through the clinic and to commercialisation.

With the first gene therapy products already on the market in Europe and the US, research and development activity into new gene medicines has increased significantly. UniQure NV was the global pioneer with Glybera for lipoprotein lipase deficiency, which was launched in Europe in 2014. The therapy was withdrawn from the market in 2017 for commercial reasons, but that didn't stop others from progressing these novel medicines. GlaxoSmithKline Plc launched Strimvelis for ADA-SCID in Europe in 2016 and a year later, Novartis brought Kymriah to the US market for acute lymphoblastic leukaemia.

While these initial therapies are proving successful in curing a number of severe hereditary diseases, there is now a shift in research towards more common illnesses. As such therapies involve higher patient numbers and larger doses of viral vectors per treatment, a bottleneck in production is inevitable. Viral vectors are needed to deliver the drug to the target cells or tissue – and the inability to produce these vehicles in sufficient amounts to meet today's growing demand is recognised by industry experts as a 'production gap.'

Conventional methods for the production of viral vectors use adherent cell lines, such as the human embryonic kidney cells HEK293 or the epithelial cells of a cervical carcinoma HeLA. These cell lines share one disadvantage: their production capacity and scalability is significantly limited by their adherent nature as they need a solid substrate to grow on. Larger production volumes thus require increased surface areas. The cell factories with stacked plates which are used for these production processes need to be handled manually and do not allow for proper scale-up, thus severely limiting the production batch size.

By comparison, suspension cell lines do not require solid surfaces but grow in standard stirred tank bioreactors and can easily be scaled-up to industrial volumes. Our company, for example, has developed the proprietary suspension cell line CAP-GT which is a human cell line of non-tumour origin. It grows in serum-free suspension media which is adaptable to current bioreactor formats and suitable for the production of a wide range of different gene therapy vectors such as lentiviral, adenoviral and adeno-associated virus vectors.

The most commonly used vector type in gene therapy today is derived from the adeno-associated virus (AAV), a very small virus. AAVs have a number of advantages. They are not associated with a disease and in their natural lifecycle, they do not integrate non-specifically into the genome of the host cell, thus reducing the oncogenic potential of AAV-mediated gene therapies. Moreover, they are very stable and enable the targeting not only of dividing cells, but also of resting tissue (e.g. brain tissue).

Although well suited for use as gene therapy vehicles, AAVs have one big disadvantage. Current standard production processes for AAV vectors require transfection of the production cells with two or three plasmids encoding the components necessary for replication and assembly of the AAV particle. These transient transfection steps need to be repeated for each production run and require large amounts of high-quality plasmids produced according to good manufacturing practice (GMP) guidelines, adding extra costs.

Moreover, establishing a fully reproducible transient transfection process, as is required under GMP conditions, becomes increasingly difficult with higher production volumes. Alternative production methods require the addition of so-called helper viruses and are associated with several challenges including helper-virus production, removal of the helper-virus particles from the final product and extensive analytics.

## Solving the AAV production gap

As a solution for scalable AAV production, our company has taken its CAP-GT platform to the next step and created a stable AAV production system. In order to generate generic packaging cell lines, the CAP-GT cells are equipped with all of the genes required for the synthesis of the viral particle and packaging of the AAV vector. To produce a dedicated AAV vector for a specific gene therapy, only the therapeutic gene is added to the genome of the packaging cell, resulting in a producer cell. In a final step the optimal clone, the production cell line, is selected which, after chemical induction, produces the desired amount of viral vector loaded with the therapeutic gene.

With all of the required components permanently present within the cell line and without the need for further manipulation, such as transient transfection or transduction with helper viruses, industrial scale manufacturing is feasible. This can take place following standard processes that are as easy and cost-efficient as the production of recombinant proteins and monoclonal antibodies in Chinese hamster ovary cells.

For the future development of gene therapy applications, especially for indications with patient numbers exceeding the one million mark, the production of viral vectors on an industrial scale is indispensable. Conventional transient expression systems no longer meet the requirements for modern viral vector production due to process-related restrictions. We believe that our helper-virus free AAV production platform overcomes the production gap and opens the way for a broad use of modern gene therapies in a wide range of medical indications.

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