CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHTS

Downstream purification of adeno-associated virus for large-scale manufacturing of gene therapies

Mikhail Goldfarb & Orjana Terova

As the growth of the gene therapy field continues apace, it is crucial that the production of high yield, high quality viral vectors is achievable at a larger scale to meet the industry's growing needs. Validated viral clearance approaches are a key step in the manufacture of safe gene therapy products, and Thermo Fisher Scientific's combined CaptureSelect™ and POROS™ technologies offer an affinity chromatography platform that can simplify the vector purification process while also maintaining yield. A series of viral clearance studies conducted by REGENXBIO using the POROS™ CaptureSelect™ AAVX affinity resin demonstrated that the resin provided robust viral clearance, even under 'worst case' processing parameters outside of typical manufacturing conditions, as demonstrated using typical model viruses expanded to include human viruses. Additionally, minimal to no non-specific interactions were observed between the viruses and the AAV capsid, as well as between the viruses and POROS™ beads or V_HH ligand.

Cell & Gene Therapy Insights 2020; 6(7), 955–963

DOI: 10.18609/cgti.2020.106

INTRODUCTION

Significant advancements have been made in the gene therapy field in recent years, as evidenced by the increasing number of clinical trials and significant investments being made in the sector. With two therapies having already received FDA approval – Luxturna® (Novartis), in 2017 and Zolgensma® (AveXis) in

2019 – momentum in the field is building, further underpinned by the FDA announcement last year that it expects that by 2025, approvals for cell and gene therapies will rise to 10 to 20 per year [1].

Vectors such as the non-enveloped adeno-associated virus (AAV) have played a key role in this clinical success and are being engineered and used to deliver DNA to target patient cells for the long-term treatment of rare diseases. Recombinant AAV subclasses have become the vector of choice for such therapies, with excellent clinical outcomes achieved at least in part due to their particularly strong safety profile and low immunogenicity.

However, there are still challenges for the field to address. The utilization of viral vectors in the clinic risks being derailed by manufacturing issues. While the field is able to produce high quality and high yield viral vector to meet current clinical needs, as more gene therapies become commercially available and their indications expand, there is an inevitable requirement to increase the scale and productivity of viral vector manufacturing. As such, there are many efforts underway to increase titer productivity in the manufacture of AAV, including attempts to optimize cell lines and achieve cost-effective scale-up by working with higher volumes.

As these improvements occur upstream, it is also of key importance that downstream purification solutions offer the high capacity, specificity and throughput that is needed to obtain AAV products with high yield and purity. Viral clearance is a crucial consideration within downstream purification – as clearly mandated by the FDA, and ICH Q5A [2,3], "all biotechnology products derived from cell lines have to demonstrate that the products are safe, by implementing appropriate testing, and demonstrating that the manufacturing process is capable of clearing any endogenous or adventitious viruses". Validated viral clearance approaches are therefore an essential step in ensuring the safety of gene therapy products. The importance of viral clearance studies is further emphasized by the need to understand the degree to which each individual step contributes to total viral clearance. Affinity chromatography, an established platform in both mAb and AAV capture spaces, is one such step that can contribute to viral clearance.

ENABLING A PARADIGM SHIFT IN VECTOR PURIFICATION

Affinity chromatography utilizes specific ligands which are more or less redundant coupled to a solid chromatographic support, allowing products to be captured from crude material. This approach offers several benefits including highly specific separation and provides the ability to perform one-step purification from crude material to a product with high yield and purity.

To bring the benefits of affinity chromatography to the gene therapy field, Thermo Fisher Scientific combined CaptureSelect™ and POROS™ technologies (see **Box 1**) to provide a process for the purification of viral vectors. CaptureSelect™ technology, which has already transformed the purification of antibody derivatives, and recombinant proteins, is now being applied to viral vectors; it can provide a simplified approach with lower cost and complexity while delivering the high purity and high yield products that the cell and gene therapy industry needs.

Resins currently available are the POROS™ CaptureSelect™ AAV8 and AAV9, which have been specifically designed and commercialized for AAV8 and AAV9 purification, and the AAVX affinity resin, which has been demonstrated to have specificity and affinity for a wide range of AAV serotypes. POROS™ CaptureSelect™ AAV affinity resins offer superior performance in terms of specificity and binding capacity, as shown through data obtained with various collaborators in both industry and academia [4].

