CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING & ANALYTICS:
TODAY'S KEY TOOLS AND INNOVATION REQUIREMENTS TO MEET FUTURE DEMAND

SPOTLIGHT

INTERVIEW with:
Nicole Faust, CEO, CEVEC, now part of Cytiva

"The major challenge I anticipate will be to bring down production costs. The current price of gene therapies makes it inaccessible to so many patients worldwide."

Scaling AAV vector manufacture: overcoming roadblocks to the translation of gene therapies

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Q What impact are the current limitations on AAV vector manufacturing having on the translation of gene therapies, and what do you see as the key challenges when using current AAV production methods?

NF: One of the biggest challenges for manufacturing AAV gene therapies stems from the fact that the methods for producing AAV vectors were developed at the universities where the gene therapies were invented. In this setting, researchers only needed small amounts of the AAV material, and needed it quickly, so they used the methods that were at hand. As these initial therapies were all directed against ultra-rare diseases, there was no need for a huge amount of material for patients either. There simply was not a lot of pressure to develop scalable production methods.

The result was that the R&D methods from the universities were modified and adapted to be performed under GMP, and to be acceptable for clinical studies and commercial material. But they were never developed and evolved to fit production needs at an industrial scale. What we see now are more therapeutic approaches for common diseases, such as Alzheimer's and Parkinson's, moving into the clinic with much larger patient numbers and with a need for high doses of AAV particles per patient. So those adapted lab methods have really hit their limit due to the fact that they are based on adherently growing cells.

The standard cell line for AAV vectors in marketed products is adherently growing HEK-293 cells. These cells are substrate-dependent to be able to proliferate, and they can be grown either using cell culture plates, e.g., cell stacks with 10 plates on top of each other, or more sophisticated devices, like the iCELLis, where cells are grown on fibers. However, these devices have their limitations with respect to capacity and the handling of the cells. Very often serum is involved in adherent production, which is acceptable but does not really meet modern biotherapeutic production standards.

> "The predominant production method right now is still triple transfection of plasmids."

There are suspension methods being developed, and for HEK-293 cells these efforts are evolving, but what they all have in common is that the production step is always based on transient transfection using plasmid DNA, preferably of GMP grade quality. This is needed in huge quantities, so you have a significant cost factor – the plasmid cost can be up to one third of the actual batch production cost. Sourcing the plasmid can additionally become a time constraint as it can take up to half a year. You have to apply a relatively complex process involving mixing the plasmid and transfection reagent and adding that to the process. This might not seem too complex, but when you imagine doing this on a scale of several hundred liters, it can become complicated and also makes the process less robust than a standard protein production

process, for example. All of these features mean that even suspension cell production is not truly scalable.

There are some other methods based on helper virus infection of your production cells – but again, you have to produce the helper virus first, and you have to prove the absence of the helper virus at the end of the process. Methods using adeno helper virus or baculovirus are being used despite these challenges, but they are not broadly applied. The predominant production method right now is still triple transfection of plasmids.

> "Our dream was to make AAV production as easy as antibody production."

Q Could you tell us more about CEVEC, now part of Cytiva's work on viral vectors, particularly your stable AAV production platform ELEVECTA®?

NF: When we started our viral vector work, we initially asked ourselves, which viral vector types are the most important ones in gene therapy. As it turned out, the most common viral vector types are lentiviral and AAV-based vectors. We have production systems for lentiviral vectors, all based on transient transfection, with very competitive titers and yields. But we quickly realized that the real production gap is in the AAV field. Lentiviral vectors are mostly used in *ex vivo* therapy, which means you are adding them to cells that have been isolated from the patient. While this brings its own challenges, these are not so much centered on the vector itself, which is usually required in relatively low doses, making vector production less of an issue here. On the other hand, with AAV there is an increasing demand for big production batches. For this reason, we turned our focus to simplifying AAV production.

Consider monoclonal antibodies: making a monoclonal antibody was a huge challenge 25 to 30 years ago, and now antibody production services have become a commodity; something more or less every CMO can do using standard methods. Our dream was to make AAV production as easy as antibody production, and we decided that the only way to truly achieve this was to move away from transient transfection towards *bona fide* stable producer cells.

To achieve this, we had to integrate all necessary components stably into our cell line, thereby generating a true producer cell that carries everything that is required for AAV production, including the therapeutic gene. Then, all you have to do is expand your cells for the production volume, which can be whatever bioreactor format you are working with, up to several thousand liters. After expanding the cells to that volume, induce induction of the AAV, and essentially the AAV production in this culture works like for a monoclonal antibody.

