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SPOTLIGHT

Viral vector manufacturing and platform evolution

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CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

VIRAL VECTOR MANUFACTURING AND PLATFORM EVOLUTION

SPOTLIGHT

EXPERT INSIGHT

Practical process development strategies to prepare AAV downstream operations for manufacturing

Tom Elich, Kavitha Bodige, Jacob Guzman, Roberto Facendola, Shamik Sharma, and Kumar Dhanasekharan

While AAV vectors hold significant promise for gene therapy, their complex capsid structure, propensity to form empty capsids lacking a therapeutic transgene, and sensitivity to process parameters create manufacturing challenges. Urgent patient needs and accelerated timelines to reach the clinic provide limited window for process optimization, adding complexity for process developers. To navigate these constraints, we explore practical development approaches to improve downstream purification efficiency and enhance platform compatibility. We examine affinity capture chromatography by characterizing the relationship between binding capacity, feed concentration, and process time. In certain scenarios, implementing tangential flow filtration prior to affinity capture significantly benefits process time, resin utilization, and scheduling logistics. We also investigate anion exchange chromatography for separating empty and full capsids, highlighting a strong correlation between chromatogram peak area and elution pool titer. This scalable correlation supports real-time product quantification, enhancing process understanding to enable data-driven decisions during AAV manufacturing.

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INTRODUCTION

Recombinant AAV vectors have emerged as a promising delivery system for gene therapy due to their favorable safety profile, long-term gene expression, and ability to target a wide range of tissues [1,2]. Despite its clinical relevance, the vector's complex capsid structure, propensity to form empty capsids lacking the packaged transgene, and sensitivity to manufacturing process parameters can lead to production inefficiencies which increase costs and limit patient accessibility [3–5]. Such challenges

may be further compounded by accelerated process development timelines necessitated to deliver new treatments to patients as quickly as possible, providing limited opportunity for process optimization prior to cGMP production. With these perspectives in mind, it is helpful to consider practical downstream process development strategies to improve AAV purification operations at manufacturing scale.

A typical process for transfection-based expression, harvest, and purification of recombinant AAV is shown in Figure 1. Our work focused on three core downstream unit operations, with the goal of optimizing purification process efficiency, manufacturing time, and platform fit. First, we explore affinity capture chromatography, specifically characterizing the relationship between resin binding capacity, load flowrate, and feed concentration. In many cases, these parameters may contribute to unfavorable affinity processing times more than 10 hours long. To reduce capture step processing time, improve resin efficiency, and simplify scheduling logistics, next we consider whether adding a tangential flow filtration (TFF) step prior to affinity chromatography is beneficial. Here, we share a detailed technical review of commercially available TFF formats in this application, and discuss the benefits and trade-offs associated with this process-enabling technology. Finally, we consider the anion exchange (AEX) chromatography step for separation of empty and full AAV capsids. A review of chromatogram data uncovers a strong correlation between ultraviolet (UV) absorbance peak area and elution pool titer, allowing for real-time quantification of in-process product concentrations at this step. We investigate the scalability of this correlation moving from development to manufacturing and consider the practical implications of applying the correlation for rapid assessment of AEX elution pool titer.

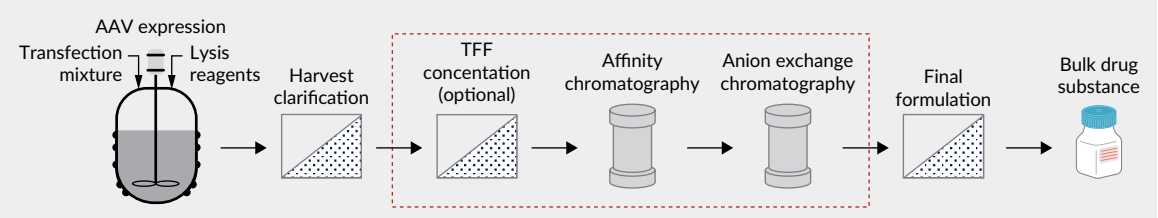
Our evaluations utilized novel AAV9-derived capsids identified using the TRACER™ capsid discovery platform previously described by Nonnenmacher *et al.* [6]. While parameters such as resin binding capacity and filter area requirements may be specific to the capsid constructs evaluated here, the fundamental principles are helpful to navigate operational bottlenecks in the current AAV manufacturing landscape.

AFFINITY CAPTURE CHROMATOGRAPHY: UNDERSTANDING THE RELATIONSHIP BETWEEN BINDING CAPACITY, LOAD TITER, & PROCESS TIME

Capture chromatography is an essential part of AAV downstream purification. This technology typically utilizes ligands with specific affinity for AAV capsid affixed to a resin stationary phase [7]. The ligands bind

FIGURE 1

A manufacturing process for transfection-based expression, harvest, and purification of recombinant AAV. The boxed region highlights the downstream unit operations discussed in this article.



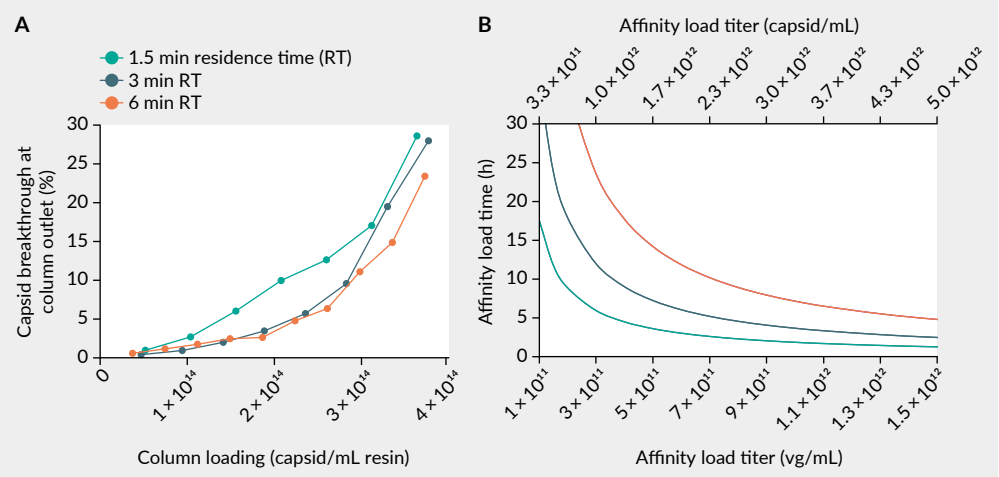
AAV under favorable loading conditions while impurities are removed in the effluent stream. After loading, the AAV capsids are subsequently eluted by modifying mobile phase conditions to reverse ligand binding, often using acidic pH [8]. Many characteristics need to be considered when developing an affinity chromatography step for optimal purity and yield, as detailed in prior literature [9,10]. From an operational and scalability perspective, we find that it is especially important to understand the relationship between affinity resin binding capacity, load flowrate, and overall process time.

We investigated the capacity of Poros™ CaptureSelect™ AAV9 resin to bind a novel AAV9-derived capsid referenced as Capsid A packaged with Transgene 1, or CapA:Tg1. The resin was challenged with clarified lysate feed which had been processed by TFF diafiltration to replace cell culture media with a neutral pH buffer for improved feed stability. Dynamic binding capacity (DBC) testing was performed at three different load flowrates to achieve residence times of 1.5, 3, and 6 minutes, and capsid breakthrough was measured at the column outlet over the course of the load phase (Figure 2A). The resin's DBC was identified as the loading at 10% total capsid breakthrough. Note that while in-process vector genome titer can be measured by PCR, quantifying total capsids in the affinity load material is more complex. In this case, analytical ultracentrifugation was used to assess the distribution of full and empty capsids in the affinity eluate. The total capsid titer was then estimated by dividing vector genome titer by the full:empty capsid ratio, which was assumed to remain constant for load, flowthrough, and eluate pools.

Data in Figure 2A shows that the short 1.5-minute residence time offered the lowest DBC (approximately 2.1×10^{14} capsid/mL resin), which is indicative of diffusive mass transfer properties typically observed for resin-based stationary phases [11]. The data show increased binding capacity at longer residence time, as both the 3 and 6-minute conditions achieved a DBC near

FIGURE 2

(A) Capsid breakthrough as a function of affinity column loading at 1.5, 3, and 6 minute residence times. (B) Affinity load time to achieve 7.1×10^{13} vg/ml resin (equivalent to 2.3×10^{14} capsid/mL at 30% full) for various load titers and residence times.



Load titers are provided as both vg/mL and capsid/mL.

2.9×10^{14} capsid/mL resin. This indicates that a 3-minute residence time is sufficient to maximize binding capacity, with slower flowrates providing diminishing returns. For scale-up, it is common to apply a safety factor to target 80% of the DBC [12]. Therefore, our target loading for the CapA:Tg1 construct was 2.3×10^{14} capsid/mL resin at a 3-minute residence time, or approximately 7.1×10^{13} vector genomes (vg) per mL resin for a feed containing 30% full capsid.

To understand total processing time of the affinity operation, one must also consider load titer in addition to the binding capacity and residence time. As expected, affinity process time is heavily dependent on the load titer and load flowrate parameters, in addition to binding capacity (Figure 2B). For relatively dilute affinity load titers ($<1.7 \times 10^{12}$ capsid/mL, or 5×10^{11} vg/mL at 30% full), more than 10 hours of load may be needed to maximize resin binding capacity at a 3-minute residence time. This reality needs to be considered during process development, especially in early-stage programs where strategies to accelerate clinical timelines for the benefit of patients may not allow for extensive upstream optimization. Note that in addition to bioreactor titer, one must also consider the addition of lysis and nuclease buffer components, as well as buffer flush of clarifying depth filters, which contribute to volume expansion and titer dilution prior to affinity capture. From an operational perspective, long affinity load times can create scheduling challenges during manufacturing campaigns, or force under-loading of costly affinity resin in an effort to reduce the total process time. Our characterization of the relationship between resin binding capacity, residence time, and load titer highlights an opportunity to apply TFF prior to affinity capture for reduced column process time while maximizing dynamic binding capacity utilization.

TFF: AN ENABLING TECHNOLOGY TO EXPEDITE AFFINITY LOAD TIMES, IMPROVE RESIN BINDING EFFICIENCY, & DE-COUPLE UPSTREAM & DOWNSTREAM OPERATIONS

From Figure 2B, affinity load titer concentrations $\geq 5 \times 10^{11}$ vg/mL are ideal to ensure the affinity operation can fit comfortably in an 8-hour working shift, while also maximizing binding capacity at a 3-minute residence time. In cases where sufficient affinity load titers cannot be readily achieved upon bioreactor harvest, TFF can be used to increase concentration of the affinity load intermediate pool. Several TFF formats and molecular weight cut-off (MWCO) options are available for biopharmaceutical applications. For viral vectors such as AAV, TFF is commonly performed with either flat sheet cassette or hollow fiber device format [13]. Depending on the process objectives one may select 30, 100, or 300 kD MWCO to retain AAV while permeating buffer species and small impurities through the TFF membrane [14,15].

Our pre-affinity TFF development work focused on a comparison of a 30 kD flat sheet cassette, 100 kD flat sheet cassette, and 100 kD hollow fiber with 20 cm length. Clarified lysate was used as feed material for TFF experiments. First, flux excursion experiments were performed in recirculation mode to understand filtrate flux (expressed as liters per minute and m^2 filter area, or LMM) as a function of trans-membrane pressure (TMP) and feed pump crossflow (CF) rate. Flat sheet membranes were evaluated at a constant feed CF rate while varying TMP, while the 100 kD hollow fiber format was evaluated at a constant 5 psi TMP while varying feed CF rate (Figure 3). The specific CF and TMP setpoints were selected based on previous experience with the MWCOs and formats evaluated, with the goal of identifying curvature in the filtrate flux trend which may

indicate onset of significant fouling or gel-layer polarization.

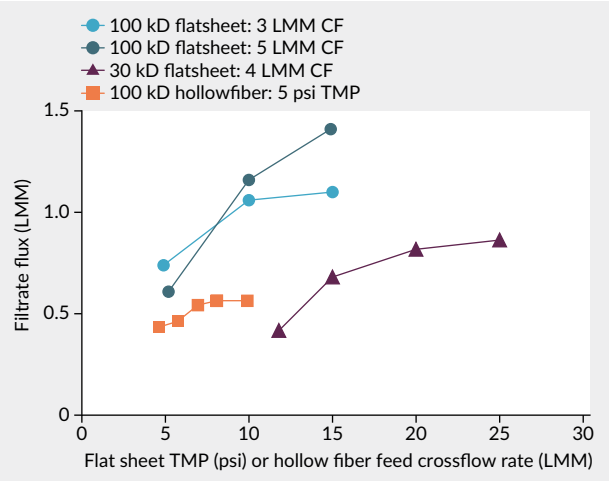
Results in Figure 3 show that the 100 kD flat sheet format offered the highest filtrate flux rate, which is advantageous to minimize process time and filter area requirements. Of the two CF rates evaluated for the 100 kD flat sheet format, 5 LMM offered higher filtrate flux compared to the 3 LMM CF rate, and also appeared to slow polarization effects near 10 psi TMP. Considering this, a 5 LMM CF rate and 7 psi TMP was selected as operating setpoints to balance filtrate flux and loading on the 100 kD flat sheet. Comparatively, the tighter porosity 30 kD flat sheet exhibited lower filtrate flux, with asymptotic curvature above 20 psi TMP indicating pressure-independent behavior. Finally, based on prior experience, the 100 kD hollow fiber was evaluated at 5 psi TMP to reduce membrane fouling effects. At this pressure, a filtrate flux near 0.6 LMM was achieved at approximately 8.5 LMM feed CF rate, with higher CF rates offering minimal improvement in filtrate flux. Since crossflow rate for hollow fiber devices is often reported as shear rate, it is worth noting that the 8.5 LMM feed CF rate equates to a shear rate near 4000 s^{-1} for the tested hollow fiber.

After identifying the optimal TMP and CF rates from flux excursion results, our evaluation proceeded with small scale concentration experiments. Each TFF format was challenged with clarified harvest material using optimal TMP and CF setpoints described in Table 1, and filtrate flux was measured at regular intervals over the course of concentration (Figure 4). The results show that while each format was able to concentrate the clarified harvest 10-fold with ≥90% product recovery, there are important differences with respect to filter loading, flux, and feed pump requirements. For example, all filter formats exhibited some decay in filtrate flux over the course of concentration, but the 100 kD flat sheet format maintained highest flux up to 70 L/m² throughput. Comparatively, the 100 kD hollow fiber format required a higher feed CF rate than its flat sheet counterparts but exhibited relatively low initial filtrate flux and more pronounced flux decay near 40 L/m² throughput. This suggests early onset of membrane fouling for the hollow fiber, limiting its operational loading capacity upon scale-up. For example, consider a scenario in which a 500 L bioreactor harvest requires 450 L filtrate throughput to achieve 10-fold concentration factor. Implementing hollowfiber TFF at this scale may require >12 m² membrane area and a high-capacity feed pump, potentially creating facility-fit challenges. In contrast, the higher loading enabled by 100 kD flat sheet cassette would need ≤7.5 m² membrane area to process a 500 L bioreactor harvest, while also facilitating use of smaller pumps to deliver reduced feed CF rates.

In our assessment, the 100 kD flat sheet format offered an appealing combination of high filtrate flux and throughput while using relatively low operating pressures and feed pump flowrates. Given the

FIGURE 3

Flux excursion at constant CF for flat sheets and constant TMP for hollow fiber.



Flowrates are reported in units L/min/m² (LMM); filtrate flux values report flowrate of filter permeate, while feed CF values report flowrate of inlet feed pump.

TABLE 1

Summary of operating conditions implemented to achieve 10-fold volume reduction using various TFF formats.

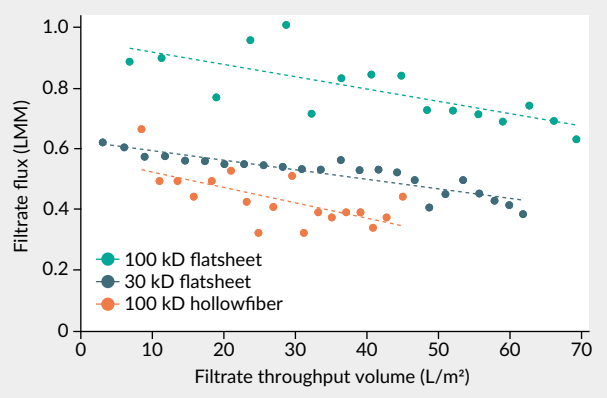
TFF format	TMP (psi)	Feed crossflow rate (LMM)	Concentration factor achieved	Vector genome step yield
100 kD flat sheet	7	5.0	10.2	≥90%
30 kD flat sheet	15	4.0	10.5	≥90%
100 kD hollow fiber	5	8.5 ± 1 (approximately 4000 s^{-1})	10.2	≥90%

average filtrate flux in the range of 0.7–0.8 LMM for the 100 kD flat sheet in Figure 4, approximately 2 hours of process time is required to achieve 10-fold concentration at 500 L scale (not including filter set-up time). Further, we have also found the performance of the 100 kD flat sheet format is maintained across various AAV9-derived capsid and transgene constructs (Table 2). This provides assurance that the pre-affinity TFF step is platform-friendly and can be applied across AAV programs with minimal additional development work.

In addition to product concentration, TFF can also be used for diafiltration buffer exchange prior to affinity capture [16]. In our experience, 3–5 diavolumes is sufficient to exchange the clarified AAV lysate into affinity equilibration buffer while removing cell culture media and lysis reagents which may contribute to pressure build-up on the affinity column during loading. Considering a filtrate flux near 0.6 LMM at the end of concentration, about one hour of additional process time is required to perform diafiltration following concentration.

FIGURE 4

Filtrate flux measured at regular filter throughput volumes over the course of 10-fold volume reduction using various TFF formats.



The chromatograms in Figure 5 present two affinity capture runs using the CapA:Tg2 construct. The chromatogram in orange used clarified harvest as affinity load material, while the blue trend employed a 100 kD flat sheet TFF cassette to concentrate the clarified lysate 7.3-fold and exchange into affinity equilibration buffer prior to column loading. In both cases the load material was filtered with a 0.22 µm membrane immediately prior to affinity chromatography, and both columns were loaded at a 3-minute residence time. Clarified lysate material was loaded to 1.0×10^{14} capsids/mL resin, and TFF-processed material was loaded to 1.4×10^{14} capsid/mL resin.

As expected, the processing times differed significantly. The load time for the clarified lysate material (orange) was over five times longer than the TFF-processed condition (270 min vs 50 min). However, despite the extended load time, the UV absorbance signal in the elution peak was lower when using clarified lysate, confirming that less AAV capsid was loaded in this run. Additionally, during loading a non-linear increase in delta pressure to 0.11 MPa was observed when using clarified harvest material. While the mechanism for pressure

TABLE 2

Comparison of 100 kD flat sheet pre-affinity TFF performance for multiple AAV9-derived TRACER™ capsid constructs.

Capsid:transgene construct	Initial filtrate flux (LMM)	Final filtrate flux (LMM)	Membrane loading (L/m^2)	Step yield (%)
CapA:Tg1	1.1	0.6	73	93
CapA:Tg2	0.9	0.6	67	90
CapB:Tg2	0.9	0.6	65	94

increase is not fully understood and might be resolved by optimization of lysis or clarification strategy, such pressure trends are a concerning indication of resin foulant accumulation which could limit binding capacity and/or make resin cleaning difficult for column re-use [17,18]. In contrast, the TFF-processed harvest enabled a much shorter cycle time with no significant pressure increase (0.04 MPa during loading). This demonstrates that TFF concentration and buffer exchange prior to affinity purification can reduce column pressure and cycle time, thereby enabling more complete and efficient use of the affinity resin's binding capacity.

To quantify the impact of pre-affinity TFF on product quality metrics, affinity eluate material processed with and without pre-affinity TFF concentration and diafiltration was further analyzed for aggregate and host cell protein (HCP) impurity content (Table 3). Product quality data indicates that pre-affinity concentration does not promote product aggregation for the tested CapA:Tg2 construct. Additionally, while the normalized HCP data shows a relatively broad range, this is consistent with typical batch-to-batch and assay variability. Our data indicates that TFF is not contributing to HCP clearance in a meaningful way, and therefore pre-affinity concentration should not be considered a purification step on its own.