For example, one end user reported that their standard purification process using three anion exchange chromatographic steps resulted in low yield and long process development times. When utilizing the POROS™ CaptureSelect™ AAV affinity resin, their product yield

DOI: 10.18609/cgti.2020.106

BOX 1

CaptureSelect™ Affinity technology

The proven CaptureSelect™ affinity technology enables the purification of antibodies, antibody fragments, recombinant and plasma proteins, and viral vectors. These products enable increased purity and yield in a single purification step and are designed to simplify workflows and reduce time and cost in biopharmaceutical drug development. CaptureSelect™ affinity resins are manufactured in an animal origin-free production process making these resins suitable for process-scale bioseparations for a wide variety of biotherapeutic compounds.
The technology is based on a variable domain of Camelid heavy-chain only antibodies, or V_HH (Figure 1). V_HHs are the smallest antigen binding fragments, of only around 15 kilodaltons, which allows binding to epitopes that may be difficult to reach, leading to unique affinity for the target molecule. These ligands are very robust, and can withstand the various conditions used during chromatography. Several V_HH ligands have been identified and developed against a broad range of AAV subtypes, as well as chimeras, allowing for effcient AAV purification.

The POROS™ backbone

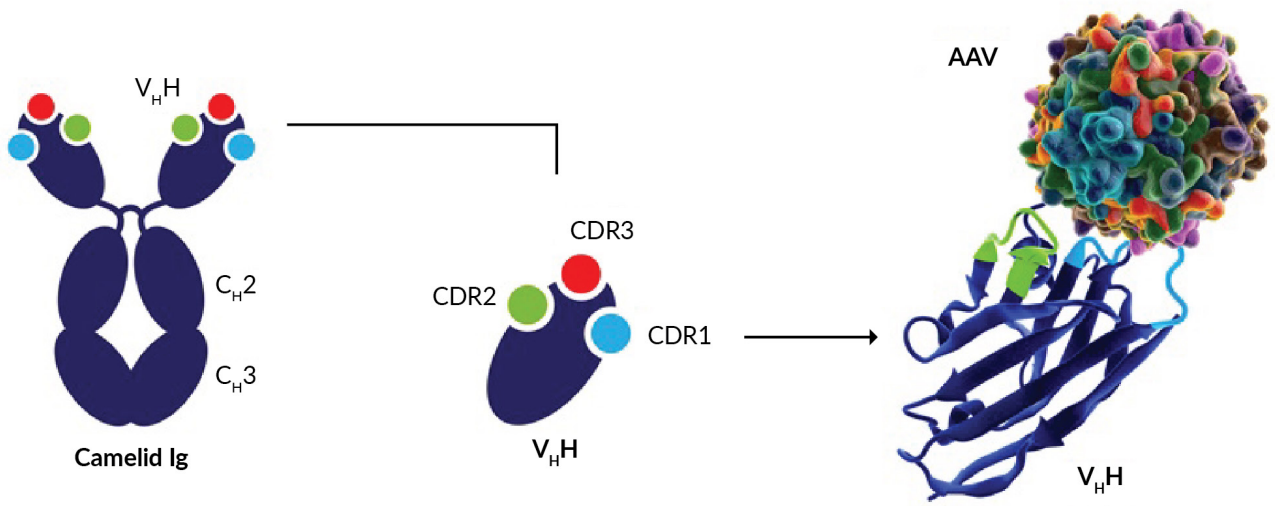
In order to make this purification process suitable for the capture of large biomolecules, such as AAV vectors, a large through-pore matrix support is used. The POROS™ technology offers three key advantages:

- **Poly(styrene-divinylbenzene) backbone:** resulting in linear and scalable performance. The beads are highly robust and chemically stable allowing for stringent cleaning when needed.
- **Large throughpores:** resulting in a reduced mass transfer resistance compared to other available resins. Capacity and resolution are maintained over a wide range of linear velocities, making the purification process more efficient.
- **50-micron bead size:** resulting in superior resolution. The smaller particle size (50 micron) results in tighter peaks and smaller elution volumes, helping to overcome tank size limitations at larger scale.