This makes it sound easy – but of course in reality it was not so simple! Our platform provides the only genuine stable AAV producer cell lines. Components required to make the AAV vector, like the AAV rep genes and some of the adenovirus helper genes, are quite toxic to cells. You thus have to make the system inducible, and due to the molecular setup of AAV, this is not trivial. But we have succeeded – and the result is our ELEVECTA® platform, which allows us to

> "Our platform provides the only genuine stable AAV producer cell lines."

generate producer cells for a desired AAV vector irrespective of the serotype-specific capsid or gene of interest and then help our clients produce their specific therapeutic AAV vector using these producer cells.

We are not only making the process truly scalable because you can run the upstream production process in the big bioreactor format, but this production is now also much more reproducible and robust than what you get from a transient transfection. For example, from a quality perspective, it is important to have a certain amount, ideally a high amount, of full particles. You always have empty particles that do not carry the therapeutic gene in the preparation, but you want that number to be relatively small, and you want it to be constant. That's something you can achieve with a clonal stable cell line, but is very hard to do with transient transfection.

Q What challenges can viral vector production pose at the different stages of gene therapy manufacture, and how are you working to meet those challenges?

NF: The biggest challenge is at the commercial stage, because you have to secure supply for a sufficient number of doses. When you submit your market application to the regulatory authorities, you have to outline how you can guarantee supply of your product.

In earlier project phases, time appears to be a key factor – ideally you want to be the first in the clinic with your product, and you want to be the first to get to market. We see this with clients who have their AAV vector developed: they know what it has to look like, they have convincing animal data, and now they need cGMP material quickly to go into the first clinical phase. The times it takes to make a stable cell line – about a year – can be considered too long in such cases. We are addressing such constraints by offering a fast route to GMP material by moving back to transient production for the first clinical phases. This uses our so-called Alpha cell line, the precursor of the final producer cell. In this way we can deliver clinical GMP material relatively fast while the cell line development is ongoing. For the next clinical phase, our client will have material from the stable cell lines which is very closely related to the cell line that was used for the transient production, thereby avoiding a major platform switch.

At the other end of the development pathway, we also hear from larger Pharma companies who run into obstacles at a later stage – they have just licensed a project which looks very promising after the initial clinical trial, but they now find the production platform being used will not work at larger scales. When they look at production for Phase 3 and beyond, they realize using transfection of adherently grown HEK-293 cells is not going to be a viable option. This is a totally different category of clients we are working with, and who we are helping to make the transition to our ELEVECTA® platform.

Q Looking at the area of viral vector manufacturing as a whole, what are your predictions for the field over the next 5–10 years?

NF: I predict in the AAV field, that for *in vivo* gene therapy we will see many new serotypes, with specific tissue tropisms in order to help direct the therapy to the target tissue. There are a number of companies out there screening for and developing new AAV serotypes. The good thing is that production technologies are adaptable to all such serotypes; if you use transient transfection for serotype 1, it will also work with serotype 2. The same is true for ELEVECTA® – it has worked consistently well with all the different serotypes we have so far tested.

The major challenge I anticipate will be to bring down production costs. The current price of gene therapies makes it inaccessible to so many patients worldwide. Increasing the number of patients does mean you can lower product prices, but if the production costs are too high, there is no way of lowering prices sufficiently. Therefore, we need better, more affordable production methods.

Q You also provide production cell lines for adenoviral vectors. Could you tell us about the interest you've had in utilizing these for the potential production of a COVID-19 vaccine?

NF: When COVID-19 started to become a major health threat, our first thought was to find a way to contribute, but initially we drew a blank. Then, we learned that a number of companies are developing COVID-19 vaccines based on non-replicating adenoviral vectors presenting Sars-CoV2 antigens on their surface. The advantage is that the adenoviral vector itself is harmless, but it boosts the immune response. As of this morning, eight COVID-19 vaccines are in clinical trials, and two of them are based on adenoviral vectors.

Our cell line was originally made to produce adenoviral vectors, and it is designed in such a way that it cannot accidentally generate replication-competent adenovirus. With the standard producer cell line, which again is HEK-293, the genetic setup is such that accidental homologous recombination can happen leading to up to 100 replication competent adenoviruses in 1010 particles. That does not sound like a big number but it is a significant risk as such replication-competent adenoviruses may replicate within the patient's body. Since safety is crucial for a vaccine, which is after all given to healthy people, we receive requests from companies working on such COVID-19 vaccines to use our platform which avoids the risk of replication-competent adenovirus formation.

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AUTHORSHIP & CONFLICT OF INTEREST

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PREMIUM TECHNOLOGY FOR PREMIUM THERAPIES

ELEVECTA® STABLE AAV PRODUCER CELL LINES

STABLE PRODUCER CELLS

All components for AAV production stably integrated into one producer cell

CONSISTENT QUALITY

Consistent AAV quality across batches – no transfection steps

FULL SCALABILITY

Scaling up from shake flask to large scale Bioreactor without drop in productivity

ROBUST PROCESS

Reliable AAV production in suspension bioreactors and serum-free media

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