CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL:
PURIFICATION

INTERVIEW

Addressing challenges presented for downstream purification by changes upstream

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Q Please can you give us some brief background on the Orth manufacturing facility and its capabilities?

MB: The facility has a long history in Austria. It has more than 30 years of experience and a proven track record in clinical as well as commercial manufacturing, beginning with the viral vaccines before moving on later to recombinant proteins for global supply.

Then 5 years ago, we initiated gene therapy development, with the aim to leverage these proven capabilities in product development for biologics. And since 2015, we have been involved in GMP production of adeno-associated-vector (AAV)-driven gene therapy products.

Regarding our core capabilities, they stem from the process development of HEK 293 cell lines for AAV production. We have also both upstream and downstream process development capabilities ranging from very small scale – approximately 15 mL – up to 200 liter. We also perform non-GMP production for preclinical studies.

Building on our knowhow from the viral vaccine downstream bioprocess side, we developed the ultracentrifugation step for the separation of full and empty AAV.

We also realized that the formulation buffer for current AAV manufacture is not ready for commercialization, so we developed proprietary formulations buffer where AAV can be stored for a few months at +5°C without loss of activity. This formulation also allows for the lyophilization of the AAV drug product, which is particularly important with commercialization in mind.

From the GMP perspective, we have a GMP AAV platform consisting of 200-liter single-use and 500-liter single-use bioreactors. Our downstream capabilities consist of fully closed and automated ultracentrifugation skids. We also have the different Ultra diafiltrations and chromatography systems. One very important thing for us is that we have already demonstrated our lot-to-lot consistency with our AAV manufacturing platform.

From the analytical perspective, we have more than 50 established methods, the majority of which are performed in-house allowing us to accelerate not just process development, but also product characterization and clinical release.

Q And what are you working on at the moment?

MB: From the product development perspective, we are working on three programmes in pre- and clinical phases and we have several more undisclosed projects currently in research. These internal projects are complemented by co-development or manufacturing programmes with external partners.

Q What are the approaches and tools you currently employ for vector purification, and what are their chief benefits and limitations?

MB: Our AAV vector purification starts with a depth filtration step. The key benefit there is really that this is a single-use system concept. The negative side of using a single-use system is that when you are running a longer campaign, you might see variability in the filter lot

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that you are using due to utilization of a new filter for each new batch, with potential differences in recovery rate.

Moving further downstream, we come to the Ultra diafilitration steps. The main benefit there from our perspective is that it's the best way to concentrate and change buffer in downstream of biological products, certainly in the GMP environment. From the manufacturing perspective, not using the single-use system does tend to prolong the cycle times at this step. Another limiting factor can be the need to utilize different systems at different scales.

Next is the immuno-affinity step. This is the most important step in downstream bioprocessing for the product purity itself, and there are currently resins commercially available which are capable of binding to different AAV subtypes. Regarding limitations, this commercial resin for the different AAV subtypes is used in a very harsh elution. We had to develop our elution conditions and our proprietary buffer because we didn't want to use the harsh conditions that might impair product biopotency.

Further on, we have the ultracentrifugation step, which we consider to be the best step for separation of the full and empty AAV particles. Some think that ultracentrifugation is not a closed system, but this is in fact not necessarily true – it depends which ultracentrifugation we are using: if we are using large-scale ultracentrifugation, which is fully automated, this is a fully closed system from the GMP perspective – I think this is a key benefit of using large ultracentrifugation, which can handle large volumes.

Regarding further purification, we use ion exchange chromatography, particularly for the polishing step. The key benefit of this step is it is also used for pre-formulation of the drug substance – in other words, the formulation that allows long-term storage of the AAV. An additional benefit of ion exchange chromatography is that you can run it in either positive or negative modus, depending on the specific downstream step for which you are utilizing it. The chief limitation is that it is not as selective as an affinity step. Consequently, from our perspective, it is best to combine ion exchange chromatography and immune-affinity for product purity, if feasible.

Q If you could request one specific improvement to the current vector purification toolbox, what would it be?

MB: What we have seen during process scale-up and the transition to the GMP environment in downstream processing is

that one tends to deal with far larger volumes in other biologic areas compared to gene therapy. In gene therapy, the volumes are significantly lower both for the intermediates and for the drug substance. So I do think there is a potential niche in the market for chromatography systems, for example, that are capable of running with lower volumes and lower flow rate, but that are still GMP compliant. Currently, some of the systems just don't have the sort of validated ranges that are needed for gene therapy.

Q How do you seek to address or control the challenges that may be presented downstream following upstream bioprocess changes?

MB: The standard approach in the industry is performing process risk assessment and following Quality by Design principles. If you are changing the upstream process, everybody is obliged to check and to make a risk assessment as to whether this upstream change has any impact on the downstream steps. It's vitally important that this process risk assessment is performed.

Then secondly, it's about robust process design. To return to the example we discussed earlier, this could mean utilizing different purification principles. When you have an ion exchange chromatography separation step which works by AAV surface charge, complemented by the immuno-affinity which utilizes a different principle for separation based on the surface epitopes of the AAV, and then you have the ultracentrifugation step, which involves separation based on the size and molecular weight of the AAV, then your downstream process is very robust and capable of dealing with any variation coming from upstream.

The last element that we are also implementing in our production is process control. Proper analytics is the key to how we can monitor and control the process. From the downstream perspective, control of your total protein coming from the upstream is one of the important elements. Next very important element – measuring your product yield at the various steps. And yield not only with the vector genomes, but complemented by the AAV ELISA, for instance, to indicate whether you're losing only full or empty particles.

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And for the end product control, we've seen that analytical ultracentrifugation is of huge benefit in terms of controlling not only full and empty AAV particles, but also effectively characterizing that the product does not

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contain different AAV subtype populations that are bearing the truncated transgene DNA.

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