Therefore improving the ability to separate proteins and obtain effective impurity removal.
For additional literature see [6].

FIGURE 1

CaptureSelect™ technology is based on Camelid-derived single domain [V_HH] antibody fragments, the smallest antigen binding molecule.



The small size of V_HH fragments (~15kD) allows binding at difficult to reach epitopes. Overall, V_HH fragments offer high specificity, affinity and stability.

increased from 20% to 60%, and reduced costs by a factor of six.

ENSURING VIRAL SAFETY

A viral safety program focuses on three core areas: prevention, detection and removal. Prevention concentrates on avoiding adventitious viruses from entering products by selection of virus-free cell lines and raw materials, and by using closed process steps and single-use systems where possible. However, it is impossible to guarantee that no adventitious viruses have entered a process: closed processes are never completely closed, and you cannot fully control raw materials in your supply chain.

Therefore, an important component of a viral safety strategy is to implement a testing program to detect viruses at critical points in the production processes, such as in cell substrates, harvest, and bulk drug substance. Testing for specific viruses that have a high likelihood of being present in the product, such as any endogenous viruses which are known to be in the cell lines, or testing for general viruses such as retroviruses, are all required. However, there is an issue of limit of detection for each assay, and viruses may fall beneath that limit and thus enter the final product.

The third focus of the viral safety strategy is aimed at demonstrating that the purification process can clear any residual viruses possibly ending up in the product despite implementing prevention and detection strategies. In this step, the capacity to remove viruses is demonstrated by executing viral clearance studies which evaluate the removal of spiked viruses via downstream steps such as chromatography, filtration, or other steps that have the capacity to inactivate or remove viruses.

The importance of viral clearance: a case study from REGENXBIO

REGENXBIO is a gene therapy company that has developed a proprietary NAV® technology platform utilizing AAV vectors for delivery of various gene targets. Additionally, REGENXBIO has multiple in-house therapeutic programs predominantly utilizing AAV8 and AAV9 serotypes for gene delivery. As such, continuous development efforts are ongoing to optimize platform processes for AAV8 and -9 production, targeting high yield and high product purity. The processes utilize HEK293 producer cells adapted for suspension culture, modified to generate AAV with a gene of interest in a triple transfection process.

Rationale for choosing an affinity resin

Effective downstream purification to generate a clinical product of high titer, potency and purity is essential while maintaining high AAV capsid recovery. AAV capsids comprise less than 0.1% of the proteins and nucleic acids that are generated by HEK293 cells, with most non-product related impurities being host cell proteins, host cell nucleic acids, plasmid DNA, media and feed components [5]. Therefore, in order to achieve such significant impurity clearance an effective affinity resin is required.

As discussed above, demonstrating effective viral clearance during the purification process is an integral part of an overall viral safety program. In order to better assess the effectiveness of the POROS™ CaptureSelect™ AAVX affinity resin, a series of viral clearance studies were conducted.

Study design

Viral validation for chromatography procedures is routinely performed by spiking a model virus into the load material and performing the chromatography procedures under scaled-down conditions that are considered representative of manufacturing scale. Both the load and the step products are evaluated for virus titers and the viral titer reduction across the purification step is measured in terms of the log reduction value (LRV), which can be expressed using the following equation:

DOI: 10.18609/cgti.2020.106

$$LRV=⟦Log⟧_10 \frac{(\text{Total virus in Load})}{(\text{Total Virus in Product})}$$

Due to assay variability, an LRV of less than one is considered negligible and cannot be counted towards the overall viral clearance. Clearance levels of 1 to 3 LRVs are thought of as contributing, and anything larger than 4 represents a robust and effective viral clearance step.

As no known viruses are present in the cell line and raw materials used in this study, a typical set of non-specific model viruses representing the four virus species categories were selected: enveloped and non-enveloped DNA viruses were represented by pseudorabies virus (PRV) and minute virus of mice (MVM); enveloped and non-enveloped RNA viruses were represented by xenotropic murine leukemia virus-related virus (XMulV) and reovirus type 3 (Reo-3). As a human-derived cell line was used, two human viruses – hepatitis A (HAV) and herpes simplex virus 1 (HSV-1) – were also added, based on risk assessment of possible sources of contamination by adventitious viruses that could originate from process operators and propagate in the producer cells.