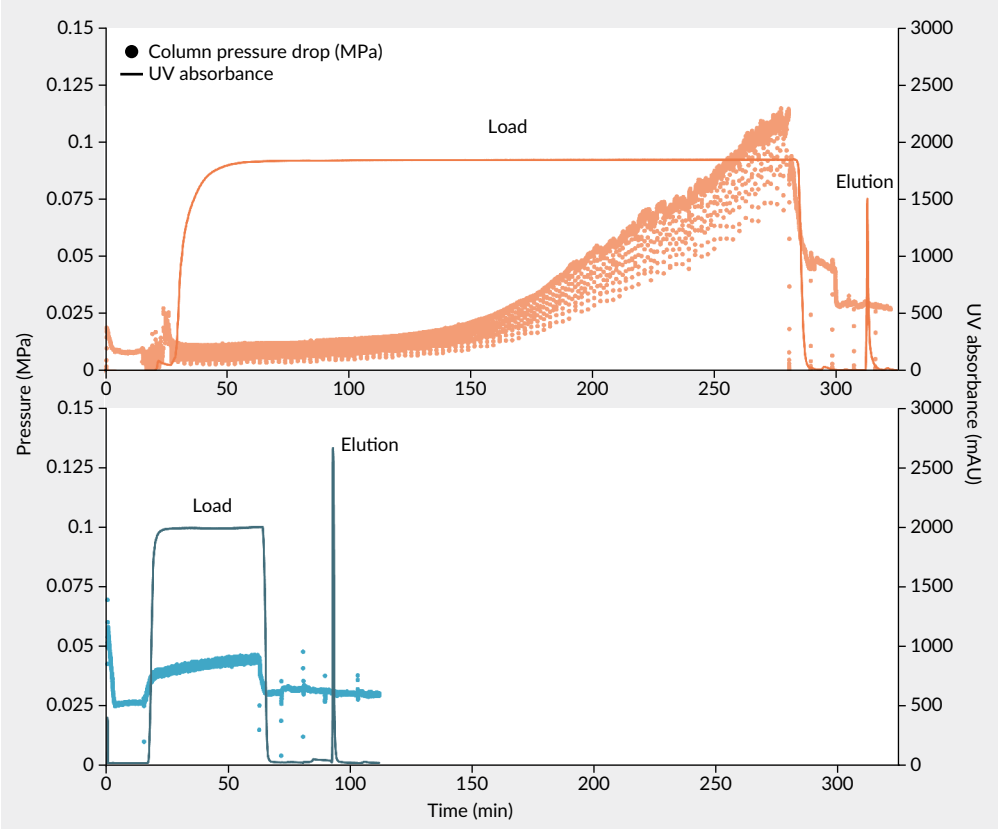
Our findings show that while pre-affinity TFF is not required to purify AAV with acceptable product quality and yield, this step can unlock three important process benefits:

- Concentration of affinity load material reduces column load times, enabling improved utilization of resin binding capacity in a standard operating shift;
- Diafiltration buffer exchange removes cell culture media and lysis buffer components from the affinity load, which may reduce column operating pressures during loading;
- The concentrated, diafiltered TFF product pool offers a convenient in-process hold opportunity to decouple bioreactor harvest steps from downstream chromatography operations. This is especially useful in situations where multiple bioreactor harvests are purified by a single purification train, or to allow scheduling flexibility for downstream preparation activities such as column packing and buffer formulation. A development team should confirm capsid freeze/thaw stability of process intermediate material prior to implementation at manufacturing scale.

We acknowledge that to achieve these benefits, one must implement a dedicated TFF unit operation which incurs added consumables cost, processing time, and some (<10%) product yield loss. Scheduling of the TFF operation should be carefully

FIGURE 5

Affinity purification chromatogram overlays using unconcentrated clarified harvest as affinity load material (orange, top), and TFF concentrated and diafiltered harvest as affinity load material (blue, bottom).



Solid lines show UV signal (mAU), and dots show column pressure drop (MPa).

coordinated with clarification operations, with attention to the stability of the clarified lysate material. The cost/benefit analysis of TFF implementation should be performed on a case-by-case basis, and the aforementioned benefits may not be compelling in all cases. Pre-affinity TFF may be most attractive for manufacture of early phase clinical assets with limited time for process optimization, as schedule flexibility gained by de-coupling upstream and downstream operations and shortened affinity load time can simplify manufacturing logistics and technology transfer. Further improvements in bioreactor titer productivity, or use of AAV affinity ligands affixed to convective chromatography stationary phases, could negate the benefits of pre-affinity TFF entirely.

ANION EXCHANGE SEPARATION OF EMPTY & FULL CAPSIDS: APPLYING CHROMATOGRAM PEAK AREA FOR RAPID ASSESSMENT OF ELUATE POOL CONCENTRATIONS

During upstream AAV production, a significant percentage of the viral capsids generated do not contain the packaged transgene of interest. These empty capsids are considered product-related impurities and must be reduced during downstream purification. Anion exchange (AEX) chromatography using either resin or monolithic stationary phases is a viable approach to separate empty and full capsids based on surface charges [19]. The AEX polishing

TABLE 3

Assessment of product quality in CapA:Tg2 affinity eluate with and without pre-affinity TFF concentration and diafiltration.

Pre-affinity TFF strategy	Aggregate content measured in affinity eluate (by SEC-HPLC)	HCP content measured in affinity eluate (ng/1 × 10^{13} vg)
No pre-affinity TFF	2.1%	914
100 kD Flatsheet	1.7%	588
30 kD Flatsheet	0.7%	753
100 kD Hollowfiber	2.3%	1775

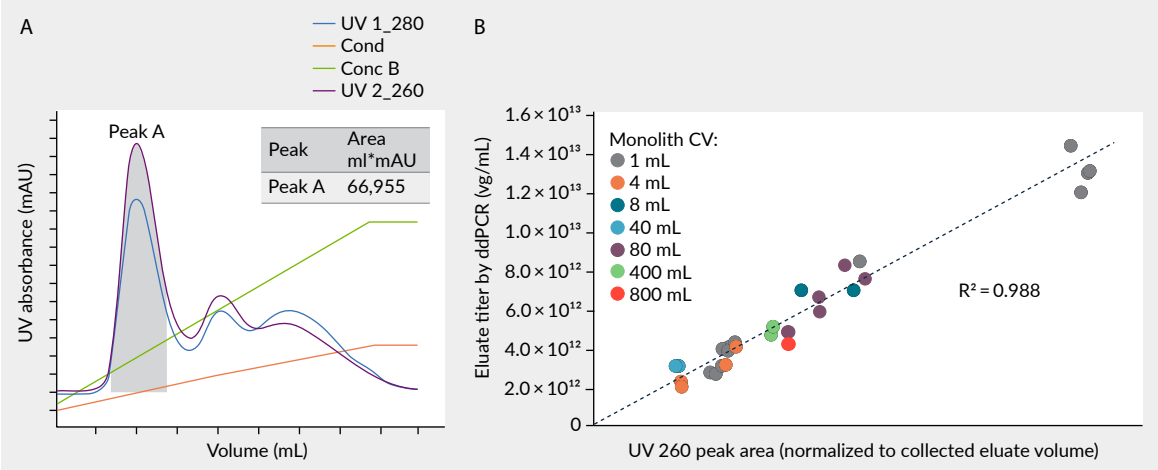
step is typically operated in a bind and elute mode, often positioned after affinity capture and prior to final concentration and formulation. As a result, proceeding from AEX elution to final formulation requires an accurate understanding of product titer in the AEX elution pool. This titer assessment can be a potential process bottleneck, as completion of typical PCR-based assay methods generally requires at least 24 hours process delay.

Our analysis of AEX gradient elution chromatogram data across development and production scales has uncovered an alternative strategy for understanding AEX elution pool titer which may alleviate current bottlenecks (Figure 6). Industry standard chromatography software enables integration of UV peak area (expressed as mAU*mL or AU*L), a parameter which is proportional to total vector genome eluted (vg), as shown in Figure 6A. Therefore, dividing the peak area by collected eluate volume provides a surrogate to PCR based titer measurement. Figure 6B characterizes the correlation between digital droplet PCR (ddPCR) and normalized UV peak area across a range of AEX column sizes (1–800 mL CV) and chromatography systems (Cytiva AKTA™ Pure, Avant, and Pilot) for the CapA:Tg2 construct. It is notable that the linear correlation is maintained across production batches, systems, and scales. The strong relationship between AEX UV peak area and ddPCR titer offers an opportunity to utilize integrated peak area for rapid assessment of in-process titers during AAV manufacturing. While not standard practice, this strategy could eliminate the process delays needed for PCR-based quantification of intermediate product pools. Further, applying chromatogram peak area correlations instead of rigorous PCR measurement to monitor AEX eluate concentration may also reduce analytical qualification requirements for this intermediate, which can be a time-consuming endeavor on the critical path to manufacturing readiness. De-risking these activities by implementing alternative in-process quantification methods, such as a peak area correlation strategy, may provide meaningful gains in speed-to-clinic timelines.

In our experience, linear slope for the correlation between UV peak area and PCR titer is heavily dependent on capsid and transgene properties. Further, as the correlation is best determined empirically, proactive characterization of peak area and PCR titer values during initial process development work can prove highly valuable upon scale-up. It is important to note that this technique was evaluated using affinity-purified material, and the correlation may not hold for alternative primary capture methods. Additionally, this approach may be best suited for gradient elution, which promotes a relatively broad elution peak with UV absorbance signal well in range of standard UV detectors. Comparatively, a step elution strategy may result in sharp peaks which saturate the

FIGURE 6

Correlating AEX chromatogram UV peak area with PCR titer measurement.



(A) Industry standard chromatography system software enables integration of UV signal peak area within a defined elution peak window. In this example, we calculate the area (mL*mAU) under the collected full capsid gradient elution peak of an AAV9-derived TRACER™ capsid. (B) Normalizing the integrated peak area to elution pool volume provides an indication of elution pool concentration. The normalized peak area is strongly correlated to PCR-based titer assays across operating scales. Column volumes ≤80 mL were operated on AKTA™ Avant or Pure; column volumes ≥400 mL on an AKTA™ Pilot. Reproduced from [20].

UV detector, potentially compromising the accuracy of the correlation.

CONCLUSIONS

Three core downstream operations—affinity capture chromatography, tangential flow filtration, and anion exchange chromatography for full capsid enrichment—were systematically evaluated to address common challenges in AAV manufacturing. Our assessment of affinity resin capacity and load flowrate showed that a 3-minute residence time was sufficient to maximize resin binding. However, in cases of low affinity feed titers ($<1.7 \times 10^{12}$ capsid/mL, or $<5 \times 10^{11}$ vg/mL at 30% full), it may be difficult to efficiently utilize this resin capacity without incurring very long cycle times. To address this, TFF was evaluated for product concentration and diafiltration prior to affinity capture. Among the formats tested, the 100 kD flat sheet cassette demonstrated the best balance of high filtrate flux, throughput, and facility fit. This format enabled a 10-fold concentration with ≥90% product recovery, reduced affinity load times, and minimized column pressure during loading. Operationally, the significant volume reduction facilitated by pre-affinity TFF offers a convenient in-process hold point to de-couple upstream and downstream activities for improved manufacturing schedule flexibility.

Our assessment of chromatogram data for the AEX full capsid enrichment step revealed a strong linear correlation between UV peak area and ddPCR-based titer measurements. This finding enables real-time, non-invasive estimation of product concentration in the AEX eluate, potentially eliminating the need for time-consuming PCR assays prior to final formulation. Such a strategy could significantly streamline in-process decision making and reduce analytical burden during scale-up and tech transfer.

Collectively, these findings support a practical and scalable framework for enabling AAV downstream operations at manufacturing scale. While specific process parameters may vary across other

AAV capsids and serotypes, the case studies presented here offer strategies to alleviate operational bottlenecks to enhance the overall manufacturability of AAV-based therapeutics.

MATERIALS & METHODS

AAV production: novel AAV capsids identified using the TRACER capsid discovery platform were produced in HEK293 cells using plasmid transfection. Upon harvest the cells were chemically lysed, treated with nuclease to digest free DNA, clarified, and filtered with 0.22 µm membrane prior to downstream experiments. Material for downstream experiments was sourced from different upstream bioreactor production runs.

TFF: TFF experiments were performed using Repligen TangenX® 100 kD HyStream modified polyethersulfone (mPES) membrane with L-Screen, MilliporeSigma 30 kD Ultracel® regenerated cellulose membrane with C-Screen, or Repligen Spectrum® 100 kD mPES hollow fiber with 0.5 mm lumen ID and 20 cm length. A Repligen KrosFlo® KR2i system was used to perform development scale TFF experiments.

Affinity chromatography: all affinity capture experiments utilized Thermo Scientific Poros™ CaptureSelect™ AAV9 resin. Affinity resin binding capacity was assessed using 200 µL RoboColumn® devices and a Tecan liquid handler. Affinity capture experiments using TFF processed materials were performed with OPUS® MiniChrom® columns packed with 1 mL or 5 mL resin volume, and were operated on a Cytiva AKTA Avant.

Anion exchange chromatography: full capsid enrichment was performed with Sartorius BIA CIMmultus® QA monoliths featuring 2 µm porosity. Experiments were performed using 1, 4, 8, 40, 80, 400, or 800 mL monolith volumes and Cytiva AKTA Pure, Avant, or Pilot systems. All AEX experiments utilized a linear conductivity elution gradient to separate full and empty capsid species. After elution, chromatogram peak area was assessed using Cytiva Unicorn™ software.

Analytical methods: genome titer was measured by ddPCR. Full capsid content was measured by analytical ultracentrifugation (AUC). The genomic titer was divided by the full capsid ratio to estimate total capsid titer. HEK293 host cell protein impurities were measured by Cygnus Technologies ELISA kit, and aggregate content was measured by size exclusion HPLC (SEC-HPLC).

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CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

VIRAL VECTOR MANUFACTURING AND PLATFORM EVOLUTION

SPOTLIGHT

INNOVATOR INSIGHT

A simplified chromatography method for robust separation of full and empty AAV capsids: a case study with AAV2

David Catalán-Tatjer, Cecile Toussaint, Barbara Paes, Eugene Sun, Robert Stairs, and Alina Venereo

The inefficiency of AAV to package therapeutic genes results in the production of numerous empty or partially filled capsids, affecting the efficacy and safety of AAV-based therapies. Developing a scalable purification method to separate full and empty AAV particles remains challenging due to their similar properties.

In this study, a streamlined AAV2 downstream was developed with particular focus on the polish step. Affinity capture resulted in 95% recovery of AAV2-GFP functional particles. Subsequently, a conductivity-based strategy with an isocratic step hold with Thermo Scientific™ POROS™ XQ strong anion exchange resin was used to increase separation between empty and full capsids. Full capsids were enriched 5-fold and reduced empty capsids 4-fold. This impurity reduction minimizes aggregation risks and immune-mediated toxicities. This approach with a strong anion exchange resin offers an easily developable, scalable, and robust solution for AAV manufacturing, essential for supporting the production of safe and effective gene therapies.

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INTRODUCTION

Gene and cell therapy has been a highly active area of research for at least the past two decades. Adeno-associated virus (AAV) became the most used delivery vector for gene therapy due to its unique safety and efficacy. As of today, seven products relying on recombinant AAVs to deliver functional genes to patients with genetic disorders such as Duchenne muscular dystrophy (Elevidys, Sarepta Therapeutics Inc) or haemophilia B (Hemgenix, CSL Behring) have been commercialized.

Over the years the number of clinical trials involving recombinant AAV has dramatically increased, as well as the demand for larger quantities of highly purified material. While enhancing the productivity and robustness of upstream processes is key to meeting demand, the development of effective downstream purification strategies is essential to produce a clinical-grade product with high titer, potency, and purity, while enabling efficient AAV recovery. One of the challenging aspects of AAV manufacturing is the inefficient packaging of the gene of interest (GOI), resulting in large quantities of empty or partially filled capsids. Moreover, clinical studies support the role of the total capsid per dose as a determinant of AAV vector immunogenicity [1]. It is crucial to develop purification processes to minimize these unwanted species from the final product.

However, the development of a robust and scalable purification process to separate empty and full AAV particles at large scale remains a challenge because of similar physiochemical characteristics of these two AAV populations. The separation of the two species has been achieved by iodixanol or cesium chloride gradient ultracentrifugation based on slight density differences between full and empty capsids [2]. However, this method is not easily scalable and is time and labor intensive. Due to a small difference in the isoelectric points of full and empty capsids, anion exchange chromatography (AEX)-based separation was found to be a scalable and robust alternative to ultracentrifugation.

Full and empty capsids are typically separated by AEX using a shallow linear gradient characterized by low peak resolution, followed by the implementation of a step gradient to enhance separation. Several groups have described these approaches using monoliths [3,4] or membrane adsorbers [5]. These studies highlighted the

FIGURE 1

VVector Bio's AAV downstream process workflow.

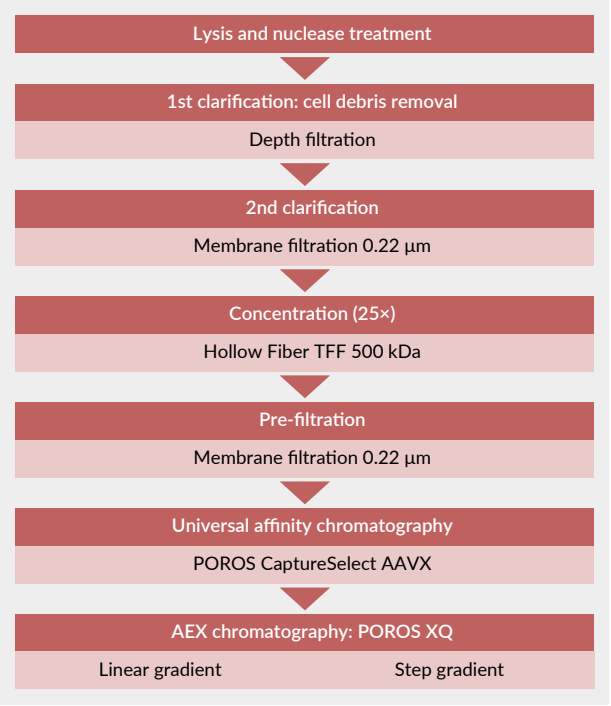


TABLE 1

Steps for the purification of AAV2 with POROS CaptureSelect AAVX Affinity Resin (5 mL).

Step	Buffer	CV	Flowrate (mL/min)
Equilibration	50 mM Tris, 0.5 M NaCl, pH 7.5	8	2.5
Sample loading	(1.6×10^{12} vg/mL-resin)	N/A	2.5
Wash	50 mM Tris, 0.5 M NaCl, pH 7.5	15	2.5
Elution	0.1 M glycine pH 2.5	3	2.5

CV: column volume, vg: viral genome.

challenges of translating linear gradient conditions to a two-step elution, requiring buffer adjustments and an additional development step to optimize step gradients.

This article describes a streamlined and scalable downstream process including affinity chromatography using Thermo Scientific™ POROS™ CaptureSelect™ AAVX Affinity Resin and AEX using Thermo Scientific™ POROS™ XQ Strong Anion Exchange Resin (Thermo Fisher Scientific) for AAV2 full capsid enrichment. The developed strategy for separating full and empty capsids enables straightforward translation of linear gradient data to a two-step gradient elution without additional optimization, allowing substantial enrichment of full capsids and efficient removal of empty ones. POROS XQ is a fully quarternized Q resin on the POROS base bead, which is significantly different to other Q resins based on agarose beads. The POROS bead's large throughpores and rigid polymer offers benefits including high resolution, scalability, and efficient mass transfer.

RESULTS AND DISCUSSION

Purification of AAV2 using POROS CaptureSelect AAVX Affinity Resin

VVector Bio's AAV downstream processing (Figure 1) starts with lysis of the cells. As the majority of AAV remains intracellularly, the cell lysis step allows the release of AAV into the supernatant. This step also releases cell debris and other impurities such as host cell proteins and DNA. The removal of cell debris is performed by either centrifugation for small-scale production or depth filtration for larger-scale production. This step is followed by a second clarification step using membrane filtration before the capture of AAV by affinity chromatography using AAVX Affinity Resin.

The AAVX resin consists of rigid poly(styrene-divinylbenzene) POROS base beads (50 µm mean particle size) functionalized with the CaptureSelect AAVX affinity ligand. The details of this affinity capture step, including the buffers used, are listed in Table 1.

Immediately following elution, the eluted sample was diluted using 10% v/v of neutralization buffer (Tris-based buffer, pH 8.8) to prevent aggregation and to stabilize the AAV.

FIGURE 2

Purification of AAV with POROS CaptureSelect AAVX Affinity Resin.

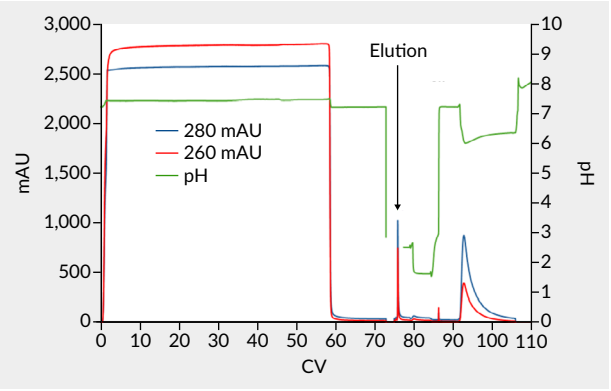


TABLE 2

Process recovery using POROS™ GoPure™ AAVX affinity column (5 mL).

Step	Volume (mL)	vg/mL	TU/mL	Total VG	Total TU	VG recovery (%)	TU	Half-life extended?
Load	294.0	2.73×10^{10}	1.75×10^{9}	8.03×10^{12}	5.15×10^{11}	N/A	N/A	1.2
Eluate	3.9	1.67×10^{12}	1.27×10^{11}	6.43×10^{12}	4.89×10^{11}	80	95	1.1

TU: functional titer. vg: viral genome. VG: viral genome titer.

