INTERVIEW

Can AAV continue to deliver the promise of gene therapy?

Charlotte Barker (Editor, BioInsights) speaks to **Ratish Krishnan** (Senior Strategy Consultant, Merck Life Sciences) and **Elie Hanania**, PhD (Vice President of Process Development Viral Vector Technologies, Avid Bioservices) about the current status and future manufacturing of AAV-based gene therapies, including how to streamline large-scale manufacturing.

Cell & Gene Therapy Insights 2023; 9(9), 1089–1096

DOI: 10.18609/cgti.2023.143

Q Does AAV still reign supreme for advanced therapies?

RK: We have reached a new era of medicine in the realm of advanced and potentially curative therapies. Adeno associated virus (AAV) has undoubtedly established itself as a leading contender for *in vivo* gene therapy due to its safety profile, efficient gene delivery, and ability to provide long-term transgene expression. It is estimated that the viral vector market is growing at roughly 30% compound annual growth rate, and AAV is used in about a third of all gene therapy clinical trials. AAV-based gene therapies have shown remarkable clinical and commercial success over the last decade with groundbreaking treatments approved for rare genetic disorders, ranging from spinal muscular atrophy, inherited retinal diseases, and most recently, hemophilia A.

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— Ratish Krishnan

However, I would be remiss if I did not mention the other viral vectors demonstrating considerable promise in clinical applications. Lentiviral vectors dominate the *ex vivo* gene therapy treatment market. They constitute about 50% of the viral vectors used in gene therapy clinical trials. They have gained significant traction because of their unique ability to transduce both dividing and non-dividing cells, along with the ability to deliver a larger payload. Like AAV, lentiviral and retroviral vectors have found considerable commercial success in treating a range of diseases.

Adenoviral vectors have also been extensively studied in clinical trials prior to the emergence of AAV. These vectors are particularly known for their robust transduction efficiency, making them suitable for applications where transient gene expression is desired. However, their immunogenicity has limited their use for long-term therapies.

In addition, herpes simplex virus (HSV) vectors can target and transduce specific nerve cells, making them potential candidates for gene therapies targeting the central nervous system. The US FDA has recently approved Vyjuvek™, which uses HSV for the treatment of wounds in patients 6 months and older with rare and serious skin disorders.

The choice of viral vector depends on several factors, and while AAV remains a front-runner, the continued advancement of other viral vectors underscores the increasing diversity in the field of viral vector-based therapies. Researchers are exploring the strengths of each vector and tailoring them to meet the unique requirements of specific indications. This expanding arsenal of vectors will unlock new possibilities for treating a wide range of diseases, providing hope for patients worldwide.

EH: I believe AAV will still reign supreme, at least for a while. As part of a contract development & manufacturing organization (CDMO), I understand why researchers favor a variety of vectors, but from the manufacturing standpoint, AAV has the advantage of being produced using transfection or infection approaches. It is also robust and can handle harsh conditions during production and purification.

I agree that it will not be the only viral vector in use, as more therapeutic targets and indications are added. Oncolytic viruses will likely be the next reigning advanced therapy, but not for some time, and these may be used in conjunction with other immunotherapy approaches. It may be that one approach may not be as effective as multiple approaches (synergestic impact), and so HSV, oncolytic, and others may increase in popularity over time.

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Q How will AAV evolve in the next 5 years?

RK: Over the past 5-year period, the AAV market landscape has undergone significant changes, mostly driven by process development improvements, commercialization efforts, regulatory changes, and increased investments in this field. The rate of progress in the gene therapy pipeline from preclinical to clinical is on course to match that of established modalities such as monoclonal antibodies. We have moved from the hype of therapeutic potential to the concrete hope of commercialization, and we have now entered a phase of reality where we are starting to see continued success in the commercialization of therapies using AAV.

In the categories of process development, commercialization, and regulation, AAV has become mainstream, with tremendous advancements in all aspects from discovery to commercialization. First, process development improvements have been a key focus, resulting in higher upstream titers and higher downstream recoveries, and more efficient and scalable manufacturing platforms like suspension cell culture are widely utilized now. These advancements have increased the efficiency of viral vector production with the ultimate goal of making these therapies economically viable to the broader population of rare diseases and moving away from the hefty price tag.