To determine the extent of viral clearance across the affinity purification step, the viral titers in load and elution were measured, and elution were measured by infectivity assay for non-enveloped viruses. Since the AAVX column elutes at low pH, and low pH elution inactivates enveloped viruses, an infectivity assay was unsuitable for demonstrating the removal of these viruses by the chromatography step. For this reason, qPCR was used for enveloped virus detection. In addition to evaluating load and elution fractions, the rest of the column fractions were measured to perform the mass balance calculation.

To assess the robustness of the purification step, 'worst-case' conditions outside of typical manufacturing ranges were chosen to evaluate whether efficient viral clearance could still be achieved. The specific conditions chosen were:

- Higher virus load ratio: maximize amount of virus loaded at 145% of manufacturing target;
- High load residence time: allows for maximum virus–resin contact time during loading; at 170% of manufacturing target;
- High elution residence time: maximum virus-buffer contact time during elution; at 170% of manufacturing target;
- Combination (high load ratio + high load/elution residence time).

A further set of experiments were designed to evaluate product–virus interactions and non-specific interactions between viruses and resin bead as follows.

Product–virus interactions were evaluated using AAV-null load, which was generated by collecting the non-bound fraction from the affinity column run. The AAV-null load, or affinity non-bound fraction, was spiked with viruses and purified at the same conditions as the control run containing AAV8 capsids, and viral clearance was compared to the control.

Non-specific interactions between viruses and resin beads were also evaluated by using resin consisting of POROS™ beads without V_HH ligand and evaluating viral clearance across that column. Lastly, interactions between virus and camelid-derived V_HH ligand were assessed using a V_HH ligand with alternate CDR regions (not targeting AAV capsids).

RESULTS

Viral clearance

The results of the viral clearance runs evaluating performance of the AAVX affinity resin at both standard manufacturing process conditions and at worst case conditions are summarized in Table 1.

The POROS™ CaptureSelect™ AAVX affinity column showed robust clearance at manufacturing target parameters for most of the tested viruses: under target manufacturing

TABLE 1

POROS™ CaptureSelect™ AAVX Affinity resin viral clearance study results: process space.

Run description	RNA enveloped	DNA non-enveloped	RNA non-enveloped		DNA enveloped	
	XMuLV	MVM	Reo-3	HAV	PRV	HSV-1
Manufacturing process conditions	>6.4	4.4	2.7	>4.9	4.0	3.1
Higher residence time	5.9	4.6				
Higher load ratio	4.3	3.7				
Higher load ratio + residence time	4.6	3.6	2.5	5.0	3.8	3.6
Clearance level	Effective	Effective	Contributing	Effective	Effective	Contributing

The extent of viral clearance achieved for each tested virus is color coded as follows: **Green = effective (or higher than 4 LRV)**, **Yellow = contributing (1 to 3 LRV)**, and **Red = negligible clearance** (which was not observed for any of the viruses).

process conditions, XMuLV, MVM, HAV, and PRV all demonstrated clearances of ≥4 LRV. Of these, MVM is the most problematic virus to clear due to its similar size to AAV and its high resistance to inactivation. The POROS™ CaptureSelect™ AAVX affinity column also contributed to the clearance of Reo-3 and HSV-1 (≥2.5 LRV). However, as Reo-3 is larger than AAV, it can be separated through filtration, whilst HSV is enveloped and can be inactivated by detergent or low pH.

The run assessing higher residence time demonstrated a comparable level of viral clearance to that achieved at manufacturing process parameters. With an additional 45% viral load, viral clearance was reduced slightly; however, in conditions of combined higher load and reduced flow rate, similar viral clearance was observed that were within 1 LRV of the clearances achieved with manufacturing target conditions.

When studying interactions between viruses, the product, and resin ligand beads, only two test viruses were selected due to study capacity constraints. XMuLV and MVM were chosen as they are commonly used in viral clearance studies, with XMuLV representing large enveloped retroviruses, and MVM representing small, hardy, non-enveloped DNA parvoviruses.