Figure 2 shows the affinity capture chromatogram demonstrating a successful AAV purification with AAVX Affinity Resin in a 5 mL pre-packed column (Thermo Fisher, Cat. A36651). In comparison to the traditional gradient ultracentrifugation method, chromatography is scalable, highly robust, and suitable for GMP manufacturing.

The viral genome titer and the functional titer of the affinity chromatography load and elution samples are presented in Table 2. A recovery of 95% of AAV functional particles was achieved, demonstrating that the process can maintain the functionality of the AAV particles. Moreover, the data show approximately 1 log difference in titer between the viral genome titer (VG) and functional titer (TU). This variation was expected, and the purification process did not change the ratio of VG to TU. The difference between functional titer and VG titer seems highly dependent on the serotype and GOI (data not shown).

SEPARATION OF FULL/EMPTY AAV2 WITH POROS XQ STRONG ANION EXCHANGE RESIN

Linear gradient development

Prior to the separation of empty and full capsids, the purified AAV sample was buffer exchanged to AEX equilibration buffer using a PD-10 desalting column (Cytiva). The material was then loaded onto an anion exchange 1 mL column pre-packed with POROS XQ resin, which contains fully quaternized amine functional groups (Thermo Fisher, Cat. A25812). The details of this chromatography step are described in Table 3. In brief, after column equilibration and material loading, the elution was performed using a shallow linear gradient from 50 mM to 500 mM of sodium acetate in buffer A (20 mM BTP, 10 mM MgCl2, 0.01% Pluronic, pH 9.0) over 40 CV.

The linear gradient elution performed to separate full and empty particles is presented in Figure 3. The chromatogram displayed two overlapping peaks. A qualitative analysis based on UV absorbance at 260 nm (A260) and 280 nm (A280) suggests that the first peak is comprised primarily of empty particles and the second peak consists mainly of full particles, as an increase in the ratio A260:A280 indicates an enrichment of full capsid containing DNA. Moreover, preliminary analysis of functional titer quantification of the fraction collected from the two peaks showed that the fraction from the second peak consisted of an approximate 25-fold increase in functional particles on the fraction from the first peak (data not shown). These data suggest that empty capsids elute first, followed by elution of the full capsids.

These results also confirmed that the buffer formulation used during the process

TABLE 3

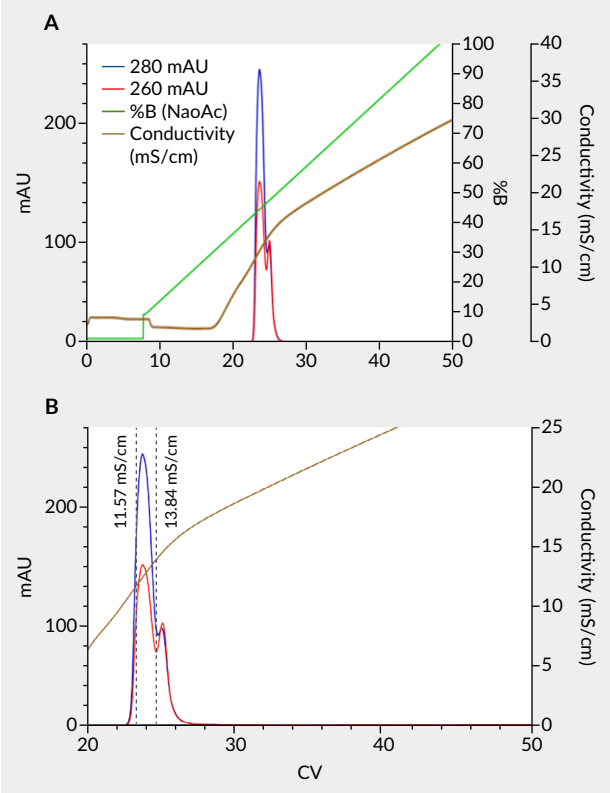
Steps for the separation of full/empty AAV2 with POROS™ GoPure™ XQ (1 mL).

Step	Buffer	CV	Flowrate (mL/min)
Equilibration	10 mM NaOAc, 20 mM BTP, 10 mM $MgCl_2$, 0.01% Pluronic, pH 9.0	5	1
Load	Purified AAV2, approximately 1.2×10^{12} vg/mL-resin	7	0.5
Elution	A: 20 mM BTP, 10 mM $MgCl_2$, 0.01% Pluronic, pH 9.0 B: 500 mM NaOAc in Buffer A	40 linear gradient 10–100%B	0.5

CV: column volume.

FIGURE 3

Linear gradient elution for the separation of full/empty AAV2 with (A) POROS XQ resin and (B) a detailed view of conductivity changes.



is suitable for the separation of full and empty capsids. This chromatogram shows that the empty capsids started to elute at a concentration of 216.5 mM sodium acetate (NaOAc) and 11.57 mS/cm. The peak maximum was found at 12.57 mS/cm and 222.5 mM NaOAc. The full capsids started to elute at 232.5 mM NaOAc and 13.84 mS/cm. It is worth noting that there was a difference of only 2.27 mS/cm between the start of the elution for full and empty capsid peaks.

A closer look at the chromatogram showed the slope of the linear gradient and the conductivity are different, meaning that conductivity increased faster than the NaOAc concentration. This phenomenon is expected based on the equipment configuration. There is a delay volume between the %B and conductivity outputs. The reason is that the % B is determined at the mixing pump, upstream of the column, while the conductivity is measured downstream of the column.

Considering the high sensitivity of the AAV elution peaks to conductivity and challenges preparing buffers within a tight range, it was decided to develop a separation based on conductivity rather than molarity to increase the resolution between the empty and full peaks.

Optimization of linear gradient including an isocratic step hold

To improve the separation between empty and full capsid peaks a hybrid elution strategy was adopted, which combined a step elution and a linear gradient with a manual hold of the system. Hold-step gradients are commonly employed to enhance peak resolution between empty and full AAV capsids [6,7]. However, various strategies can be used to define the initiation of the hold step. For instance, Aebischer *et al.* (2022) describe a method for AAV8 empty/full capsid separation in which the hold step is triggered at specific %B values; four different %B settings were tested to optimize resolution [6]. For AAV5, Lavoie *et al.* (2023) implemented a strategy based on UV absorbance, triggering the hold step when the empty capsid peak reached a defined percentage of total absorbance (85% vs 95% were tested) [7]. Several studies have employed monolith columns to enrich full capsids of AAV2 [3] as well as AAV5, AAV6, AAV8, and AAV9 [4]. These investigations highlighted the challenges of implementing step-gradient elution protocols based solely on %B and salt concentration, as such approaches often necessitated extensive buffer screening. Notably, in the case of AAV9, monolith-based chromatography proved ineffective, and resin-based chromatography was required instead.

Together, these findings demonstrate that translating conditions from

linear gradient experiments to step-gradient elution using %B and salt concentration demands iterative, trial-and-error optimization to identify suitable parameters

Another promising approach involved the use of a conductivity-based step gradient to separate full and empty AAV5 capsids using Mustang Q membrane chromatography [5]. In this study, as linear gradient failed to achieve sufficient separation, a multi-step gradient protocol was developed consisting of nine steps, each with a 1 mS/cm conductivity increment. This method was subsequently refined to a five-step gradient and finally, to a two-step protocol. However, the authors concluded that further optimization of buffer conductivity and composition was necessary to enhance resolution. These findings underscore the critical role of conductivity in capsid separation; however, in the study presented here, a significantly simpler conductivity-based step gradient that eliminates the need for additional buffer optimization is proposed. For this work, the linear gradient and the buffer composition used was the same as those described in Table 3. The timing of the isocratic step hold was based on the data collected from the first linear gradient (Figure 3B) and accounted for the equipment configuration, where the conductivity sensor was downstream of the column. Once the isocratic step hold was started, conductivity continued to increase before stabilization due to the holdup volume between the mixing chamber and the conductivity sensor. It was critical that the conductivity stabilizes before reaching the value of 13.58 mS/cm as this triggers the elution of the full capsids. Based on this data, the linear gradient was held once the conductivity reached 11.57 mS/cm and until the UV absorbance returned to baseline. The linear gradient was then resumed to allow the elution of the second peak corresponding to the full capsids.

The resulting chromatogram (Figure 4) shows that the isocratic step hold initiated

FIGURE 4

Combination of linear and isocratic elution for the separation of full/empty AAV2 using POROS XQ resin.

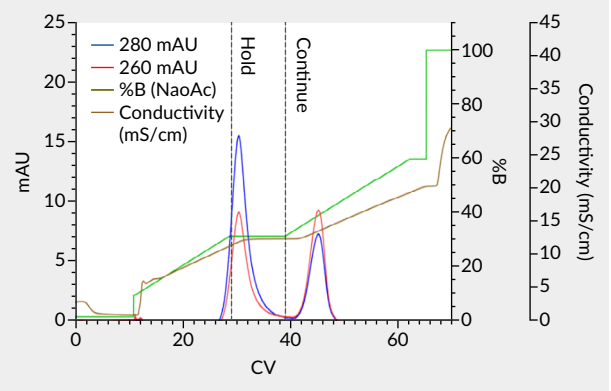


TABLE 4

Process recovery and AAV particle characterization of load and elution fractions using the AEX pre-packed column POROS GoPure XQ 1 mL.

Step	Volume (mL)	Total VG	Total TU	VG recovery (%)	TU recovery (%)	Log (VG/TU)	Full capsid (%)
AEX load	3.5	2.15×10^{12}	7.18×10^{10}	N/A	N/A	1.5	14
1st peak (empty peak)	6.0	3.20×10^{10}	8.94×10^{8}	1.5	1.2	1.6	~1
2nd peak (full peak)	5.5	1.30×10^{12}	3.30×10^{10}	60.7	46.0	1.6	75

TU: functional titer. vg: viral genome. VG: viral genome titer.

at 11.57 mS/cm resulted in conductivity stabilization around the target conductivity of 12.5 mS/cm. The UV traces also showed this elution strategy resulted in two fully resolved peaks. As expected, the second peak displayed a higher ratio of A260:A280 reflecting an enrichment of AAV capsids containing DNA. The elution of the first peak started at 11.57 mS/cm as expected. However, the concentration of NaOAc was determined to be 158 mM (31.6 %B) while the previous separation showed an elution at 217 mM NaOAc for the same conductivity of 11.57 mS/m. These data showed that conductivity is a more robust criteria for the elution of the AAV2–GFP than salt concentration.

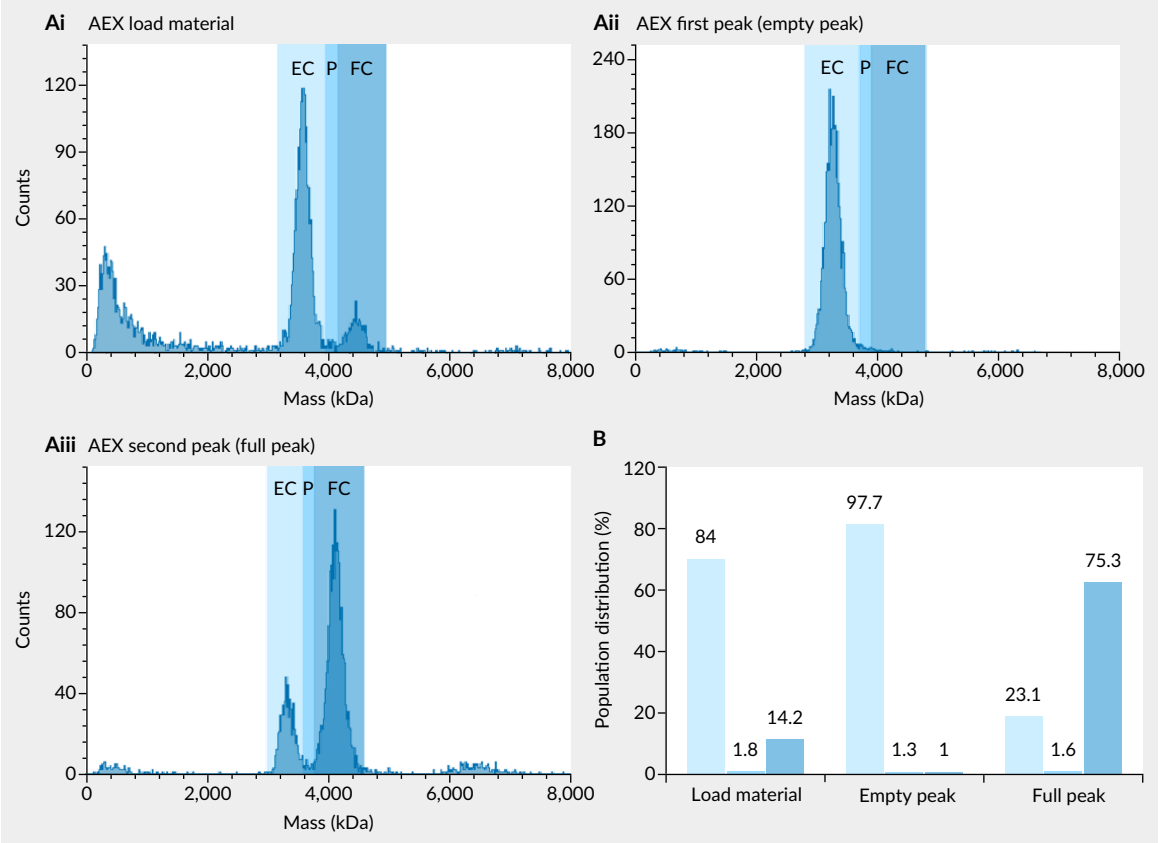
These results demonstrate a direct, one-step translation of linear gradient data to a two-step gradient elution method, without the need for further optimization or buffer modification.

DETERMINATION OF FULL CAPSID ENRICHMENT USING MASS PHOTOMETRY

Although analytical ultracentrifugation (AUC) is currently considered the gold standard for quantifying the proportions of full and empty capsids, mass photometry (MP) was employed in this study. Previous studies have demonstrated that MP is a rapid, high-throughput, and effective

FIGURE 5

The mass distributions of three fractions (load, empty, and full peak) comprising different proportions of AAV subpopulations.



EC, P, FC represent empty capsid, partially filled capsid, and full capsid respectively (Ai, Aii, Aiii); proportion of AAV2 subpopulation (EC, P, FC) from AEX load material (affinity-purified material), first peak (or empty peak), and second peak (or full peak) (B).

method for determining the relative abundance of capsid populations [8,9]. Notably, MP shows the strongest correlation with AUC measurements (R^2=0.9963) compared to TEM or UV-spectrometry [9]. However, MP provides slightly lower resolution in distinguishing partially filled capsids. An important advantage of MP is its significantly lower sample material requirement compared to AUC. The characterization of the fractions collected from the hold-step elution in term of viral genome and functional titer is shown in Table 4.

The column and method used for the separation of full and empty capsids demonstrated a recovery of approximately 60% of viral genomes while the recovery of functional particles is approximately 46%. The recovery of the full capsid fraction yield could be further improved by optimizing buffer conditions.

Figure 5 shows that the anion exchange load material was primarily composed of empty capsids (84%) while full capsids represented only a small portion, approximately 14% of the total amount of particles. The empty peak was comprised of mostly empty capsids (98%) while a proportion of full capsids marginally remained at approximately 1%. These data demonstrate efficient empty capsid removal while minimizing full capsid loss. Finally, the characterization of the full peak shows a composition of 75% full capsids and only 23% empty capsids. The separation method developed here achieved a full capsid enrichment factor of 5-fold and a 4-fold reduction in empty capsids compared to the anion exchange load material. Achieving a high concentration of AAV can be challenging due to potential aggregation. Thus, the removal of empty capsid offers the possibility to further concentrate full capsids carrying the therapeutic gene while minimizing the risk of aggregation. This is particularly important as total capsid dose appear to be responsible for immune-mediated toxicities/ Removing empty capsid with no therapeutic benefit is therefore crucial [1].

CONCLUSION

VVector Bio has developed a simple and scalable process for efficient separation of full and empty AAV2 using anion exchange chromatography with POROS XQ resin after affinity purification with POROS CaptureSelect AAVX Affinity Resin. The advantage of this method is the easy and direct translation of the linear data to the two-step gradient, allowing a high resolution without any further optimization or buffer adjustments. We hypothesize that this approach can be successfully applied to other AAV serotypes. The enrichment of full particles is crucial to achieve highly concentrated AAV preparations, which is a critical factor to obtain an effective response *in vivo*. Reducing the empty capsids in final preparations can also reduce the risk of aggregation if the sample needs to be further concentrated. This work offers detailed insights into the operation protocol for separation of empty/full capsid and emphasizes conductivity as the primary factor to consider when developing such separation methods.

ANALYTICAL METHODS

As part of the dosing and potency analysis of AAV vectors, the determination of the VG titer needs to be accurately quantified. This titer refers to AAVs that package the GOI either in full length or truncated form. Droplet Digital PCR (ddPCR) can provide an absolute count of viral nucleic acids, enabling the precise quantification of AAV vectors containing the GOI. However, not all full AAV particles are functional due to the insertion of host cell DNA or truncated GOI in the capsid. The viral genome, estimated as vg/mL, does not always directly translate into functional titer or transduction units (TU).

Hence, the titration of functional particles is critical to determine vector efficacy. A gene transfer approach was developed in house to detect the expression of the transgene. To determine the transduction efficiency or the potency of AAV2–GFP vector, a cytometry-based assay was developed to quantify the percentage of GFP expressing cells.

ddPCR

ddPCR was used to quantify the concentration in vg/mL of all relevant samples as previously described [10]. Briefly, the High Pure Viral Nucleic Acid Extraction kit (Roche Diagnostics, Switzerland) was used to extract the viral DNA corresponding to the AAV following manufacturer's instructions. Then, with forward primer 5′-CTGCTGCCCGACAACCAC-3′ and reverse primer 5′-TCACGAACTCCAGCAGGAC-3′ obtained from Integrated DNA Technologies (USA), a PCR was carried out with a preincubation step at 95 °C for 15 mins, followed by 40 cycles at 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min with a 5 min final extension at 72 °C. The plates were scanned on a QX100 droplet reader (Bio-Rad, USA), and the analysis was carried out with QuantaSoft software (Bio-Rad, USA).

Potency assay

A transduction assay was performed in which samples containing AAV were added to HEK293 cells previously transduced with Adenovirus 5 as previously reported [10]. At 24 h post-transduction, cells were harvested and fixed in 2% paraformaldehyde at 4°C for 30 mins, followed by two analyses: firstly, in BD Accuri C6 flow cytometer (BD Biosciences, USA) to account for all cells, and secondly, with BD Accuri C6 Plus Analysis Software (BD Biosciences, USA) to quantify eGFP expression. The linear range of quantification was established between 2% and 20% of GFP-positive cells. The functional titer was calculated as transducing units (TU)/mL.

Mass photometry

The ratio of full and empty capsids was determined by mass photometry as reported elsewhere [11]. Briefly, a SamuxMP Auto instrument (Refeyn Ltd, Oxford, UK) was used according to the manufacturer's instructions utilizing the Refeyn MassFerence P2 as calibrant. The samples and calibrant were automatically processed and the resulting data analyzed using the system's software.

POROS: Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only. CaptureSelect: For Research Use or Further Manufacturing. Not for diagnostic use or direct administration in humans or animals.

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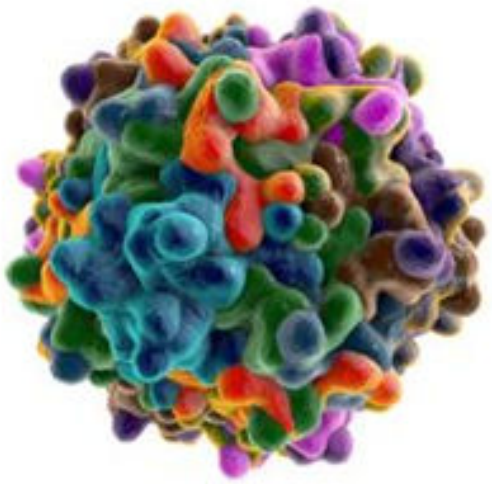

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VIRAL VECTOR MANUFACTURING AND PLATFORM EVOLUTION

SPOTLIGHT

EXPERT INSIGHT

Increased efficiency and productivity in rAAV upstream processing

Ashutosh Gupta

With the approval of multiple gene therapies in recent years, recombinant AAV (rAAV) has been proven to be the most safe and effective delivery vehicle. With the usage of rAAV for clinical and commercial manufacturing for indications in multiple therapeutic areas, the high cost of rAAV production has become a barrier for these therapies to be able to reach a wider population and its application for diseases with fewer number of patients. This review focuses on the current challenges in the upstream manufacturing of rAAVs and recent progress in addressing some of these challenges.

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INTRODUCTION

Over the last decade, recombinant AAV (rAAVs) have emerged as one of the gene therapy delivery vehicles for treatment of genetic diseases with great potential. With the increased use of rAAV in clinical and commercial manufacturing for various diseases; in addition to safety and potency concerns; complex production processes, process robustness, and high cost of production have emerged as a barrier for the further adoption of this delivery vehicle for other diseases with high unmet needs. This article provides an overview of the current state of rAAV upstream production process and provides insights into the future direction of the field. This article also summarizes current upstream production platforms, advantages, and challenges of using each of these platforms for various phases of development and how route of administration and target indication are important aspects of the platform selection process.