Second, commercialization efforts have intensified, with successful clinical trials and regulatory approvals attracting substantial interest from pharmaceutical companies and biotech firms. This surge in interest has led to increased investments in manufacturing infrastructure, expanding production capabilities to meet the growing demand for viral vectors, and large biotech corporations have been continuously exploring merger and acquisition deals with smaller gene therapy biotechs.

Third, regulatory changes have played a critical role in facilitating the transition of AAV research into clinical applications. Regulatory agencies worldwide have recognized the potential of AAV therapies and have worked to establish clearer guidelines for their development and approval. The Center for Biologics Evaluation and Research (CBER) at the US FDA has led the way in this aspect. Looking into the future, we can expect even more exciting developments in the AAV market. There will be a range of therapeutic applications as researchers explore treatments for more prevalent diseases like neurodegenerative disorders.

Advancements in AAV manufacturing and plasmid engineering will enable personalized AAV therapies tailored to individual patient needs, thereby improving treatment efficacy and safety. Ongoing investment in the AAV space will continue to drive its growth. Venture capital funding partnerships and collaborations will fuel further research and development, expanding preclinical and clinical pipelines.

EH: It is important to state that when viral vector technologies emerged, the basic technologies available at that time were designed for monoclonal antibodies. The manufacturing and purification processes were not ideal, and hence I believe that as time passes, there will be improvements in these processes. The ultimate goal is of course to increase titer, yield, and purity of our product.

As Ratish mentioned, we are not just dealing with pediatric hereditary monogenic disorders. Now, researchers are ambitious in trying to tackle more complex disorders, so there is an increased demand for large amounts of AAV with higher titer. Having the infrastructure for scaling up is critical. Getting better plasmids is also important, especially if triple transfection is considered as the primary mode for AAV production. There are now many more transfection reagents on the market that have improved and selective characteristics, resulting in higher titer.

To further tackle the yield issue, innovative approaches, such as producer cell lines, are required. This is a holy grail for researchers, and we have seen great strides forward, but we are not there yet. Finally, the characterization of the AAV is becoming quite important. We need improved assays with superior specificity to enable proper assessment of titer, overall yield, and purity. These are some areas that I foresee becoming more dominant in the AAV field—in production, purification, and characterization.

Q As the use of AAV continues to grow, what challenges stand in its way?

RK: At the macroscopic level, manufacturing scalability remains a critical hurdle to meet the increasing demand for AAV vectors for diverse therapeutic applications. There are also lingering concerns around immunogenicity and host immune responses, which may limit the effectiveness of some AAV therapies, especially in cases of repeat dosing. Lastly, the regulatory landscape, and specifically the harmonization of guidelines across regions, will pose a challenge to the global development and commercialization of these AAV-based therapies.

EH: The biggest hurdle will be scale-up. We already mentioned operational challenges, but as we begin systemic delivery of these large doses, we must be cognizant of the safety profile. AAV is still in its infancy, so we do not have an extensive safety profile. So far it has been tolerable, but with much higher doses, we must be prepared for potential side effects, some of which may be serious. It is something to be aware of.

In addition, when it comes to AAV, we always talk about full capsids versus empty capsids, but of course, now we are aware that this is not black and white. There are also a wide range of partials that have not been extensively researched. We do not know what percentage of full versus empty we truly need to achieve the desired therapeutic effect, or what the impact is of injecting some of the partials will be.

Q What will be the most promising innovations in AAV production to emerge over the next 5 years?

EH: In upstream production, continuous manufacturing and intensification in cell culture will become dominant factors. Using chemically defined media, additives, and boosts, can yield high density of cells, which is critical to produce more virus.

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— Elie Hanania

In downstream processing, continuous purification with chromatography and tangential flow filtration will emerge.

In addition, the design of plasmids used during transfection will improve in terms of size (smaller plasmids would be a plus) and removal of redundant or non-essential sequences.

As we now have more defined therapies, scientists can consider alternative approaches to produce the required AAV other than triple transfection (such as coinfection using baculovirus or HSV). These may be less expensive for campaign runs, easier to manage, and achieve greater consistency. Scientists are looking at the AAV particles generated by these different approaches to learn how they compare to those produced by triple transfection.