The control run was performed at target manufacturing parameters. The AAV-null run, which was loaded with material containing the spiked virus but without AAV capsids, showed similar clearance to the control, indicating minor-to-no interactions between the capsid and the virus. One can conclude that neither the absence nor presence of AAV8 capsid in the load had a significant effect on the LRV achieved.

As shown in Table 2, further runs testing virus–V_HH ligand and virus–POROS™ bead non-specific interactions demonstrated

TABLE 2

POROS™ CaptureSelect™ AAVX Affinity resin viral clearance study results: interactions.

Evaluated interaction	Run description	Virus	LRV	ΔLRV to control
	Control run	XMuLV	>6.4	
		MVM	4.4	
Virus product	AAV-null load	XMuLV	5.1	>1.3
		MVM	4.0	<1
Virus alternative V_HH	Resin with alternate V_HH ligand	XMuLV	5.1	>1.3
		MVM	3.8	<1
Virus-POROS™ beads	Base matrix resin, no ligand	XMuLV	5.8	>0.6
		MVM	3.6	<1

Conclusion: no significant interactions between viruses and resin/ligand given minor difference in LRV results relative to control.

DOI: 10.18609/cgti.2020.106

clearances that were similar to the control run, indicating that the modifications implemented during those runs did not influence the extent of the observed viral clearance.

When observing the mass balance, the results were in line with expectations: the majority of viruses were in the non-bound fraction (Table 3). Due to assay variability, it is difficult to achieve results with enough accuracy for the mass balance to add up to 100%, but by observing the quantitative trends, non-bound fractions contained most of the virus, and the amount of virus binding and remaining on the resin in the strip fraction was significantly less than 1%.

In the case of HSV, this is an enveloped virus that should be effectively inactivated during low pH hold. The low pH hold can be added at the AAVX column elution stage as AAV capsids have been observed to be stable while held at lower pH conditions.

For XMulV and MVM viruses, minimal interactions were observed between AAV capsids and the viruses. This may present a case for bridging viral clearance results generated with one AAV8-based product to other AAV8-based products, providing that inserting a different transgene into the capsid does not affect these interactions.

CONCLUSIONS

The results indicate that the POROS™ CaptureSelect™ AAVX resin is an effective resin for viral clearance, with robust clearance of XMuLV, MVM, HAV, and PRV viruses achieved under manufacturing conditions. Of particular benefit is the effective clearance of MVM, for which there are currently limited clearance options (outside of chromatography). MVM is particularly problematic owing to its similar size to AAV and being highly resistant to inactivation steps such as incubation with detergents or at low pH conditions.

The two viruses that had clearances below 4 LRV (Reo-3 and HSV) still demonstrated significant reductions of more than 2.5 LRV at target manufacturing conditions. The industry-accepted safety target for processes without endogenous viruses is 6 LRV, which is likely to be achieved by a further chromatography step in the downstream process. If necessary, a viral filtration step could also be implemented, as these viruses are significantly larger than AAV.

TRANSLATIONAL INSIGHT

As demand for viral vectors in the gene therapy field increases and large-scale manufacturing capabilities grow ever more important, improved downstream purification solutions are a key consideration when aiming to produce high yield and high purity vector products.

The POROS™ CaptureSelect™ AAVX affinity resin has demonstrated robust viral clearance during vector purification across a range of those AAV serotypes that are core to current gene therapy approaches. The POROS™ CaptureSelect™ platform provides a simplified and highly effective means of optimizing this critical downstream bioprocessing step, which is central to ensuring the safety and efficacy of gene therapies.

The POROS™ CaptureSelect™ platform provides a simplified and highly effective approach to achieving effective viral clearance, and can help optimize this critical downstream bioprocessing step, central to ensuring the safety and efficacy of gene therapies.

TABLE 3

POROS™ CaptureSelect™ AAVX Affinity resin viral clearance study results: mass balance analysis.