Gene therapy typically involves repair, replacement, suppression, or overexpression of a disease causing gene [1]. Although there have been many delivery vehicles used to achieve this goal, rAAV have been proven to be amongst one of the safest and most efficacious delivery vehicles for treatment of monogenic genetic diseases [2,3]. Although treatments for some genetic diseases such as sickle cell (LYFGENIA),

beta-thalassemia (ZYNTEGLO), and cerebral adrenoleukodystrophy (SKYSONA) have been recently approved by the US FDA, their uptake has been limited for commercial, safety, and logistical reasons. Lentivirus is the delivery vehicle in these therapies, which integrates into the host genome and has potential safety concerns [4,5]. On the other hand, rAAVs exist in the nucleus through episomal persistence after transport of genetic material to the nucleus and transduce both dividing and non-dividing cells, enabling their long-lasting expression. Since rAAVs integrate DNA into host genome at very low frequency and are designed to not integrate into host genome, they are considered relatively safer delivery vehicles as compared to retroviruses such as lentivirus. rAAVs relative safety and efficacy as a delivery vehicle has also been shown through commercially successful therapies such as Zolgensma, which have been used to treat thousands of patients worldwide. With the success of Covid vaccines in recent years, lipid nanoparticles have also emerged as an effective delivery vehicle. Although mRNA has been successfully used as genetic material for covid mRNA lipid nanoparticle vaccines, its use for treatment of rare diseases or other diseases with large patient population is currently not proven.

After the initial success of rAAV for treatment of diseases with low doses and fewer numbers of patients, such as Luxturna for retinitis pigmentosa, rAAV is now being studied for the treatment of diseases requiring higher doses and administration to larger patient populations, which has placed renewed focus on the potency and cost of manufacturing of rAAV gene therapies [6,7]. For example, while the recommended dose for Luxturna for retinitis pigmentosa is 1.5×10^{11} vg; recently approved therapy for Duchenne muscular dystrophy, Elevidys, has a recommended dose of 9.31×10^{15} vg for patients weighing 70 kg or greater; a ~60,000 fold dose difference dependent on indication. In addition to the complexity of manufacturing rAAV therapies, other factors driving the cost of production are batch to batch variability, batch success rates, raw material costs, and processes which were not designed for late phase clinical and commercial production.

This review focuses on all aspects of the upstream process including production platforms, plasmids, triple transfection, production media, suspension platform, process scalability, empty/full vectors, and process analytics.

PRODUCTION PLATFORMS & PROCESSES

Typical upstream process for rAAV production is HEK293 cells based transient transfection. It involves thawing and growing the HEK293 cells up to a cell density for transfection with three plasmids using a chemical transfection reagent. Afterwards, there is a clarification step to remove large cell debris as well as further downstream steps to remove process and product related impurities. This is the preferred platform for clinical and commercial production because of its applicability to different serotypes and its flexibility in generating material for early pre-clinical and clinical studies quickly. Another factor in widespread use of this platform is also that it fits well in terms of being able to produce the first clinical batch quickly, as well as amount of vectors needed regardless of route of administration and target indication, which may not be true for some of the other platforms.

For the HEK293 cell based platform, it is important to select a cell line that has been optimized for AAV production in terms of titer, potency, as well as ratio of full particles, and has documentation of cell line generation and clonality, which can support regulatory filings. Some commercial cell lines available for transient transfection platforms are Gibco™ VPC and

VPC2.0 from Thermo Fisher Scientific and VirusExpress® AAV production platform from MilliporeSigma.

Other production platforms that have been used for rAAV production are rHSV-based infection of HEK293 or BHK cells and recombinant baculovirus based infection of the insect cells. Although these viral infection-based platforms have potential cost and scalability advantages, drawbacks of these platforms include potential potency concerns and long lead times needed for generation of viral seed stocks. In the last few years, there have been a few baculovirus-based FDA-approved therapies (Hemgenix and Roctavian for hemophilia), which have helped to alleviate some of the concerns associated with the insect based platform.

Producer cell lines such as the ELEVECTA™ platform from Cytiva or the TESSA system have also been recently developed for production of rAAVs. Earlier efforts to develop such stable cell lines were hampered by cytotoxicity of viral proteins observed during the rAAV production. ELEVECTA uses a tetracycline based inducible system using either CAP or HEK293 cell line; while TESSA uses HEK293 cell line, doxycycline inducer, and adenoviral elements for helper function. Although these new platforms offer COGs and scalability advantages, challenges with these platforms include developmental timeline challenges for ELEVECTA and safety concerns associated with the TESSA system compared to the transient transfection platform [8–10]. HeLa cells based producer cell lines typically use wild type adenovirus for induction of production. Safety concerns for HeLa cell line based platform include presence of oncogenes in HeLa cell lines as well as need for removal of adenovirus to acceptable level in downstream steps.

In the next few sections, this review will provide a summary of the key aspects of the upstream production process for transient transfection-based production of rAAV.

IMPROVEMENTS IN CRITICAL RAW MATERIALS

Plasmids used in the triple transfection process are a critical part of the production process. Currently, most plasmids are produced using *E. coli*-based fermentation process with subsequent purification steps to remove impurities. This production process involves multiple downstream steps to remove impurities such as endotoxins, host cell DNA, and host cell proteins, along with lower overall yields, making plasmids an expensive raw material and representing a significant portion of the raw material and batch cost. There have been various attempts to improve the design of the plasmids [11–13] with the objective of improving rAAV yields as well as attempts to combine the three plasmids into one [14] or two plasmids [15,16] to lower the raw material costs as well as product related impurities for transient transfection based process. Although these are significant improvements in plasmid design for rAAV production, these platforms have yet to be adopted widely for clinical and commercial manufacturing. Similarly, for insect cell-based systems, instead of using three baculoviruses, a single baculovirus platform such as Monobac has been proposed. To overcome the baculovirus stability challenges typically seen in insect cell-based platforms, virus banking methods such as Baculovirus Infected Insect Cell (BIIC) have been implemented.

Production media is another area where improvements and optimization could be helpful. Historically, media used for many of the earlier clinical and commercial manufacturing batches contained serum or animal derived components. Although these components provide higher yields, these are considered complex media components with undefined composition and have safety as well as batch to batch reproducibility issues for commercial manufacturing and are not desired. Protein hydrolysates such as yeast

and soy extracts can replace animal derived components and alleviate the safety concerns associated with them. Although we now have chemically defined media formulations available from various vendors for triple transfection based rAAV production utilizing HEK293 cells as well as for insect cell based rAAV production platforms, these formulations may not be optimal for each cell line or serotype and are often proprietary, which leads to increased manufacturing costs and reduced usefulness. Given the limitations of available media formulations, there have been many attempts to improve titers of the triple transfection process and gain better understanding [1,17–20]. Compared to optimized media formulations for other modalities such as monoclonal antibodies, rAAV growth and production media still needs further improvements, so it can support the intensified processes [8,21] and result in higher yields.

Transfection reagents are another critical raw material for transfection based rAAV production. Cationic polymers such as polyethylenimine and FectoVIR® are typically used for clinical and commercial manufacturing. These transfection reagents may not be compatible with all media formulations, so their empirical evaluation for production media compatibility is generally necessary. Since these transfection reagents are expensive, the choice of transfection reagent, its quality and quantity are important determinant of the productivity and cost of raw materials for the transfection based rAAV production. In addition to transfection reagents, AAV production enhancer RevIT™ and Transfection Complex Stabilizer VirusGEN® is available from Mirus Bio to further optimize the transfection step.

ADHERENT & SUSPENSION CELL CULTURE

The platform used for the rAAV production is extremely critical for the long term manufacturing strategy. Since initial successes in the field were for indications with small patient populations, initial commercial platforms such as cell factories for adherent cell based production or wave bioreactors for suspension cells could support such indications [22]. Even with the improvements to increase the surface area, better process parameter control and better gas exchange rates [22–24], these adherent cell based systems are not comparable to the suspension cell based platforms in terms of process robustness and control. For indications such as Duchenne muscular dystrophy, which require a ~10^{15} vg dose for a young patient, such earlier platforms are not scalable to support such indications. In addition, indications with large patient populations make it impossible to use adherent cell based platforms. Suspension based platforms and bioreactors provide a proven, scalable, and robust platform which has been used for manufacturing commercial therapies for other modalities for many years. Suspension based platforms can support production at a very small scale using Ambr250 or similar bioreactors as well as large scale bioreactors of thousands of liters in size. Another important consideration is whether to use single-use bioreactors and accessories or stainless steel bioreactors. Although single-use bioreactors offer many advantages in terms of flexibility and timelines for multi-product clinical manufacturing facilities for bioreactor volumes of up to 5000L, their benefits are not as significant for larger scale batches or for single product commercial manufacturing facility.

PROCESS OPTIMIZATION

There are many steps in the triple transfection process for rAAV production that should be optimized to improve the yields and robustness of the process. Some recent efforts on upstream process optimization have focused on optimization of

the overall process with a holistic view [25], while others have focused on specific steps.

Usually, the first step in the production process is the cell expansion step, where it is important to ensure that cells are growing actively at each passage and are in exponential phase. After cells have reached the desired density, cells are ready for triple transfection. This is the most critical step in the process. There have been many recent reports on optimization of transfection step [19,26–29], which have looked into optimizing this step from various aspects including optimization ratios of three plasmids to transfection reagent, the transfection reagent itself, transfection complex preparation process, transfection volume, and transfection complex formation duration. In addition, there have also been attempts to optimize the rAAV production process through mechanistic and other modelling based methods [30,31]. Another avenue that has been explored recently for improving the rAAV productivity is perfusion based intensified process similar to processes used for other modalities [21,32–35]. To further improve the yields, there has recently been a number of attempts to use omics-based tools such as RNA-seq to gain further insight into the AAV production kinetics. These efforts have shown that some small molecule cell cycle modulators (histone deacetylate inhibitors etc.) can further increase the rAAV production.

During process optimization for rAAV yield improvement, it is also important to optimize the number of full particles, as a larger fraction of empty particles, in addition to not being desired from a safety and regulatory point of view, are also harder to purify during the downstream steps [36,37]. The use of producer cell lines can lead to a higher percentage of full particles in the upstream steps, thus leading to higher full to empty particle ratio in the drug substance.

After the rAAV production, the next step is typically cell lysis and DNA digestion by triton/endonuclease treatment to digest the host cell DNA, to inactivate enveloped viruses, and to release the rAAV in the harvest. Benzonase has been used as the endonuclease for most processes, but recently high salt tolerant endonuclease, DENARASE® was introduced by c-LEcta and provides more process flexibility. Similarly, because of environmental concerns associated with use of Triton X-100, other alternative detergents such as Deviron® 13-S9 and Nereid® have recently been proposed.

Another important aspect of upstream process optimization is also the reduction of process and product related impurities. Selection of a platform that produces a lower level of these impurities in the upstream process, specifically impurities which are packaged inside the rAAV such as encapsidated host cell DNA and encapsidated plasmid DNA, significantly helps in reducing immune response and enhancing the safety of AAVs. For example, the ELEVECTA producer cell line significantly reduces host cell DNA through cell line engineering. Such designs inherently lead to safer and better quality of the rAAV vectors produced. In addition to the encapsidated DNA, other process and product related impurities are minimized through upstream process design and through their reduction in downstream steps to an acceptable level.

ANALYTICAL TECHNOLOGIES

Improvements in analytical technologies used to measure rAAV titers as well as empty to full capsid ratios are necessary for efficient upstream process development. While the accuracy and robustness of the measurement techniques is critical for efficient optimization of upstream process steps, the amount of sample used for measurement, time to process sample,

and high throughput nature of the technology/instrument is as important. While it has been typically challenging to measure rAAV titer and total capsid quantity in the crude lysates because of lower concentrations and presence of process and product related impurities, some technologies [38–40] have recently been available commercially in recent years, which have helped in fast and efficient upstream process development [41–43].

POTENCY & POST-TRANSLATIONAL MODIFICATIONS

Potency and yield of the rAAV vectors produced using different platforms has been a topic of discussion in the gene therapy field. There have been many studies comparing whether suspension vs adherent platform or HEK293 vs insect cell platform are more efficient or better in terms of potency or yields for rAAV vector production [44–49]. As therapies produced using either of these platforms have shown to be safe and effective, with appropriate process optimization and process controls in place resulting in robust and consistent processes–most of these platforms can produce safe and effective therapeutics.

Similarly, post translational modifications in different production platforms are another topic of discussion in the field in recent years. Other than the host cell platform chosen for production, upstream and downstream processing conditions can influence the post translational modifications on the rAAV vectors [44,50]. With tight control of production conditions, robust and reproducible production of clinical and commercial grade rAAV vectors with consistent quality can be ensured.

Potency of rAAV vectors is also important from safety and lowering the COGs point of view. Higher potency vectors can lower the dose required for same therapeutic effect, which in turn lowers the cost of manufacturing for one dose and improves safety of the product. Lowering of dose required for therapeutic effect can also result in lower number of empty vectors dosed and potentially lower immune response. On the other hand, higher potency vectors can enable higher therapeutic effect for the same dose. Potency of rAAV vectors can be primarily increased through capsid selection, design of plasmid vector, and optimization of gene of interest.

SUMMARY

This review summarizes the current upstream production platform for rAAV production for clinical and commercial manufacturing. In addition, the review focuses on selecting and designing the production platform, which can produce clinical grade material quickly with acceptable quality, while also keeping in mind that same platform is robust, scalable, and can support later commercial production.

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CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

VIRAL VECTOR MANUFACTURING AND PLATFORM EVOLUTION

SPOTLIGHT

INNOVATOR INSIGHT

Regulatory path transitioning from transient to stable cell lines for AAV manufacturing

Emmanuelle Cameau, Evelyne Nguyen, Andreia Pedregal, Jacob Staudhammer, and Catherine Colandro

AAV manufacturing is transitioning from transient transfection to stable producer cell lines for improved scalability and cost–effectiveness. This framework addresses regulatory considerations for implementing this manufacturing change during clinical development. Risk assessment encompasses technical, regulatory, and business factors. Early-stage transitions pose lower risks with safety-focused comparability studies, while late-stage changes require extensive data addressing both safety and efficacy. Key recommendations include comprehensive cell line characterization with genetic stability evaluation, leakiness assessment, and tier-based acceptance criteria. Master cell bank testing should monitor cell viability, expression levels, titer, and capsid ratios through stability studies. Proactive regulatory communication with agencies like FDA's CBER is essential for successful implementation, particularly for late-stage manufacturing changes, to ensure regulatory approval while maintaining product quality.

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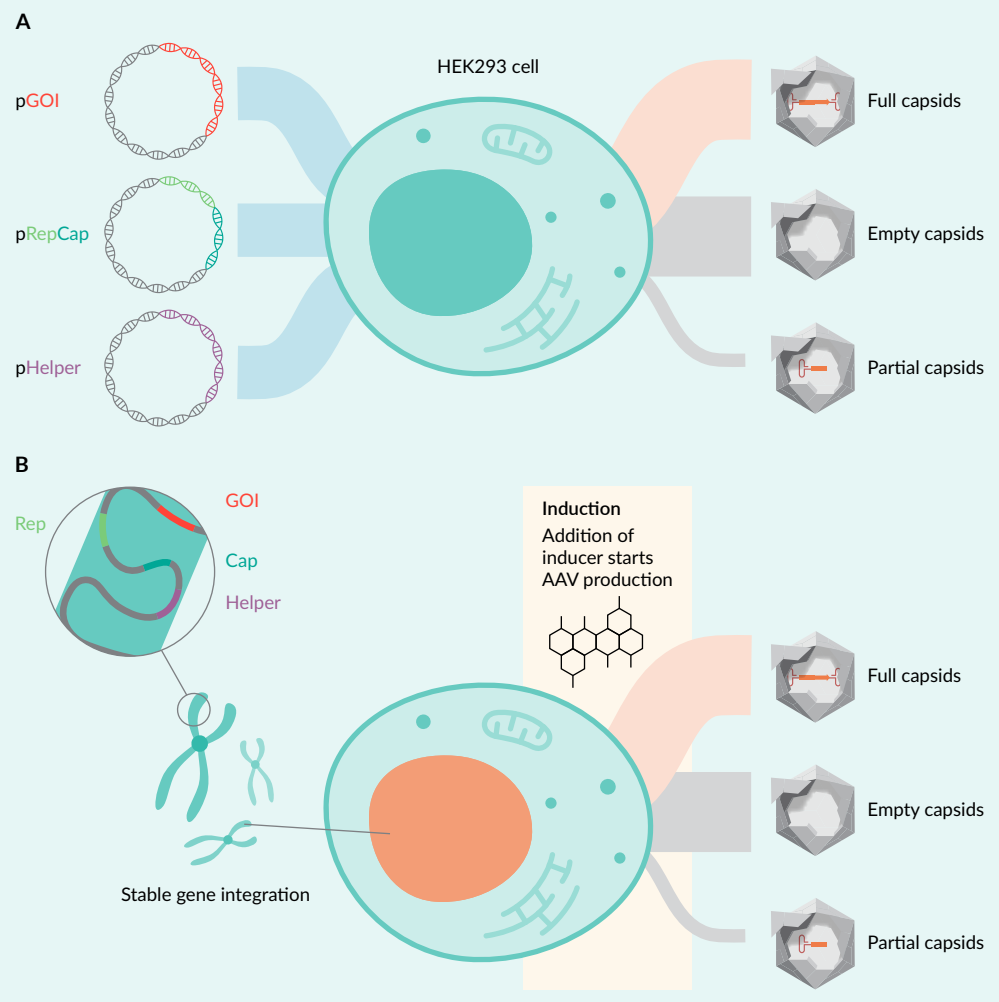
INTRODUCTION

Adeno-associated virus (AAV) is a gene therapy delivery tool often generated via cell transfection-based methods. Although this approach can be fast and flexible for early-stage research and construct optimization, and lead to high titers under optimized conditions (1×10^{13} vg/L to 2×10^{15} vg/L; [1–3]), it is generally considered less efficient and more costly compared to producer cell lines. (Figure 1). Recent advances in the gene therapy field demonstrate successful achievement of high AAV titers using stable producer cell lines, with yields reaching up to $>2 \times 10^{15}$ vg/L [4,5]. These stable producer cell lines typically harbor all relevant components for AAV production stably integrated in their genomes, enabling high-titer AAV production upon treatment with the inducing agent.

FIGURE 1

Schematic explanation of transient cell line manufacturing, compared to stable producer cells.



(A) Transient AAV manufacturing with triple transfection. (B) Stable manufacturing with HEK293 stable producer cell line.

The Alliance for Regenerative Medicine (ARM) outlines in their regular snapshots that the gene therapy landscape will continue to grow and address more prevalent or systemic indications, particularly with AAV-based therapies [6]. This growth suggests that a significant portion of these therapies will need to be manufactured at a scale appropriate to supply larger quantities of vector per year (up to ~10^{20} vg/year potentially). Stable cell lines provide significant cost savings when used for large-scale production. Indeed, in a recent cost modelling study published by Saltarin *et al.*, in the case of a therapy requiring 2×10^{19} viral genomes per year, the manufacturing costs can be decreased by up to 64% compared to transient transfection [7].

Moreover, transitioning from plasmid-based transfection to a stable producer cell line for rAAV manufacturing can offer advantages in scalability, consistency, and regulatory compliance. Large scale plasmid transfection requires substantial quantities of GMP-grade plasmid DNA and transfection reagents, which adds process complexity. By eliminating these components, the process reduces the introduction

of raw materials variables and lowers the risk of plasmid-related contaminants, such as residual host or plasmid DNA, leading to potential improvements in product purity and safety.

Stable producer systems can also help reduce batch-to-batch variability by ensuring consistent expression of Rep, Cap, helper, and GOI. Moreover, they would be expected to simplify technology transfer (process and supply chain complexity) and ultimately facilitate regulatory approval, as these systems are easier to characterize, validate, and scale across manufacturing sites. Fewer process variables make stable platforms more suitable for late-stage development and commercial production of rAAV-based gene therapies.

The purpose of this document is to summarize a comparability framework to support the transition from transient cell line to producer cell line to manufacture AAV during clinical development of AAV-based therapies.

The scope of the framework includes the following assumptions:

- Stable producer cell lines will incorporate all required components for rAAV production (transgene flanked by the inverted terminal repeats (ITR), rep genes, viral proteins genes, and helper functions). Integration frequency and site within the genome are assumed to be random;
- No change in sequence of the rep, cap, or transgene (ITR to ITR) will occur;
- Stable producer cell line clones will be isolated and screened for desired properties prior to selection of the final pre-Master Cell Bank (MCB; e.g., copy number, AAV productivity, AAV quality, etc.);
- Regulatory risks are gauged against US FDA requirements;
- The manufacturing processes and in-process testing for transient HEK293 and stable producer cell line systems have no significant differences, except where required for differences between transient transfection versus induction.

The assessment considered three categories of risks for sponsors seeking to change their manufacturing process from transient transfection to a stable producer cell line: Technical, Regulatory and Business risks.

RECOMMENDATIONS

Stable producer cell line process change risk assessment

Starting a program with a stable producer cell line at the pre-clinical stage generally poses a low risk to comparability, provided that the stable cell line has been used for Investigational New Drug (IND)-enabling non-clinical studies. Transitioning from a transient to a producer cell line early in clinical development carries a risk and requires thorough comparability data focused on safety and manufacturing consistency. Switching during late-stage clinical or commercial development involves a significant higher risk and demands extensive comparability data.