RK: AAV production is on the cusp of transformation, with a lot of innovative trends that are poised to shape the landscape. As Elie mentioned, continuous bioprocessing approaches like perfusion bioreactors will improve productivity.

Improved transfection methods will also be important—the producer cell lines along with plasmid engineering and miniaturization of plasmid promoter elements will further optimize AAV vector production. Lastly, purification techniques like continuous downstream processing and advanced chromatography methods using membranes will significantly improve purification efficiency, thereby ensuring high-quality AAV vectors.

Q What innovative technologies and manufacturing strategies should we consider to streamline AAV manufacturing for more common disease indications?

RK: Streamlining manufacturing is paramount to enhancing patient access and affordability of advanced therapies worldwide. We are seeing AAV-based therapies being approved for high-dose indications and large patient populations. Optimizing productivity, reducing cost per dose, and maintaining high-quality standards will all be important.

This can be achieved by process optimization for improved efficiency and higher yields, scalable manufacturing platforms for adaptability, automation and robotics for reliable production, robust supply chain management, modular facilities for resource optimization, and CDMO partnerships for expertise and cost-effectiveness. These collective efforts will drive the transformation of advanced therapies, making them more accessible to patients in need.

EH: Regulatory agencies will probably have more stringent requirements when it comes to AAV production and purification for relatively prevalent neurological and oncological disorders. Most of these regulations focus on the safety and efficacy of the final product. However, I think some of the requirements need to be vetted by scientists to make sure that they are critical for the process and the product, since these requirements ultimately have an impact on production cost and timeline.

Furthermore, testing and characterization improvements are required to develop precise assays with extended ranges. We also need to have orthogonal methods of testing to be able to confidently affirm that we are generating and delivering what we say we are. Overall, all these approaches need to be robust, scalable, and able to consistently generate the required purity and quality, batch after batch.

BIOGRAPHIES

RATISH KRISHNAN is a Senior Strategy Consultant in the Novel Modalities BioProcessing group for the Americas. A process development scientist by background, he has over 13 years of experience in vaccines, monoclonal antibodies, and viral vector modalities from preclinical to late-stage process characterization, validation, and commercialization activities such as BLA authoring. Before joining Merck, Ratish managed process development teams at Novartis and Pfizer. Now, he serves as a global subject matter expert for viral vector manufacturing and provides strategic guidance to internal stakeholders and key customers. He holds a master's degree in Biotechnology from Pennsylvania State University.

ELIE HANANIA has led the process development teams at different cell and gene therapy CDMOs including Fujifilm Diosynth Biotechnologies (College Station, TX), Millipore Sigma (Merk KGaA, Carlsbad, CA), and Progenitor Cell Therapy (Hitachi Company, Mountain View, CA). Elie's expertise spans academic, clinical, and biotech, with diverse experience in viral vectors, molecular and cell biology, virology, and characterization assay development with implementation of empowering technologies in advanced cell and gene therapies.

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DOI: 10.18609/cgti.2023.143

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.
Acknowledgements: None.
Disclosure and potential conflicts of interest: The authors have no conflicts of interest.
Funding declaration: The authors received no financial support for the research, authorship, and/or publication of this article.

Article source: This article is based on a podcast with Ratish Krishnan and Elie Hanania which can be found here.
Podcast recorded: Aug 8, 2023; **Revised manuscript received:** Aug 3, 2023; **Publication date:** Sep 13, 2023.

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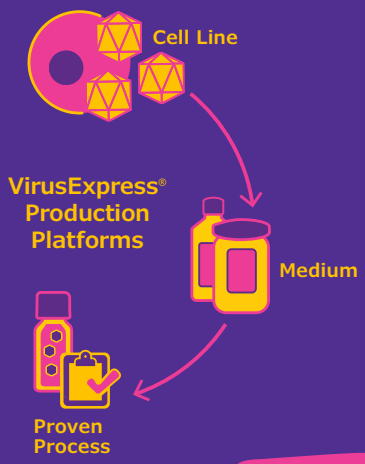

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