Column fraction	XMuLV	MVM	Reo-3	PRV	HSV-1	HAV
Non-bound	165.8%	66.1%	83.7%	64.7%	79.3%	25.8%
High-salt wash	3.1%	1.0%	1.1%	1.4%	0.7%	1.2%
Low-salt wash	0.7%	0.2%	0.1%	0.3%	0.2%	0.01%
Product elution	<0.00004%	0.004%	0.2%	0.01%	0.1%	0.0004%
Low pH strip	<0.0002%	0.08&	0.03%	0.002%	0.02%	0.006%

REFERENCES

1. Statement from FDA Commissioner Scott Gottlieb, MD, and Peter Marks, MD, PhD, Director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies: https://www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-and-peter-marks-md-phd-director-center-biologics

2. Q5A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q5a-viral-safety-evaluation-biotechnology-products-derived-cell-lines-human-or-animal-origin

3. ICH Q5A (R1) Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin: https://www.ema.europa.eu/en/ich-q5a-r1-quality-biotechnological-products-viral-safety-evaluation-biotechnology-products-derived

4. Toueille M, Dejoint L, Attebi E *et al.* Development of purification steps for several AAV serotypes using POROS™ CaptureSelect™ AAVX affinity chromatography. *Cell Gene Ther. Ins.* 2018; 4(7), 637–45.

5. Fraser Wright J. Product-Related Impurities in Clinical-Grade Recombinant AAV Vectors: Characterization and Risk Assessment. *Biomedicines* 2014; 2, 80–97.

6. Terova O, Soltys S, Hermans P, De Rooij J, Detmers F. Overcoming Downstream Purification Challenges for Viral Vector Manufacturing: Enabling Advancement of Gene Therapies in the Clinic. *Cell Gene Ther. Ins.* 2018; 4(2), 101–111.

AFFILIATIONS

Mikhail Goldfarb
Principle Scientist, Downstream Process Development, REGENXBIO

Orjana Terova
Senior Product Manager, Bioproduction Division, Thermo Fisher Scientific

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All 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: *The authors would like to thank Texcell.*

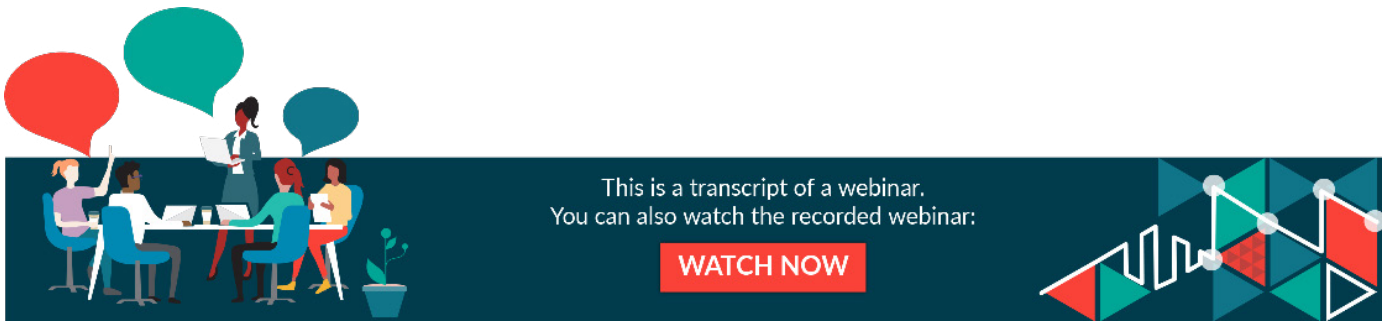
Disclosure and potential conflicts of interest: *The following materials and services were provided free of charge for this work: ThermoFisher's chromatography resin, REGENXBIO's AAV materials, and Viral clearance studies conducted at Texcell.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