- Technical risks:
  - Product quality changes (e.g., changes in product safety and efficacy due to changes to critical quality attributes)
  - Process changes (e.g., potential impact on unit operations, process parameters, hold times, etc.)
  - Potential impact on analytical methods and assay qualification/validation status

- Regulatory risks:
  - Safety and sourcing of raw materials
  - Impacts on clinical and commercial regulatory submissions
  - Requirement for comparability studies
  - If comparability cannot be successfully demonstrated and accepted by the regulatory authority:
    - Clinical data from early-stage study cannot be pooled with clinical data from late study to support licensure
    - Potential need to submit a new IND or generate additional clinical data if the regulatory authority determines that the drug product is not comparable
- Business risks:
  - Manufacturing cost of goods related to operational, material, equipment, and facilities changes
  - Potential delays to clinical development (e.g., availability of clinical batches)
  - Timeline to create and screen the top selected clones to identify the best producer cell line

Whatever the cell line (transient or stable), the MCB and Working Cell Banks (WCBs) should be generated under appropriate conditions and tested according to relevant regulatory guideline [8].

Table 1 shows an example of a WCB testing panel. The full panel of tests will depend on what has been done for the MCB.

The long-term stability of MCB is typically performed on an annual basis after the first year. However, an increase in the frequency during the first year (e.g., months 3, 6, 12) is recommended to demonstrate that there is no change in critical attributes.

Stability testing of the MCB/WCB should be performed in accordance with the applicable regulatory agency guidance [9], and additional test methods specific to a stable cell line should be added, including, at a minimum, those listed in Table 2 below.

Genetic stability evaluation

Evaluation of genetic stability of each custom producer cell line MCB is required to demonstrate that there is no change to the integrated sequences. Cell genetic stability is evaluated through a representative number of cell passages, greater than or equal to the maximum number of passages that would occur in the typical manufacturing process. End-of-production cells (EOPC) testing may include testing of Critical Quality Attributes (CQA)s such as those listed in the stability testing panel in addition to the following methods at both release and EOPC passage:

- Number of copies per inserted gene (cap, rep, helper, therapeutic transgene)
- Karyology (G-banding)
- Insertion site analysis (NGS-based)

Leakiness evaluation

Here we define 'leakiness' of stable or producer cell lines as the amount of production of inducible proteins during standard growth conditions, when no protein expression is intended to occur. Stable cell lines can potentially have some level of leakiness, even if this will have been reduced to a minimum by the cell line provider. The concern is usually not at the transgene level, as the transgene might not be inducible (in most systems it's not). If the transgene is under a cell specific promoter, it might not be expressed. However, it can be under a constitutive promoter. The levels of transgene

expression will be relevant to determine if the transgene is toxic to the cells, or in case of toxic transgenes to make sure these are not expressed in the cell when not desired (i.e., during cell growth).

The main concern will usually be for the Rep, Helper and Capsid genes, as their expression is toxic to the cells, and could lead to production of a small amount of unintended AAV particles production when not desired during the growth phase, prior to induction. The expression of these proteins should therefore be tested in the presence and absence of induction, and the risk of any background leakiness should be considered and managed.

TABLE 1

WCB testing panel.

Test	Method
Identity (cells)	DNA fingerprinting or STR analysis
Identity (product-specific)	PCR-based identity of transgene, NGS, or similar
Cell viability	Exclusion dye, Flow cytometry
In vitro virus test	14 or 28 days in vitro cell-based detection method using 3 detector cell lines (typically, MRC5, Vero, Hela)
Sterility	USP <71>
Mycoplasma	USP <63>

NGS: next generation sequencing. PCR: polymerase chain reaction. STR: short tandem repeat. USP: United States Pharmacopeia.

TABLE 2

MCB stability testing of producer cell lines.

Cell viability	Automated cell counter (via dye exclusion)	
Cell growth rate	Automated cell counter	3, 6, 12 months and annually thereafter
Rep, Cap, helper, transgene expression with induction agent	PCR based (and/or ELISA, flow cytometry, Western blot, etc.)	
Rep, Cap, helper, transgene expression without induction agent	PCR based (and/or ELISA, flow cytometry, Western blot, etc.)	
Titer (vg/mL)	qPCR or ddPCR (and/or capsid ELISA, spectrophotometry, etc.)	
Empty:full capsid ratio	AUC, cryoTEM, CDMS, MP, etc.	
Sterility*	USP <71>	
Mycoplasma*	USP <63>	
Container closure integrity testing	Vacuum decay, dye penetration, etc.	

*Sterility and mycoplasma testing is not required at 3 and 6 months and it is recommended to be performed on an annual basis. AUC: analytical ultracentrifugation. CDMS: charge detection mass spectrometry. cryoTEM: cryogenic transmission electron microscopy. ddPCR: digital droplet polymerase chain reaction. MP: mass photometry. NGS: next generation sequencing. PCR: polymerase chain reaction. qPCR: quantitative polymerase chain reaction. USP: United States Pharmacopeia. vg: vector genome.

For late-stage process characterization, sponsors may also perform stress studies in the absence of induction agent to evaluate the full possible range of process parameters (temperature, cell density, glucose level, CO_2, cell passages, etc.) to determine impact to leakiness and relevant CQAs.

Recommendations for additional characterization of stable producer cell lines are outlined in Table 3.

Developing a comparability framework and meeting FDA expectations

Comparability is essential when a sponsor seeks to implement a manufacturing change that may negatively impact product quality. Comparability study design should consider whether or not the sponsor intends to pool clinical data from pre- and post-change batches to support a BLA. Efficacy data for cell and gene therapy products typically derives from Phase 2 and Phase 3 studies but can also come from Phase 1 studies in rare and ultra-rare gene therapy programs. The amount of supporting information, analytical evaluation, statistics, and discussion needed generally increases with the potential impact on quality and progression in clinical and product development. Ultimately, the risk and study design for a change to a producer cell line depends on the product and clinical development context. In 2023, the FDA released a draft guidance document on Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products [10].

Figure 2 outlines a decision tree that can be used to inform when comparability study will be needed to support a manufacturing change. The pause symbol indicates that if the changes are not supported by a comparability protocol and study appropriately powered to support the change, a clinical hold may be given to the sponsor. Additional details on the approach for early and late phase manufacture are described in the 'Early phase comparability framework' and the 'Late-stage comparability framework' sections below.

TABLE 3

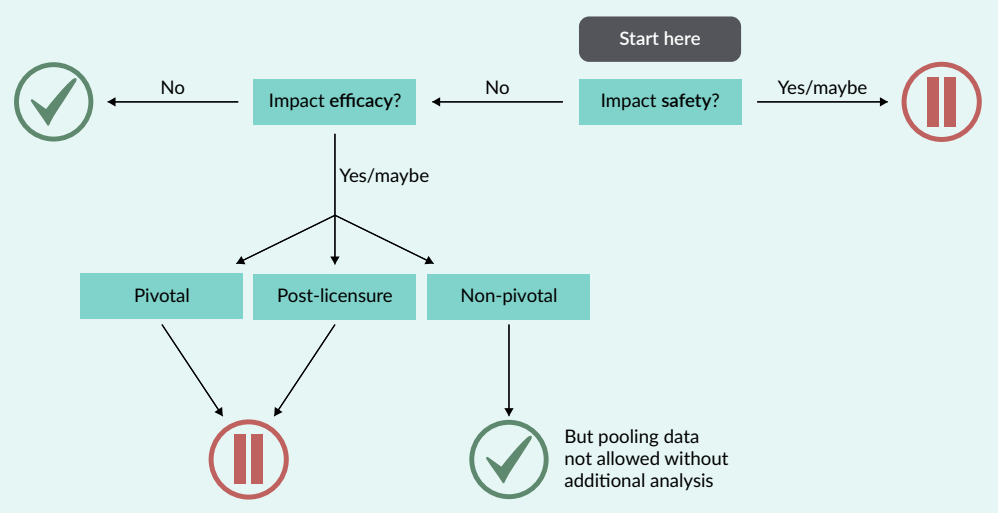
Additional characterization tests for stable producer cell lines.

Test	Method
*Rep, Cap, helper, transgene expression with induction agent	PCR based (and/or ELISA, flow cytometry, Western blot, etc.)
Rep, Cap, helper, transgene expression without induction agent	PCR based (and/or ELISA, flow cytometry, Western blot, etc.)
Titer (vg/mL)	qPCR or ddPCR (and/or capsid ELISA, spectrophotometry, etc)
Empty:full capsid ratio	AUC, cryoTEM, CDMS, MP, etc.
Function (potency)	Product-dependent (as transduction assay, *in vivo* potency, qPCR)
Packaged sequence integrity and heterogeneity	NGS
Capsid composition (VP1/VP2/VP3 ratios)	SDS page

AUC: analytical ultracentrifugation. CDMS: charge detection mass spectrometry. cryoTEM: cryogenic transmission electron microscopy. ddPCR: digital droplet polymerase chain reaction. MP: mass photometry. NGS: next generation sequencing. PCR: polymerase chain reaction. qPCR: quantitative polymerase chain reaction. vg: vector genome.

FIGURE 2

Clinical study phase-dependent comparability decision tree.



Risk assessment methodology and comparability study design for all clinical phases

The potential impact of the transition from transient to stable cell lines on drug product CQAs and stability should be evaluated regardless of the drug development phase. As defined by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, risk can be described using qualitative terms such as 'high/substantial', 'medium/moderate', or 'low/minor', which need to be clearly defined [11–14]. It can also be quantified using a 'risk score' through risk ranking. Sponsors should provide a detailed risk assessment to evaluate detectability, probability, and severity for each CQA (Table 4). The results from this risk assessment should inform the design of the comparability study.

A risk priority number (RPN) is determined for each effect by multiplying the severity, occurrence, and detectability risk scores. The higher the RPN, the greater the risk [11–14].

A risk mitigation strategy is established for each risk identified. When recommended hazard controls and mitigations are implemented, risk levels are generally reduced, and a post-mitigation Risk Priority Number (RPN) is calculated. It is possible that a high risk can't be reduced immediately and will require time to put in place the necessary mitigations. In general, the severity of any failure mode remains unchanged. However, there have been examples of risk severity being reduced as more scientific experience and understanding were gained.

Attributes may be categorized into tiers (high, medium, low or tier 1–3), and acceptance criteria for comparability should be set aligning with the criticality of the attribute. An example of a tier-based acceptance criterion is as follows:

- Tier 1: high-risk attributes may be evaluated using the most stringent approach (e.g., stringent quality range or equivalence range)
- Tier 2: medium-risk attributes may be evaluated using a moderately stringent quality range approach (e.g., mean ± 3STD) or release specifications

TABLE 4

Severity, probability, and detectability for process change risk assessment.

	Risk assessment
Severity (S)	Severity of the harm; if a failure were to occur, what effect would that failure have on the product quality (CQA) and on the patient safety (if any)?
Probability (P)	How likely is it for a particular failure/harm to occur (probability of occurrence)?
Detectability (D)	What mechanisms are in place (if any) to detect a failure if it were to occur?

- Tier 3: low-risk attributes may be evaluated against release specifications or for information only (FIO)

Method equivalence

If the pre- and post-change lots are tested using different methods, equipment, or at different sites, method equivalence/bridging data for the comparability study may be necessary. The comparability protocol/report should include a table summarizing methods and results from studies supporting changes to methods or testing sites. Some methods for early-stage changes should be qualified and validated, such as dosing assay (titer) and safety assays if non-compendial (e.g., non USP sterility). Methods for late-stage changes generally must be qualified or validated (depending on the method and stage of development), especially if the change occurs post-licensure or after pivotal studies but before BLA submission.

Results & report summary

A summary table should include test results for each lot, summary statistics, release specifications (if applicable), comparability acceptance criteria. Calculated results should be compared against the comparability acceptance criteria and release specifications using appropriate statistical methodologies (e.g., confidence interval, tolerance interval, two-one sided t-test), and the conclusion (comparable yes/no).

The visual presentation of data should include a dot plot for each quantitative CQA, regardless of the acceptance criteria approach. The dot plot should indicate the release specification limit as well as the comparability acceptance criteria (if different from the release specification). If an equivalence range is used, provide bar-like plots that include the equivalence range and confidence interval for each relevant CQA.

For the discussion of results in the comparability report, the conclusions from the study should be clearly stated and any divergence from the acceptance criteria should be justified. If additional data is available to strengthen your conclusion, include it in this section.

Early phase comparability framework

The change from transient transfection to stable producer line is considered a significant change, and the product quality should be assessed before and after the change to ensure that there is no impact to safety or efficacy of the product (Table 5). Therefore, this change would need to be supported by comparability analysis with a focus on safety at early-stage development (i.e., prior to initiation of studies intended to provide evidence of efficacy).

Late-stage comparability framework

Like in early stage, the change from transient transfection to stable producer line is considered a significant change, and the product quality should be assessed before

TABLE 5

Acceptance criteria for early phase comparability.

Tier	Stringency	Comparability acceptance criteria approach
Tier 1	+++	Quality range based on clinical experience or manufacturing experience (e.g., $Mean^{Ref}$ ±3*STD*, CQA range shown to be safe and effective in patients)
Tier 2	++	Release specification
Tier 3	+	FIO

FIO: for information only.

TABLE 6

Acceptance criteria for late-stage comparability.

Tier	Stringency	Comparability acceptance criteria approach
Tier 1	+++	Equivalence range (TOST) or quality range based on clinical experience
Tier 2	++	Release specification or quality range based on manufacturing experience (e.g., $mean^{Ref}$ ±3*STD*)
Tier 3	+	Release specifications, or FIO for characterization attributes

FIO: for information only. TOST: two one-sided T-tests.

and after the change to ensure that there is no impact to safety or efficacy of the product (Table 6). Therefore, this change would need to be supported by comparability analysis with focus on both safety and effectiveness at late-stage development (i.e., after initiation of studies intended to provide evidence of efficacy). Sponsors should evaluate a significant enough number of lots pre and post change, to ensure sufficient statistical power is achieved. The use of a smaller number of lots than suggested by the power analysis is a risk to take and will require regulatory authority's flexibility. A scale-down model may be used if the model is qualified and shown to be representative of the full-scale process.

Implementing a change to a producer cell line during late-stage clinical trials does inherently carry more risk but may be mitigated with a comprehensive and well-powered study design that can detect statistically significant changes in CQAs. This will likely require at least several post change lots, which will require significant investment in resources and cost and timeline delays to generate enough data to support this significant change.

CONCLUSIONS & RECOMMENDATIONS

Transitioning from transient transfection to a producer cell line during pre-clinical or early clinical development poses fewer risks than doing so in pivotal or late clinical phases. A comprehensive risk assessment should guide the rigor for each attribute affecting AAV product safety and efficacy. Using the risk assessment's output to inform the design of the comparability study should ensure the appropriate rigor in case of late clinical phase changes.

In summary, as part of the process for the regulatory path transitioning from transient to stable cell lines for rAAV

manufacturing, it is recommended that a technical assessment including functional testing of rAAV expression and bioactivity, long-term stability studies confirming consistent performance across multiple passages, and comparability analyses showing equivalence to reference material in critical quality attributes is undertaken. The collective results should support the robustness and reliability of the cell line for future development, scale-up and AAV manufacturing.

Finally, it is advisable to establish a proactive communication strategy with regulatory authorities. For example, for drug development in the USA, FDA recommends sponsors to discuss in advance significant manufacturing changes with Center for Biologics Evaluation and Research (CBER), especially if the manufacturing change is to be implemented during later stages of the rAAV development phase [10]. Sponsors can request FDA to comment on information submitted in an IND amendment or BLA, or can request a formal meeting. A variety of meeting types (e.g., B, B end of phase, C or D) may be suitable for this purpose, depending on sponsor circumstances [15].

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CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

VIRAL VECTOR MANUFACTURING AND PLATFORM EVOLUTION

SPOTLIGHT

INNOVATOR INSIGHT

How far have AAV platforms taken us? Lessons from a CDMO on accelerating time-to-clinic

Ify Iwuchukwu

Time to clinic remains a critical success factor for both emerging biotech's and established pharmaceutical organisations in the cell and gene therapy (CGT) sector. Driven by competitive pressures and milestone-based funding models, alongside a commitment to deliver life-changing treatments to patients in need, the urgency to advance novel therapies into the clinic has never been greater. In response, a range of adeno-associated virus (AAV) platform technologies have emerged, each aiming to streamline development and reduce timelines. This article explores how far platform-based approaches can go in accelerating time to clinic. Drawing on real-world insights from a contract development and manufacturing organisation (CDMO), we examine key process and technology innovations, the pivotal role of analytics in expediting development, and the future evolution of AAV platforms.

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As the field of gene therapy continues to advance, the urgency to bring safe and effective treatments to patients faster is intensifying. However, the path from preclinical development to first-in-human trials remains fraught with complex technical, regulatory, and logistical hurdles. This is especially true for adeno-associated virus (AAV)-based therapies, where serotype variability can significantly delay progress. In response, platform technologies have emerged as a strategic solution. The first commercial AAV platform technologies entered the market almost a decade ago, with REGENXBIO's NAV® Technology Platform being a notable example [1]. This approach was soon embraced by leading CDMOs such as Catalent, ThermoFisher, and OXB, who recognised the potential of platforms to streamline development of viral vectors.

Over the past decade, platform technologies have played a pivotal role in advancing gene therapies from preclinical stages through to commercialisation. This approach should be leveraged by drug developers bringing in multiple genomic drugs to their pipeline. Impact on timelines

has been significant; for example, OXB's inAAVate™ platform can now release a GMP batch in as little as 7 months from feasibility study. With such tangible improvements already realised, continued platform innovation is expected to drive further acceleration in time to clinic in the years ahead.

THE UNIVERSAL CHALLENGE FOR PLATFORM TECHNOLOGIES: BALANCING SPEED WITH CONSISTENCY IN A COMPLEX THERAPEUTIC LANDSCAPE

While platform technologies are often lauded for their ability to accelerate timelines, speed alone is not sufficient. In the context of an increasingly diverse pipeline of gene therapy candidates, true progress requires both rapid development and consistent, high-quality output in parallel.

To meaningfully shorten time to clinic, platform technologies must deliver reliable performance in key quality attributes, such as vector titer, purity, and potency, across scales and serotypes. Achieving this consistency demands highly robust upstream and downstream processes that are not only scalable, but also broadly applicable across multiple AAV serotypes with minimal tweaking. Crucially, these processes must function effectively without the need for time-consuming development of each new program.

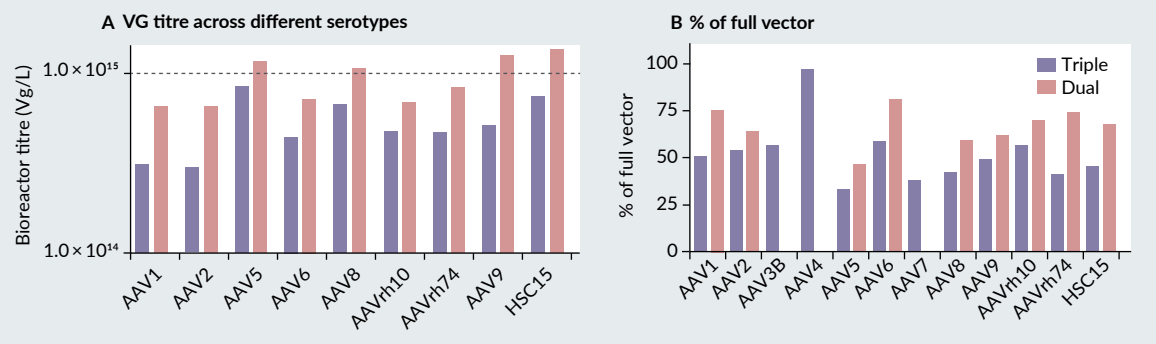
UPSTREAM INNOVATIONS: HIGH TITERS WITHOUT THE TRADE-OFFS

A major technological advancement in the upstream development of AAV platform technologies is the shift from traditional triple-plasmid transfection to dual-plasmid systems. This new innovation has significantly simplified and streamlined the transfection process by reducing the number of plasmids required, thereby lowering the risk of variability in plasmid ratios and enhancing batch-to-batch consistency. So far, dual-plasmid platforms have been announced by OXB with the InAAVate platform and Ascend Advanced Therapies with the EpyQ™ platform. OXB has spent nearly a decade refining and optimizing their upstream process to deliver high titer and improved packaging efficiency. Construct design, transfection density, DNA amounts, plasmid ratio as well as process and transfection parameters were explored. This was then optimized for multiple serotypes and gene-of -interest to establish what they call a global optimum for different vector types. This eliminates the need for upstream development each time a new construct is introduced to the pipeline. Furthermore, dual-plasmid systems have demonstrated improved productivity and packaging efficiency across multiple AAV serotypes and genome constructs (Figure 1), making them an increasingly preferred choice for platform-based manufacturing where reliability and reproducibility are critical [2]. The dual plasmid system consists of the gene-of-interest and Rep/Cap in our plasmid resulting in transfection with 2-plasmid instead of the traditional 3-plasmid transient transfection for AAV production.