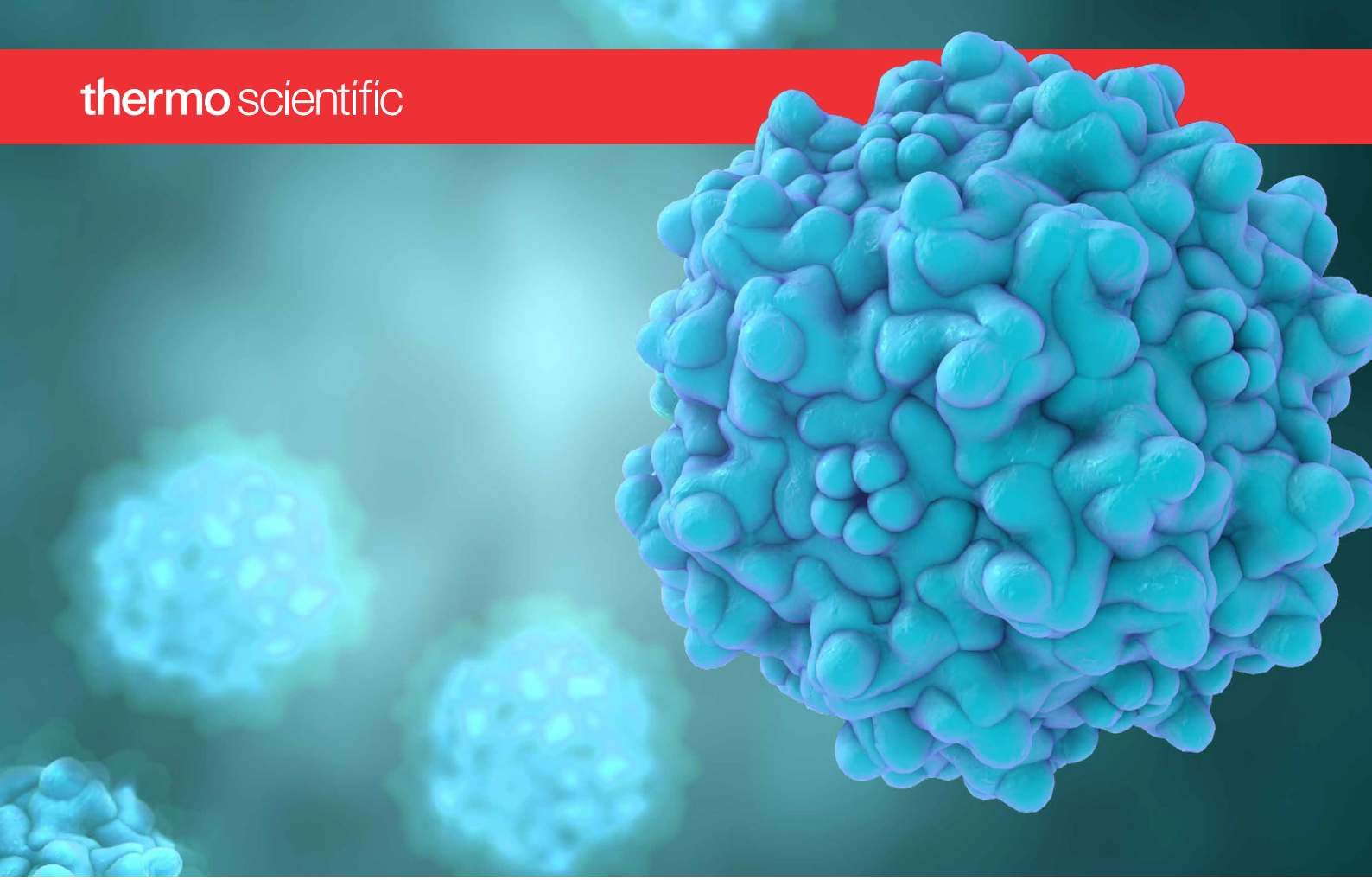
Article source: *This article is a transcript of a previously published webinar, which can be found here.*

Webinar published: *May 20 2020;* ***Publication date:*** *Sep 28 2020.*



DOI: 10.18609/cgti.2020.106

thermo scientific



Accelerate advancement in gene therapy

Improve productivity of your AAV downstream process

Reduce purification steps and increase throughput in your viral vector purification workflow. Thermo Scientific™ POROS™ CaptureSelect™ AAV affinity resins allow you to:

- Maximize downstream process efficiency
- Achieve high purity and yield in a single purification step
- Scale up to clinical and commercial gene therapy manufacturing

To learn more, visit **thermofisher.com/aav-purification**

ThermoFisher SCIENTIFIC