In parallel, ongoing optimization of process parameters has also driven significant gains in upstream performance. At OXB, the inAAVate platform has achieved a 3.2-fold increase in vector titers through targeted refinement of transfection reagents (TFX), plasmid ratios, and the introduction of novel process additives. These enhancements contribute to greater consistency and output across serotypes. Figure 2 illustrates the impact of these optimizations, comparing titers across multiple AAV serotypes at the 2 L bioreactor scale before and after the implementation of these process improvements [3].

FIGURE 1

(A) A comparison of viral vector titers across multiple serotypes for triple versus dual plasmid transfection. (B) Improved packaging efficiency for dual plasmid for different serotypes demonstrated by analytical ultracentrifugation percentage full from affinity purified vectors.



DOWNSTREAM ROBUSTNESS: ELIMINATING DEVELOPMENT OF AEX CHROMATOGRAPHY FOR DIFFERENT SEROTYPES

Downstream processing remains one of the most resource-intensive stages of AAV manufacturing, where significant yield losses can occur. Historically, many of the purification technologies applied in gene therapy manufacturing were adapted from processes originally developed for traditional biologics, rather than being designed specifically for viral vectors. This has posed unique challenges, particularly for adeno-associated virus (AAV), where an expanding repertoire of naturally occurring and engineered serotypes demands broad process compatibility.

To address this complexity, platform developers have focused on building flexible, adaptable purification solutions. At OXB, the inAAVate platform incorporates a strategically designed downstream process developed through comprehensive Design of Experiments (DoE). This approach has enabled the creation of a robust, multi-serotype toolbox that minimises the need for program-specific development, and in turn reduces development times. It is well known that different gene-of-interest and different serotypes results in variation in charge and conductivity of a vector that is crucial for AAV AEX purification process.

One of the key outcomes of this optimization is the definition of an optimal anion exchange (AEX) chromatography operational space, applicable across multiple AAV serotypes with minimal tweaking of process parameters. Most serotypes tested within this defined range have consistently demonstrated >90% full capsid content, significantly reducing development timelines for new vector programs. Importantly, this high level of performance is achieved regardless of whether the upstream process uses dual- or triple-plasmid transfection systems, highlighting the modularity and scalability of the platform.

Figure 3 illustrates how the pre-defined AEX operational window supports high full-to-empty capsid ratios across six AAV serotypes, underscoring the value of a platform-based approach in accelerating development while maintaining product quality.

Formulation is a key element in AAV development that is often underappreciated, yet it plays a vital role in maintaining vector stability, preserving biological activity, and ensuring therapeutic potency. Within a robust AAV platform, an optimized formulation strategy can enhance cold chain stability, extend shelf life, and support flexibility across multiple routes

FIGURE 2

A comparison of viral vector titers across multiple serotypes before and after process parameter optimizations using the inAAVate platform.

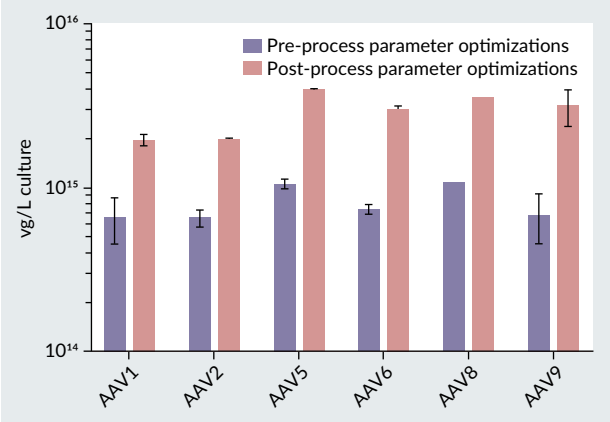
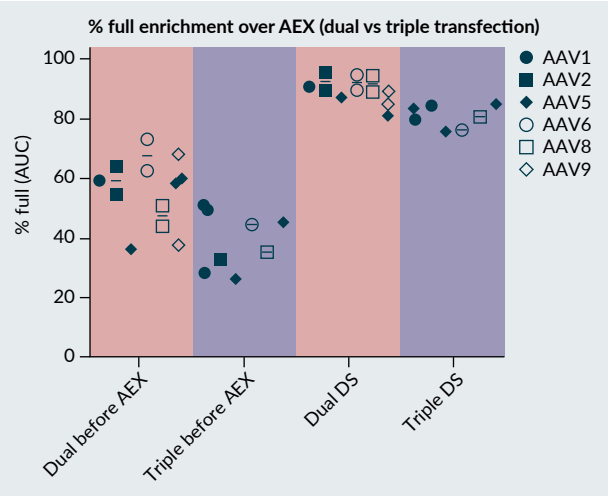


FIGURE 3

Percentage full capsids obtained after AEX for multiple serotypes. Differences in percentage full for triple and dual are as a result of improved packaging in dual plasmid transfection system. Furthermore, variation observed in drug substance percentage full are as a result of differences in construct design and gene-of-interest.



of administration. OXB's formulation have been tested in wild type serotypes like AAV1, AAV2, AAV5, AAV8 and in multiple novel or engineered capsids. Figure 4 demonstrates the stability that can be achieved with an optimized formulation over an 18-month period across both vector genome titer and relative potency. A drop in potency for accelerated stability but no changes in vector genome titer demonstrates that vector genome titer is not a stability indicating assay.

CDMOs and other developers that prioritise formulation development, recognising its downstream impact on product handling, storage, and clinical performance, provide a significant advantage to their partners. This strategic foresight is essential for ensuring long-term success beyond initial manufacturing.

Downstream example: this example demonstrates how downstream robustness allowed for the enhanced recovery of a challenging engineered capsid. A client approached OXB after encountering consistent challenges with an engineered AAV capsid, achieving no or low product recovery and no more than 35% full vector despite working with three different CDMOs. With preclinical animal studies looming, the need for a rapid and reliable solution was critical. The underlying challenge stemmed from the atypical charge and conductivity profile of the engineered capsid, which had fallen outside the narrow anion exchange (AEX) operating ranges used in previous attempts, resulting in poor recovery rates.

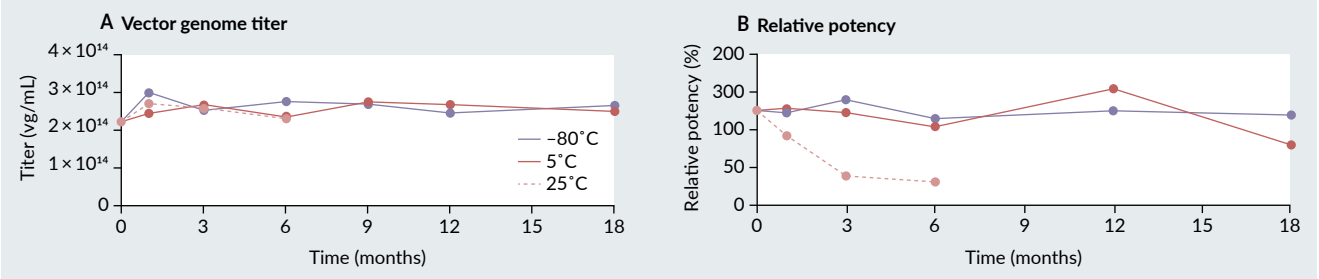
Leveraging the inAAVate platform's flexible AEX operational space, OXB was able to fine-tune process conditions to accommodate the unique properties of the vector. Through isocratic elution, the team successfully achieved 92% full capsids in addition to improved recovery within just 6 weeks. This rapid turnaround enabled the client to meet their timelines and advance the program into IND-enabling studies.

ANALYTICS AS AN ACCELERATOR: NOT AN AFTERTHOUGHT

In the early days of AAV platform development, analytics often represented the

FIGURE 4

Stability of AAV9 vector over an 18-month period at -80 °C, 5 °C, and 25 °C demonstrated for vector genome titer and relative potency.



primary bottleneck on the path from preclinical studies to first-in-human trials. Despite advances in upstream and downstream processing, timelines remained constrained by lengthy assay development, particularly for functional potency testing, which could take up to 18 months or longer to complete. For too long, analytics was treated as an afterthought, limiting the true potential of platform technologies to compress development timelines.

This paradigm has shifted. By integrating early-phase product characterisation into the front end of development, platform-based programs can now identify and address critical quality issues before they become costly downstream delays. For example, high-resolution techniques such as analytical ultracentrifugation (AUC) allow for the early detection of subtle yet impactful issues like genome truncations or minor overpacking. Proactively resolving these challenges avoids the need for time-consuming re-optimization later in development [4]. Today, key assays are increasingly pre-qualified as part of the platform itself in QC-ready format to reduce tech transfer timelines and accelerate time to GMP release. Additionally, product specific assays leverage a platform approach enabling faster and more streamlined development. The inAAVate platform, for example, allows genome titer and gene expression assays to be established and operational within just 2 months, significantly reducing time to qualify release assays, and in turn reducing time to clinic. Table 1 outlines list of platform assays and outsourced assays.

OXB also is continuously advancing their analytical toolkit one example being developing a SYBR™Gold ejection assay that is fast, easy and cheap to run, as a surrogate for potency assays. This gives early actionable insights into vector potency and post translational modification, enabling faster, more informed decisions without relying on lengthy LC-MS timelines or waiting for potency assay development. Early potency readouts not only help shape the optimal development strategy but also offer clients early visibility into predicted product performance and cost per dose as they advance toward clinical trials.

Figure 5 demonstrates the close comparability of the results from the rapid SYBR Gold injection assay with the final measured potency using a model built from the OXB database, reinforcing the value of early analytical insight in accelerating time to clinic.

Analytics example: this example demonstrates how accelerated potency assay development enables rapid time to IND. To meet a critical IND filing deadline, one client required a fully qualified functional potency assay within just 6 months, a timeline that typically extends to 12 months or more across the industry.

TABLE 1

List of 90%+ analytical method performed in-house to accelerate batch release.

Method type	Method
Capsid-specific	Capsid titer
	Capsid AAV identity
	Purity
	Aggregation
	Peptide mapping/PTMs
Product-specific	VG titer
	Infectivity
	Transgene expression (%RGE)
	Potency (%RP)
Host cell impurities	% empty, % partial, and % full
	Host cell DNA
	Host cell protein
Product- and process-related impurities	10+ residual methods
Safety	Formulation contents
	Adventitious viruses
	Replication-competent AAV
	Sterility
	Mycoplasma
	Bioburden
	Endotoxin

Blue: platform with some product-specific development. Green: full development. Purple: outsourced. White: platform method.

By combining a robust, phase-appropriate development strategy with a fully cross-trained Analytical Development (AD) and Quality Control (QC) Bioassay team of scientists, OXB was able to significantly compress the GMP transfer and qualification process.

As a result, the qualified potency assay was delivered on schedule, achieving a recovery of 103%, with an intermediate precision of 7% coefficient of variation (CV) and repeatability of 9% CV. This case highlights the power of integrated analytical planning and operational agility in enabling accelerated clinical development.

FUTURE OUTLOOK: HOW FAR CAN PLATFORM TECHNOLOGIES TAKE US?

There is no question that over the past decade platform technologies have evolved significantly, from early-stage concepts into mature, reliable frameworks that accelerate gene therapy development across the industry. They've reduced time to clinic from years to months, improved yields and consistency, and enabled greater standardisation across complex programs. Looking ahead, it is clear that further innovation will continue to shape the capabilities of

platform-based approaches. We anticipate several key advancements on the horizon:

- Purpose-built downstream materials: currently, many downstream purification solutions for AAV rely on tools originally designed for monoclonal antibodies or other biologics. In the coming years, we expect to see a wave of reagents, membranes, and resins specifically engineered for AAV purification. These purpose-designed materials, tailored to the biophysical characteristics of viral vectors, will increase recovery, reduce process losses, and simplify the purification of increasingly diverse capsids, including engineered and novel serotypes;

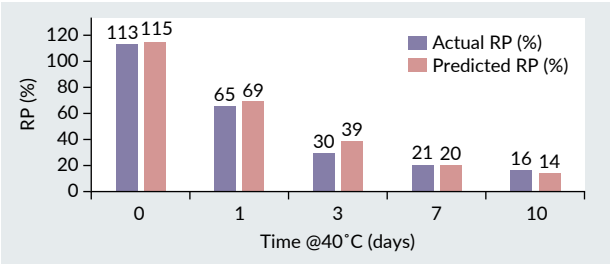
- Next-generation transfection technologies: the shift from triple to dual plasmid transfection has already improved scalability and consistency. The next leap may come in the form of plasmid-free transfection systems, synthetic biology-based expression tools, or cell line engineering strategies that eliminate the need for transient transfection altogether. These innovations could radically enhance reproducibility and reduce costs, moving the field closer to true industrialization;

- Integrated, real-time analytics: the future of platform technologies lies in tighter integration between manufacturing and analytics. Real-time, in-line monitoring tools, such as multi-attribute methods (MAM) or rapid qPCR alternatives could enable adaptive control strategies where batches are optimized in real-time, not after the fact. This would mark a significant shift from reactive quality control to proactive quality assurance, further reducing variability and development timelines;

- Modular platforms for next-gen modalities: while current AAV platforms are highly effective for single-use programs, the emergence of more complex therapeutic modalities such as dual-vector systems, capsid libraries, or cell-targeted gene therapies will require modular, adaptable platforms. These systems will need to accommodate variable payload sizes, serotype switching, and custom purification schemes without resetting development timelines, unlocking even more flexibility for advanced gene therapy pipelines.

FIGURE 5

Model predicted potency vs actual potency of an AAV vector over time for AAV accelerated stability studies at 40 °C.



One thing is clear: as gene therapy continues its journey toward full industrialisation, CDMO platform technologies offer the most effective path forward—delivering more therapies, for broader indications, and reaching patients earlier in their treatment pathway than ever before.

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VIRAL VECTOR MANUFACTURING AND PLATFORM EVOLUTION

SPOTLIGHT

EXPERT INSIGHT

Impurities in viral-based therapeutics: mastering residual hcDNA: a harmonized approach for AAV gene therapies

Reshma Sundar, Xue Mi, Ashton Lavoie, Ane Quesada Ganuza, Suna Lipp, Marija Brgles, Denise Teber, Steven Henry, Ashoka Maddur, Pepijn Burgers, Beata Adamiak, Victor Chen, Roland Pach, Haiqing Yu, Kate Groves, Nic Preyat, Parag Kumthekar, Simon Walker, Yong-Syu Lee*, Hunter Walker*, Qirong Wu*, Spyridon Gerontas*, Ying Zhang*, Sophie Cheverry*, and Ishtiaq Khaliq*

AAV gene therapy products are produced in continuous cell lines which can leave host cell DNA (hcDNA) in the final product, posing potential risks to patient safety. Regulatory guidelines keep evolving to address these risks, recommending limits on hcDNA levels and fragment sizes. To better control hcDNA impurities manufacturers rely on risk assessments, characterisation and quantitation, and removal strategies.

Based on survey insights, the BioPhorum workstream highlights differences in hcDNA reporting across the industry, proposes a standardized reporting approach by normalizing hcDNA levels to AAV genomes (vg), establishing a reference range for vg-normalized hcDNA level in AAV manufacturing processes. This supports risk assessment and better comparison across gene therapy products.

Holistic manufacturing control strategies for hcDNA are outlined, including optimizing cell line selection, robust purification techniques, and nuclease treatments.

By adopting these harmonized reporting and control strategies, the industry can master hcDNA impurities, ensuring safer gene therapy products for patients.

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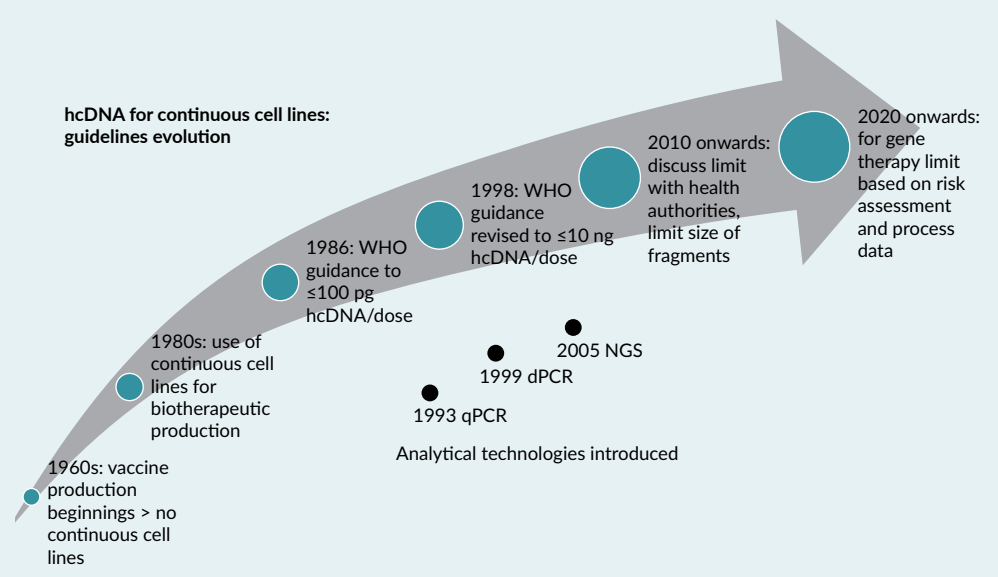
INTRODUCTION

Gene therapy products, particularly those utilizing viral vectors derived from AAV, lentivirus (LV), and adenovirus (AdV), are commonly produced in continuous cell lines (CCLs) (e.g., HEK293, HeLa, PER.C6 and Sf9) [1]. These host cells are often used for viral vector production, and residual host cell DNA (hcDNA) can be present as an impurity in the final product, posing potential risks to patient safety [2]. These risks are more prominent for viral vectors compared to other biologics such as antibodies since it is complex to separate the product (viral vector + therapeutic gene) from the vectors containing unwanted hcDNA during the purification process. Monitoring, minimizing and controlling hcDNA is essential to ensure product safety, quality, and regulatory compliance for manufactured viral vectors. Residual hcDNA can theoretically be associated with oncogenes, particularly for AAV produced with human-derived continuous cell lines [3]. Oncogenicity [4] risks arise when hcDNA contains intact genes which have the potential for the induction of cancer, i.e., oncogenes. Infectivity [5] risks arise when hcDNA contains viral genomes that could replicate, producing infectious agents. Given these risks, manufacturers must effectively control and quantify hcDNA.

The safety risks associated with hcDNA have led health authorities (HAs) to issue guidelines outlining acceptable levels of hcDNA per dose. These non-binding recommendations have evolved as scientific knowledge and biopharmaceutical capabilities have advanced (Figure 1), adapting to new insights and technologies in gene therapy production. The first recommendation on hcDNA value was given in 1986 by WHO stating that the risk associated with hcDNA in a product is negligible when the amount of hcDNA is 100 picograms (pg) or less per parenteral dose. This threshold was revised in 1998 to increase the acceptable limit to 10 nanograms (ng) per dose [6], reflecting improved understanding and assessment techniques. Another key recommendation is that hcDNA fragments should ideally be a median DNA size of 200 base pairs (bp) or lower to further minimize the risk of functional gene integration, which could contribute to genotoxicity [7]. It is important to note that guidelines were

FIGURE 1

Schematic of the evolution of hcDNA guidelines and analytical technologies.



recommendations only and always emphasized the importance of a risk assessment, taking into account specificities of the product, cell substrate, manufacturing process and size, and quantity and activity of hcDNA. In a guidance published in 2010, the US FDA advised to limit the amount of residual DNA for continuous non-tumorigenic cells to less than 10 ng/dose and the DNA size to below a median of 200 base pairs. Additionally, manufacturers are recommended to test products made in CCLs with known potential oncogenes for those specific oncogenes, e.g., 293T cells for adenovirus E1 and SV40 Large T antigen sequences, similarly products made in Hela cells for E6/E7 genes [8].

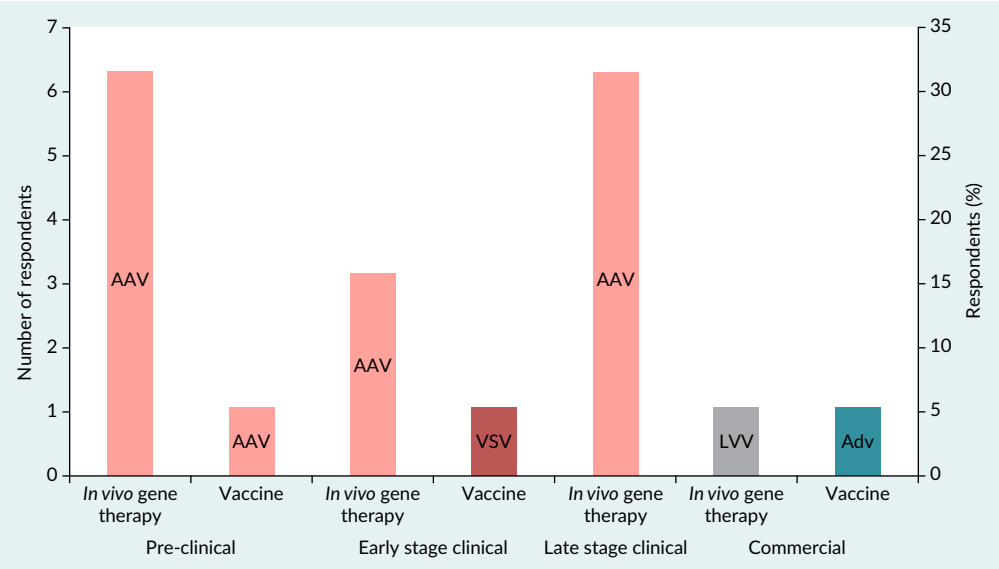
In recent guidance for gene therapies targeting neurodegenerative diseases, the FDA has purposefully not provided guidance on a specific hcDNA threshold, instead encouraging manufacturers to assess the characteristics of their production cell lines, particularly for sequences that might impact patient safety (such as presence of tumorigenic sequences) and limit residual hcDNA levels and DNA size [9]. This shift is in line with development of gene therapy domain and especially AAV-based products for which non-specific encapsidation of hcDNA is known to be resistant to enzymatic degradation [10] and also causing copurification due to similarity with the viral product, a phenomenon also acknowledged by the FDA [11]. This evolution in guidelines reflects an increasingly nuanced regulatory approach that considers the complexities of modern gene therapies and the specific characteristics of different viral vector production systems.

To support organizations developing viral-based therapeutics in a changing regulatory landscape, BioPhorum initiated the Impurities in Viral-Based Therapeutics workstream which comprised of 34 members from 20 organizations.

To assess the unmet need in this space, two benchmarking surveys were conducted to gather insights from individuals in the viral therapeutics industry. The first survey received 32 responses from 23 BioPhorum member companies and included questions relating to several process and product-related impurities. The second, hcDNA

FIGURE 2

Summary of demographic from second BioPhorum member survey focussed on hcDNA.



focused survey, received 22 responses from 16 BioPhorum member companies. It should be noted that some questions in the surveys were not mandatory, so the number of responses shown in figures, can be lower than total number of respondents. The surveyed companies primarily comprised AAV-focused entities (Figure 2). As a result, whilst the focus of this article is AAV gene therapy, many of the principles can be extrapolated to other viral vectors with similar cell lines and manufacturing processes.

hcDNA ANALYSIS METHODS

Accurate quantification of hcDNA is critical for effective monitoring and regulatory compliance. The biopharmaceutical industry primarily uses three methods: quantitative PCR (qPCR), digital PCR (dPCR, which includes ddPCR for the purposes of this article), and long-read next-generation sequencing (NGS) [12].

For hcDNA quantification various PCR targets are commonly employed, including sequences specific to E1A, E1B, 18S, and Alu genes [13,14]. These sequences differ by the occurrence frequency in the genome and this directly affects sensitivity of the hcDNA quantification method (Table 1).

qPCR

Real-time qPCR is a highly specific and sensitive test method to detect and quantitate residual hcDNA. According to BioPhorum's survey, qPCR is the preferred technique for hcDNA quantification (70% of 16 respondents). This assay quantifies hcDNA content by comparing Ct or Cp values from

TABLE 1

hcDNA method comparison summary.

Methodology	qPCR	dPCR	NGS
% of survey responses using for hcDNA quantification	70%	21%	9%
Characteristics	Quantitative (relative); targeted approach; amplification measured at each cycle; data is collected at exponential phase	Quantitative (absolute); targeted approach; measures amplification at the end of PCR cycles; data subjected to Poisson statistics	Quantitative (relative); facilitates global sequencing of DNA; short-read and long-read sequencing possible
Advantages	High throughput analysis for larger number of samples; larger dynamic range	High throughput analysis for larger number of samples; high reproducibility; less affected by PCR amplification efficiency; higher PCR inhibitor tolerance [15]; no standard curve required, potentially reducing assay cost; DNA extraction step can be avoided for some sample types, i.e., post-affinity downstream processing samples generally do not show significant matrix effect; high sensitivity	Long-read—confirmation of long complex sequences; short-read—high accuracy; no *a priori* knowledge of target DNA material required
Disadvantages	*A priori* knowledge of target DNA material required; lower sensitivity; high variability (affected by PCR efficiency, selection of DNA extraction process); prone to PCR inhibitors; sensitive to matrix interference, DNA extraction step essential; requires standard curve	*A priori* knowledge of target DNA material required; conversion required to report in ng/mL	Low throughput; high cost; advanced computational analysis required

the sample to a standard curve generated using genomic DNA (gDNA) from the target species.

Primary advantages of qPCR for hcDNA analysis are increased sensitivity in comparison to more traditional methods, like DNA-hybridization on a membrane or the Threshold® method and that it provides quantitative results directly as mass per reaction, eliminating the need for unit conversion [16]. Furthermore, the method is recognized in several pharmacopeial guidelines, which outline regulatory expectations for controls, PCR efficiency, sensitivity, and acceptable DNA recovery [17,18,19].

Method sensitivity is influenced by the selected target sequence; for instance, highly repetitive elements like Short Interspersed Nuclear Elements (SINE), e.g. aforementioned Alu sequences, can enhance sensitivity, but with regard to human residual DNA detection may increase the risk of contamination from environmental sources, such as the laboratory personnel, which may lead to a falsification of the sample result and increases the risk for positive results in negative controls.

BioPhorum's survey indicates that the 18S ribosomal RNA gene is among the most frequently used targets for human hcDNA quantification, aside from commercial kits (Figure 3).

Additionally, for hcDNA, fragment size analysis should be included, as larger fragments (median of >200 bp) may present an increased risk of infectivity or oncogenicity, whereas with a median of <200 bp there is a lower risk to have the size of a functional gene. This may be facilitated by using two primer/probe sets or by using an independent method like capillary gel electrophoresis.

Total DNA isolation prior to PCR

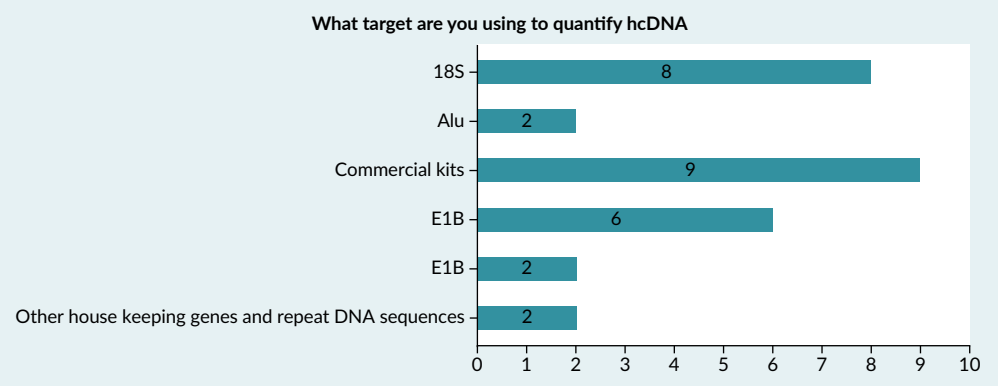
Before qPCR, gDNA extraction from viral capsids is a crucial step to DNA profiling as qPCR is sensitive to matrix interference. In general, DNA extraction involves capsid protein digestion (i.e. with proteinase K), and lysis so that encapsidated DNA is also extracted. The DNA extraction methods may have a significant impact on the qPCR results [20,21]. The downstream chromatography purification of AAV products is insufficient to remove excess plasmid DNA (pDNA) from vector samples.

dPCR

dPCR is an alternative technique for quantifying hcDNA. Unlike qPCR, which measures amplified DNA in bulk, dPCR partitions the PCR reaction mixture, including the sample, into thousands of smaller reactions. Each

FIGURE 3

Summary of responses to BioPhorum survey relating to the target used to quantify hcDNA.



"mini-PCR reaction" is analyzed individually, and the results are processed using Poisson statistics to determine the final copy number of the target gene. One of the key advantages of dPCR is its ability to provide absolute quantification [22] of DNA copy numbers without relying on a standard curve, a limitation of traditional qPCR. Despite these benefits, a recent BioPhorum survey found that only about 20% of 16 respondents currently use dPCR.

One drawback is the longer time required for assay performing and data collection, compared to qPCR. Another challenge is that the dPCR reporting unit, copy number, is not directly aligned with regulatory threshold, which is expressed in ng/dose. In the study presented by FDA scientists in 2021 FDA Science Forum demonstrating the high sensitivity and high matrix effect tolerance of dPCR for quantifying hcDNA in drugs produced in *E. coli* [23]. However, the study didn't discuss how to convert the copy number results to mass values of the hcDNA. Hussain and Bowers described a method to convert DNA copy numbers to mass (in femtograms) using a conversion factor based on the inverse slope of the standard curve [24,25]. Haiqing Yu further demonstrated that pre-treating gDNA with restriction enzymes as a reference material significantly improves the accessibility of reporter genes, resulting in a much more accurate conversion factor, going in line with previously published papers like Higashiyama *et al.*, 2023, who demonstrated the beneficial impact of restriction enzymes on dPCR results when genomic DNA is targeted [22,26]. However, in a recent BioPhorum survey, only 36% of 11 respondents reported using the restriction enzyme-pretreated DNA as the reference material.

NGS

NGS can be used to determine the presence of hcDNA in an AAV preparation by sequencing drug substance/product using both vector backbone together with gene of interest and host cell line genome libraries, allowing for identification of contaminating hcDNA sequences within the viral vector, providing a comprehensive analysis of purity and potential contaminants in the AAV sample.

When determining hcDNA content in AAV preparations, both Short-Read sequencing (SRS) and Long-Read sequencing (LRS) technologies have their advantages and limitations. SRS can be used for initial quantification, due to its high accuracy and throughput whereas LRS may then be used to resolve any ambiguous regions or verify complex rearrangements that are difficult to interpret with short reads alone.

Applying NGS data as an orthogonal analytical method, may aid optimisation of manufacturing process and comparability studies after making a process change. However, an assay validation for accuracy, precision, linearity, and limit of quantification will be necessary to establish a specific purpose of new method [12].

The set of recommendations on establishment of a GMP identity assay for recombinant AAV drug product using SRS have been described by recently published paper by BioPhorum [27]. The key parameters and practical advice to method development and validation of a transgene-specific

FIGURE 4

Survey responses indicating the range of total vector genomes (vg) administered per AAV product.

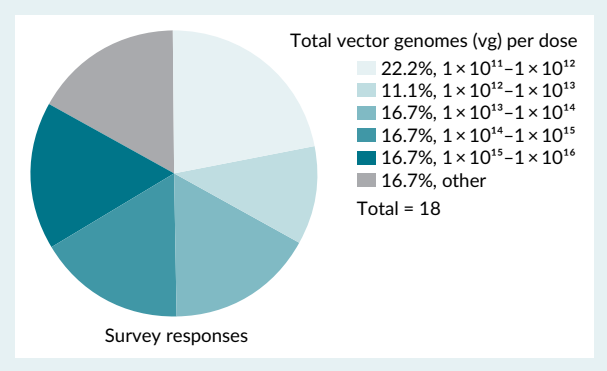
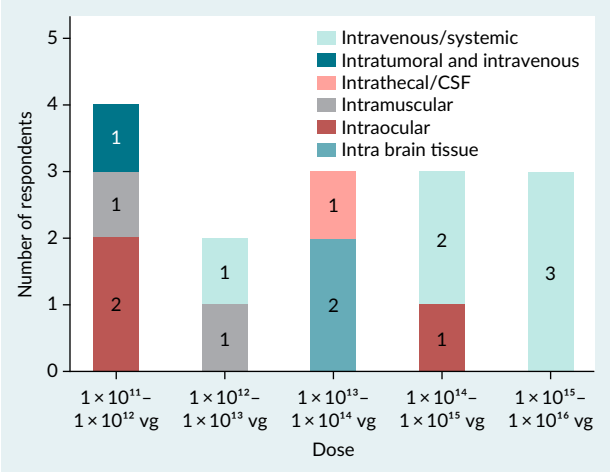


FIGURE 5

Survey responses indicating the range of total vector genomes (vg) administered per AAV product across different dose routes.



identity assay using NGS workflows in a GMP environment have been provided. Similar approach may be leveraged to method development and validation of LRS technologies for applications to characterize gene therapy products and DNA impurities.

CURRENT SITUATION

Range of dose levels and routes

AAV are administered through various routes depending on the disease type, patient age, and vector serotype-specific properties. It was found from the survey responses that the predominant dose route is intravenous/systemic (32%), followed by intra ocular (21%), intramuscular (11%), intra brain tissue (11%) and intrathecal/CSF (cerebrospinal fluid) (5% of 19 respondents).

The dosing for AAV can vary significantly, spanning from 1×10^{11} to 1×10^{16}vg [28], which is supported by the data gathered in the survey (Figure 4) and seen with commercial products [29,30,31,32,33,34]. About 22% of 18 respondents from the survey used 1×10^{11} to 1×10^{12} range of total vg to administer to the patients. This was found to be the major range of vg dose administered. It should be considered that systemic and larger organ dose routes offer potential for a higher total vg dose (Figure 5).

Levels of hcDNA

Reporting of hcDNA levels shows significant discrepancy through the industry. This discrepancy includes the units of reporting of hcDNA levels as demonstrated by the survey results (Figure 6A), which shows respondents use ng/mL, ng/ 10^{13}vg, ng/dose as well as other units (ng/10^{9} and ng/10^{12} vg) and 83% of 23 respondents made no further conversion

FIGURE 6

A: Survey responses indicating the units used for reporting hcDNA results. Those responses that fall under 'other' include ng/ 10^{9} and ng/10^{12} vg. B: Any other additional unit conversion used by respondents for reporting of hcDNA.

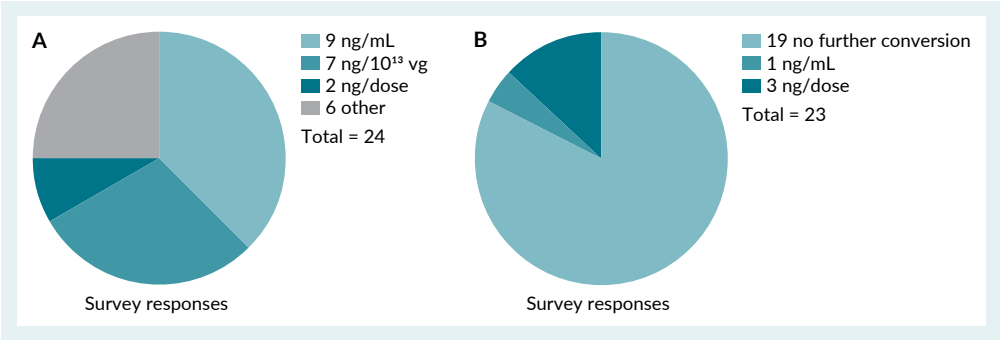
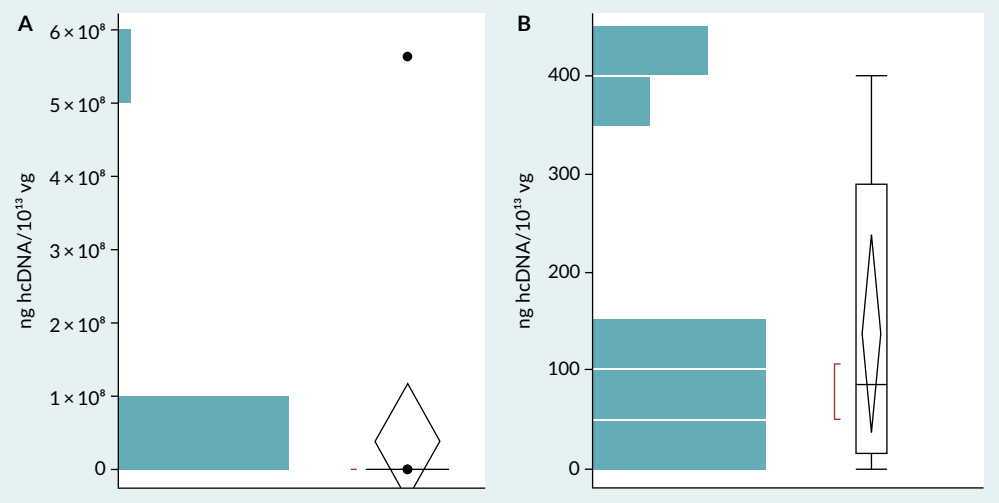


FIGURE 7

Box-and-whisker plots representing the dataset obtained from the second survey on hcDNA levels normalized by vg (ng/10^{13}vg). (A) Dataset before outlier exclusion. (B) Dataset after outlier exclusion.



of their reported results to other units (Figure 6B).

Survey respondents were asked to provide the range of hcDNA targeted in ng/dose in their respective drug products. 35% of 23 respondents indicated that a specific hcDNA target was unknown. The remaining 65% showed responses spanning multiple orders of magnitude, and only 35% indicating the WHO recommendation of ≤ 10 ng hcDNA/dose. It was hypothesized that the extremely broad distribution of hcDNA levels observed in initial survey responses, could be a function of the large dose level range as opposed to process and analytical capabilities. To confirm this hypothesis, a second survey was conducted in which respondents were asked to provide hcDNA levels normalized by vg (ng/10^{13}vg).

The dataset obtained from the second survey consisted of 15 data points. Three extreme values were excluded as outliers, using the Tukey Outlier method (≥2700ng/10^{13}vg), as they skewed the overall results. Figure 7 shows two box-and-whisker plots of the dataset before (Figure 7A) and after (Figure 7B) outlier exclusion. The post-outlier removal plot (Figure 7B) shows a more condensed distribution within the defined 95% confidence interval. This refined dataset presents a clearer view of typical hcDNA levels gathered in the survey.

Table 2 shows the summary statistics calculated for the refined dataset obtained from the second survey. The mean at 137 ng hcDNA/10^{13}vg serves as a central measure of typical hcDNA levels across samples. Considering the 95% confidence interval, it suggests that hcDNA values would likely fall between 39 and 235 ng/10^{13}vg. This range provides a probabilistic benchmark for representative hcDNA levels. The results showed above indicate a possible consistent vg-normalized hcDNA level, and confirmed the hypothesis formulated after the first survey, that the broad range of hcDNA/dose was due to extremely broad dose ranges.

To further confirm this hypothesis, the vg-normalized hcDNA level distribution was used to create a model that predicts the percent of products that could achieve the WHO hcDNA limit of ≤10 ng hcDNA / dose as a function of total vg dosed. The resulting model (Figure 8) similarly confirmed the initial hypothesis, indicating

TABLE 2

Summary statistics of the refined dataset of the benchmarking analysis.

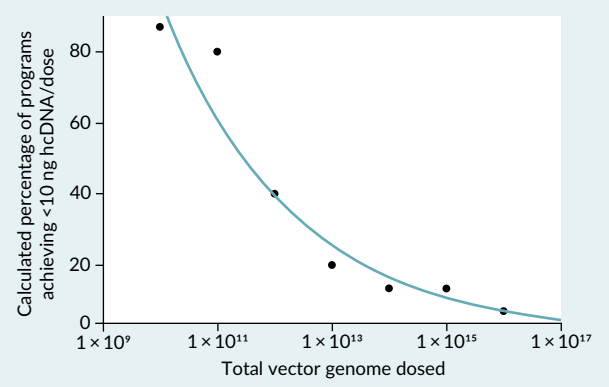
Summary statistic		Result (ng hcDNA/10^{13} vg)
Mean (average)		137
Standard deviation		154
Standard error mean		44
95% confidence interval	Upper bound	235
	Lower bound	39

that at the lower end of the dose range (1×10^{10} –1×10^{11} vg/dose), > 80 % of products could achieve the WHO limit. Beyond this low dose range, a steep decline in the proportion of products capable of achieving the WHO limit was observed, with ≤ 20% of products capable of achieving < 10 ng hcDNA/dose at 1×10^{13}vg or higher (Figure 8). These results were consistent with a prior independent assessment [5].

It should be noted that the method (including analytical platform, sample preparation, standards and target sequence) used to assess hcDNA level may impact this assessment. Additionally, a low percentage of responses represented programs in late stage clinical or commercial applications (37% pre-clinical, 21% early-stage clinical trials, 32% late- stage clinical trials, 11% commercial programs of 19 respondents), which may skew the benchmark results towards higher hcDNA levels.

FIGURE 8

Bivariate fit of calculated % of programs achieving <10 ng hcDNA/dose by total vector genomes dosed.



The equation of the calculated regression line is Log(Calculated % of programs achieving <10 ng hcDNA/dose)=4.3101504–0.1896496*Log(Total VG dosed), resulting in a R2 value of 0.958.

hcDNA CONTROL STRATEGIES

The presence of hcDNA in AAV preparations can occur in two distinct forms: encapsidated hcDNA and unencapsidated hcDNA. Encapsidated hcDNA consists of host cell genomic fragments that have been inadvertently packaged within AAV capsids. While packaging of DNA species without an AAV ITR region is likely less efficient given its key role in AAV genome packaging [35], the host cell DNA substrate exists in excess compared to vector genome DNA templates [5] resulting in significant levels of hcDNA encapsidation.

It is well known that the production of viral vectors such as AAV and AdV, induce apoptosis and a molecular cascade involving caspase-activated DNase [36]. This leads to internucleosomal cleavage of DNA in fragments of 180 bp and multiples thereof (e.g., 360 bp, 540 bp, etc.) during the upstream process. Of the hcDNA present during AAV capsid formation, these smaller fragments are much more prone to be encapsidated than larger hcDNA fragments as these quickly exceed the virion's packaging capacity [37].

Finally, the level of encapsidated hcDNA is heavily dependent on the selected production system and upstream processing conditions as many existing downstream process steps for hcDNA clearance are primarily effective in clearing only unencapsidated species.

Upstream hcDNA control strategy

The following data around upstream control strategies were collected alongside the normalized hcDNA levels in this survey:

- production system
- whether starting materials were engineered to minimize hcDNA
- maximum and minimum viable cell densities
- end of production characteristics including cell viability and vg titer, and
- production stage duration.

The data collected do not draw clear conclusions as no statistically significant correlations between survey responses and hcDNA levels were observed. While this may reflect the true nature of these production processes, it could also be due to the limited survey size. Therefore, process development studies of these and other process parameters may still be impactful.

Thorough screening of production systems that impact encapsidated hcDNA levels is particularly recommended for high dose applications [38, 39]. Additionally, the level of hcDNA degradation by caspase-activated DNase as well as the level of hcDNA encapsidation depends on various process parameters and/or material attributes determined by process characterization studies. Alternatively, the CCL could be genetically manipulated to reduce hcDNA encapsidation during AAV production [37].

While direct understanding of the impact of upstream processing steps influencing the encapsidation of hcDNA is still in nascent stages, it is anticipated that new production strategies to reduce hcDNA level will emerge. A number of effective approaches for the clearance of unencapsidated hcDNA are discussed in 'Downstream hcDNA control strategy'. However the total amount of process hcDNA generated during the upstream production will influence the downstream purification. It is therefore desirable to minimize the hcDNA impurity level where possible. Encapsidated hcDNA clearance during the downstream purification is non-specific, leading to full capsid removal as well, resulting in loss of yields.

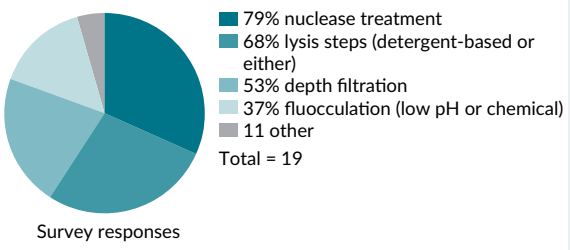
Downstream hcDNA control strategy

It was found from the survey (Figure 9) that respondents expected various process steps, conditions and addition of materials to have an impact on hcDNA levels. This included the temperature and duration of nuclease treatment, type of detergents used, pH, addition of salts, lysis buffers and $MgCl_2$, ultracentrifugation, ultrafiltration/diafiltration and modification of full/empty separation steps.

Once encapsidated, hcDNA becomes challenging to remove since it resides

FIGURE 9

Survey responses when asked what techniques are used during harvest and clarification steps that may impact hcDNA levels.



within the AAV capsid. AAV capsids containing hcDNA often biophysically (e.g., size, density and surface charge) resemble the AAV product (i.e., fully packaged AAV capsids containing the intended therapeutic genome), implying that they cannot be separated from the AAV product by any of the conventional purification techniques that are based on biophysical attributes such as size, charge or density. In contrast, non-encapsidated species are generally considered to be accessible for various purification strategies.

Thus, purification strategies for reduction of hcDNA in AAV manufacturing are primarily focused on clearance of unencapsidated hcDNA. A commonly used method to degrade hcDNA is treatment of harvest and/or other product intermediates with a nuclease to digest the hcDNA species [40]. Nuclease digestion of nucleic acid impurities is well established as a highly effective and robust step for this purpose. However, it is frequently cited as a significant contributor to high manufacturing costs for AAV production. There are also methods to clear unencapsidated hcDNA during harvest and clarification steps. Treatment of AAV production harvest at low pH to hydrolyse nucleic acid species prior to clarification allows substantial clearance of not only hcDNA, but also reduction of host cell protein impurities [41]. Additionally, techniques to utilize chromatographic clarification filters in place of traditional depth filters to reduce hcDNA species show promise in removal of nuclease treatment steps from AAV production processes [42]. Additional clearance may occur during downstream purification steps such as affinity, ion exchange, and/or ultrafiltration steps, but level of clearance can vary substantially as a function of the level of clearance observed over harvest and clarification steps. Encapsidated hcDNA has been identified in not only full capsids but also intermediate and, to a lesser extent, empty capsids [43]. This indicates the theoretical possibility that full capsid enrichment steps could contribute to additional clearance of encapsidated hcDNA via clearance of intermediate and empty capsids, though this correlation is not consistently captured in literature. The survey performed for this work included collection of information on downstream control strategies alongside the normalized hcDNA data to determine whether any industry-wide trends could be ascertained, including:

- harvest and clarification strategies,
- use of nuclease at harvest or other process intermediate,
- product capture step technique, and
- full/empty capsid separation step technique.

Similar to upstream process conditions, no statistically significant trends were observed in this analysis, though a controlled experiment directly comparing these strategies could provide more insight into the impact of downstream process conditions on hcDNA level.

Overall, for definition of a process control strategy for hcDNA it is recommended that risk assessments be performed early in clinical development to determine a process development path. Generally speaking, the upstream and downstream controls for hcDNA levels are deemed sufficient to enable dosing with hcDNA maintained below the WHO established limit for "low dose" *in vivo* AAV gene therapies, defined in this work as $< 1 \times 10^{13}$ total vg, and thus low risk. Programs requiring higher doses, often accompanied by higher hcDNA levels, should consider extended time for evaluation of manufacturing strategies that reduce hcDNA levels, particularly those focused on preventing non-discriminant hcDNA packaging including novel production systems. Enhanced characterization of

hcDNA species present should also be considered at high dose levels.

Analytical hcDNA control strategy

As discussed above, hcDNA can be controlled by having a low amount, degrading it and/or making it inactive. In 'hcDNA analysis methods' various analytical tools are identified, which can confirm the amount and possible degradation.

Either of the qPCR and dPCR methods could be part of the release panel to confirm the amount of extraneous hcDNA present. A common, and well accepted by HAs, practice for biological products is that hcDNA removal (spiking performed if needed) studies are executed and that consistent hcDNA removal is confirmed during process performance qualification (PPQ). If this consistent hcDNA removal is demonstrated, often no hcDNA quantification test is required as part of the release panel [9]. However, post-approval monitoring may still be requested for a certain number of batches dependent on the route of administration and amount of hcDNA administered.

According to the BioPhorum survey, 58% of 19 respondents monitor the size of hcDNA amplicons, and half of the respondents reported that up to 40% of their total hcDNA consisted of fragments larger than 200 bp. PCR methods, agarose gel and long range NGS can be applied to assess size distribution. Careful consideration should be made if such a control is required depending on the amount of extraneous hcDNA present. If based on the indication, dosage and the route of administration the amount of extraneous hcDNA is deemed too high, a certain level of hcDNA reduction and/or degradation may be required from a safety perspective. This can be assessed based on supportive characterization studies.

If a CCL is chosen, the oncogenicity and/or infectivity of possible genes (e.g., E1A/E1B in HEK 293 cell line) needs to be evaluated. A specific qPCR or dPCR method may be required then as part of release unless a correlation can be demonstrated between removal and/or degradation of those specific genes (including potentially encapsidated) and total hcDNA. This is likely the case as the removal and/or degradation is often non-specific.

Of note, during development it is standard practice that a quantitative hcDNA method is part of the release panel and hcDNA size and oncogene/infectivity genes quantification methods are part of the characterization panel. Furthermore, the hcDNA analytical control strategy is also dependent on the indication, dosage and the route of administration.

For reasons of safety, it is crucial to understand what hcDNA is encapsidated. This can be assessed via NGS with, e.g., appropriate separation prior to the sequencing so that only the encapsidated hcDNA is sequenced. A risk assessment can be performed to determine if any intact oncogenes or infectivity genes are carried over and assess their potential impact on safety. Based on the outcome of the risk assessment certain manufacturing process and/or analytical controls may be required.

Oncogenicity studies can be performed for a CCL to determine if it is oncogenic based on various residual hcDNA concentrations [44]. These data can then be extrapolated to determine toxicological levels for specific oncogenes/infectivity genes based on how many gene copies are present, the size of the genome and the amount of residual hcDNA injected which did not cause tumor formation. Such studies were previously performed for the PER.C6 cell line [45], which was constructed in a similar fashion as the HEK293 cell line [46].

Infectivity, i.e., the presence of retrovirus genome in the host cell DNA, can be assessed by assessing the presence of endogenous and/or latent retroviruses in the cell bank. To thoroughly demonstrate that there are no retroviruses endogenously present in the cell bank, a fluorescent

product enhanced reverse transcriptase (F-PERT) assay can be conducted on the cell bank. The presence of latent retroviruses can be assessed by chemical induction with 5-iodo-2-deoxyuridine (IdU) or 5azacytidine (AzaC) followed by co-cultivation with detector cells and F-PERT, as well as transmission electron microscopy (TEM).

TRANSLATION INSIGHT

Proposal for harmonized reporting approach

As shown through the benchmarking surveys presented in this work, assessment of hcDNA levels in AAV-based *in vivo* gene therapies represents a challenge particularly for high dose applications (i.e. ≥ 1×10^{13} vg/dose). Administration of dose levels with differences across many orders of magnitude have resulted in an unclear understanding of what is achievable with respect to hcDNA levels, limiting practical assessment of hcDNA-related risks early in clinical or even pre-clinical development.

Given the regulatory expectation that hcDNA levels should be justified in a product-specific manner, particularly in scenarios where hcDNA levels result in >10 ng hcDNA administered per dose, it is likely that a significant number of *in vivo* gene therapy programs will independently need to justify hcDNA levels above the historical WHO limit. It is implicit that for any individual program, the total hcDNA dosed along with characterization of the hcDNA impurities species present is the most relevant means of assessing the safety risk for patients.

In cases involving rare and ultra-rare diseases where potentially revolutionary treatments are being developed for populations with significant unmet needs, there may be situations where the considerable benefits outweigh the increased risks posed by high impurity levels, resulting in an overall improvement for patient communities. In these scenarios, manufacturers should aim development to minimize hcDNA levels and other impurities to the extent feasible by the existing industry best practices for AAV manufacturing. While the industry benchmark cannot and should not be used independently from established safety data or total hcDNA levels dosed for a particular manufacturing process, it can aid in determining whether drug substance or drug product hcDNA levels could feasibly be reduced further with targeted process development. When further reduction in hcDNA levels is improbable through additional process development, the industry benchmark can serve as a valuable support for a current process range, unless new innovations emerge to challenge established best practices.

Second, a key challenge in innovation and process improvement for *in vivo* gene therapies is identifying when real improvements above existing best practices are observed. In the context of process improvements that reduce hcDNA, head-to-head comparisons of hcDNA levels on a per dose basis across multiple manufacturing processes where dosing can vary by multiple orders of magnitude does not allow for true assessment of overall process capabilities. As a result, proposed novel process improvements that aim to reduce hcDNA or other process impurity levels are limited to assessing fold-changes without reference to an industry benchmark. This limitation reduces the ability to identify whether improvements are observed in real-world applications. Given the need for elevated dosing levels, coupled with the low likelihood of achieving <10 ng hcDNA/dose at and above 1×10^{13} total vg dosed, further innovation in this arena could provide strong impact to patient communities, and potentially improve safety profiles such that higher dosing to improve effectiveness could potentially be enabled through lower risk from high hcDNA level.

In support of this justification, harmonizing reporting industry-wide to not only include total hcDNA per dose administered, but also normalized hcDNA level per vg will allow head-to-head comparisons of levels measured (encompassing hcDNA generation, encapsidation and associated clearance and degradation) for a specific manufacturing process compared to the established industry benchmark. The proposed calculation for vg-normalized hcDNA level is shown in the followoing equation:

$$\text{hcDNA}\left(\frac{\text{ng}}{\text{vg}\times 10^{13}}\right)=\frac{\text{hcDNA concentration}\left(\frac{\text{ng}}{\text{mL}}\right)\times 10^{13}}{\text{vg titer}\left(\frac{\text{vg}}{\text{mL}}\right)}$$

Combining this assessment with safety assessments in regulatory submissions can strengthen justification, particularly if normalized hcDNA levels are within or below the industry benchmark (39-235 ng/10^{13}vg). As differences in analytical procedures used for quantification of hcDNA and vg could contribute to the variability observed across these harmonised measurements, industry standardization in analytical procedure may further support comparisons.

Furthermore, continued efforts to innovate novel means to reduce hcDNA levels in AAV preparations can broadly benefit from enabling vg-normalized hcDNA comparisons to ensure that any reduction in hcDNA levels are not a result of either final vector concentration or total vector modelled for administration.

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CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

VIRAL VECTOR MANUFACTURING AND PLATFORM EVOLUTION

SPOTLIGHT

REVIEW

Process and analytical development during AAV manufacturing platform evolution

Jack Jinqi Ren and Ning Ding

Over the past three decades, AAV manufacturing has progressed significantly, from low-yield, adherent cell cultures to scalable, suspension-based systems and single-use bioreactor platforms. Despite these advancements, key challenges remain, including low upstream productivity, inefficient separation of full and empty capsids during downstream processing, and a lack of standardized analytical methodologies. This review summarizes the evolution of AAV production platforms and examines the impact of emerging technologies on process and analytical development. It also highlights current gaps in AAV vector processing and evaluates the importance of platform-based approaches and real-time analytics in improving reproducibility and commercial readiness of gene therapy products.

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THIS HISTORY OF AAV MANUFACTURING PLATFORM DEVELOPMENT

AAV manufacturing platforms have evolved significantly over the past 30 years in response to increasing demand for gene therapies, regulatory expectations, and the need for scalable, high-yield, and high-purity production. As outlined in Table 1, AAV production has evolved from small-scale, low-yield adherent HEK293 transfection systems in the 2000s, to larger-scale suspension cultures using bioreactors in the 2010s, addressing scalability but facing purity and full/empty capsid separation challenges. In the 2020s, the focus has shifted to suspension-adapted cell lines with single-use bioreactors. A comparison of adherent and suspension AAV production systems is shown in Table 2. In addition, stable cell lines and perfusion systems are widely explored in the early and late stages of clinical manufacturing and trials, although intellectual property (IP) constraints and regulatory challenges remain key hurdles.

One of the most critical hurdles limiting AAV gene therapy is its inherently low productivity and high COGs. Most AAV-based programs suffer from narrow profit margins,

TABLE 1

History of AAV manufacturing platforms.

Era	Cell type	Production method	Scale	Key limitations
2000s	HEK293 (adherent)	Triple transfection	T-flask/roller bottle	Low yield, not scalable
2010s	HEK293 (suspension)/Sf9	Transfection or baculovirus	Bioreactor (50–2000 L)	Purity, full/empty separation
2020s	Suspension lines, stable lines	Transfection, stable, perfusion	Bioreactor, single-use systems	IP, regulatory hurdles, impact to timeline

which significantly discourage sustained investment from biopharmaceutical companies and venture capital, thereby slowing the pace of clinical and commercial advancement. Addressing these challenges is essential to unlock the full potential of AAV gene therapies. To overcome this, a few strategies are under active development:

- Improving vector yield, and
- Reducing the required dose through the development of more efficient capsids.

Another critical hurdle is the safety concern associated with AAV-based gene therapies and the evolving regulatory landscape. One notable example is the recent safety incident involving Sarepta Therapeutics, where a patient death occurred during a clinical trial of their gene therapy product, raising serious concerns about the risk of adverse immune responses, liver toxicity, and other systemic effects linked to high-dose AAV administration. This review will primarily focus on current industry trends aimed at addressing CMC concerns, with particular emphasis on process and analytical development throughout the evolution of AAV manufacturing platforms.

MANUFACTURING PLATFORMS: ADHERENT VERSUS SUSPENSION SYSTEMS

AAV manufacturing platforms can be broadly categorized into adherent and suspension-based systems, each offering distinct advantages and limitations in scalability, operational efficiency, and yield. Adherent systems, typically utilizing cell factories or multilayer hyperstacks, are constrained by surface area and manual labor requirements, making large-scale production challenging. These setups commonly produce between 5×10^{15} and 1.2×10^{16} genome copies (GC) per batch. In contrast, suspension systems employing stirred-tank bioreactors allow true volumetric scaling, up to 1000 L or beyond, with reduced operator dependency and automated control. Yields from suspension processes generally range from 1×10^{16} to 2×10^{16} GC per batch, representing a notable improvement in productivity and dose efficiency. Transitioning from adherent to suspension HEK293 systems has therefore markedly enhanced manufacturing throughput, simplified media handling, and enabled consistent performance at clinical and commercial scales. These advances have been pivotal in lowering the cost per dose and supporting the industrialization of AAV-based gene therapies.

UPSTREAM PROCESS DEVELOPMENT TO INCREASE MANUFACTURING PRODUCTIVITY

Cell line development

Over the past decade, significant research has focused on enhancing upstream AAV

TABLE 2

Comparison of adherent and suspension AAV production systems.

Production system	Culture platform	Scale limitation	Performance/operation	Harvest yield (GC/mL)	Total yield per batch (GC)
Adherent system	Cell factory or hyperstack	Scale-up limited to ~36-layer hyperstack; scale-out up to ~120 hyperstacks (≈500 L harvest volume); further expansion constrained by facility space and operator requirements	Labor-intensive during upstream transfection and cell harvest; media changes often required; difficult to scale up efficiently due to manual handling	1×10^{10}–1×10^{11}	5×10^{15}–1.2×10^{16}
Suspension system	Stirred-tank bioreactor	Scalable up to 1000 L or more; easy scale-out capability	Easier operation and automation; no media change required; efficient upstream and harvest processes	1×10^{10}–2×10^{11}	1×10^{16}–2×10^{16}

production to improve vector yield, and product quality. HEK293 cells remain the industry standard due to their robust productivity and regulatory acceptance [1,2]. In addition, HEK293F suspension cell lines are widely explored due to their capacity for high-density culture and improved scalability [3]. The development of stable producer cell lines has emerged as another promising strategy, capable of eliminating the need for plasmid transfection altogether. Inducible stable HEK293 lines stably expressing Rep genes allows controlled AAV production without the need for costly transient transfection [4]. Stable lines offer the benefits of reduced batch-to-batch variability, simplified production, and better process consistency [5]. Additionally, stable lines can face reduced productivity due to cell burden from constitutive or semi-constitutive expression of toxic Rep proteins. Techniques like CRISPR/Cas9-mediated genome editing are increasingly applied to customize host cell metabolism, further improving AAV yields in suspension culture systems [5,6].

In addition to HEK293 cells, HeLa-based producer lines have been explored as alternative hosts due to their favorable proliferation characteristics and inherent differences in post-translational modifications. For instance, Ultragenyx Pharmaceutical Inc. developed a HeLa-based Producer Cell Line (PCL) for large-scale, high-quality production of AAV gene therapy vectors. This platform typical yields exceed ~1×10^{11} GC/mL, with a high ratio of full to empty AAV capsids.

PLASMID & TRANSFECTION OPTIMIZATION

In parallel, significant progress has been made in plasmid design and transfection optimization. The traditional triple-plasmid transfection systems are labor-intensive, costly, and prone to variability. Emerging single, or two-plasmid systems (such as AAVOne™ and comparable platforms) consolidate the essential components, Rep-Cap, helper functions, and transgene, into a single or two constructs. This simplification not only reduces the quantity of plasmid DNA required but also improves transfection efficiency and significantly lowers production costs [7]. This single-plasmid system can achieve ~2–4-fold yields relative to conventional methods, while minimizing contamination from residual plasmid DNA.

Advances in transfection reagents and protocols have further enhanced upstream productivity. The development of novel cationic polymers and lipid-based reagents, specifically optimized

for suspension-adapted HEK293 cultures, has improved DNA uptake efficiency and reduced cytotoxicity, leading to higher vector titers. For example, optimized polyethylenimine (PEI) formulations and lipid nanoparticles are now commonly used in large-scale transient transfection protocols. Concurrently, process improvements such as optimized DNA-to-cell ratios, controlled feeding strategies, and enhanced mixing protocols in stirred-tank bioreactors have contributed to improved volumetric productivity.

In addition, the use of chemical additives like histone deacetylase (HDAC) inhibitors and cell cycle modulators has been explored to create intracellular conditions favorable for AAV production. These additives can enhance plasmid transcription, prolong the productive phase of host cells, and reduce cellular stress, thereby further improving yields [8].

Overall, the integration of single-plasmid systems, advanced transfection reagents, and strategic process enhancements represents a critical strategy for addressing AAV production bottlenecks and achieving more scalable and cost-effective manufacturing.

DOWNSTREAM PROCESS DEVELOPMENT TO IMPROVE THE PROCESS IMPURITIES

From a downstream processing perspective, one major CMC focus is improving the percentage of full capsids, as empty capsids not only dilute potency but also contribute to immunogenic risks. Optimizing full/empty capsid separation is therefore essential. Currently, three primary platforms are used for the separation of full and empty AAV particles (Figure 1). These platforms include:

- Cesium chloride (CsCl) gradient ultracentrifugation, as described by Wright *et al.* [9];
- Iodixanol (IDX) density gradient ultracentrifugation, adapted from the method reported by Lock *et al.* [10]; and
- Anion exchange chromatography (AEX), which is currently the most widely used approach in the industry.

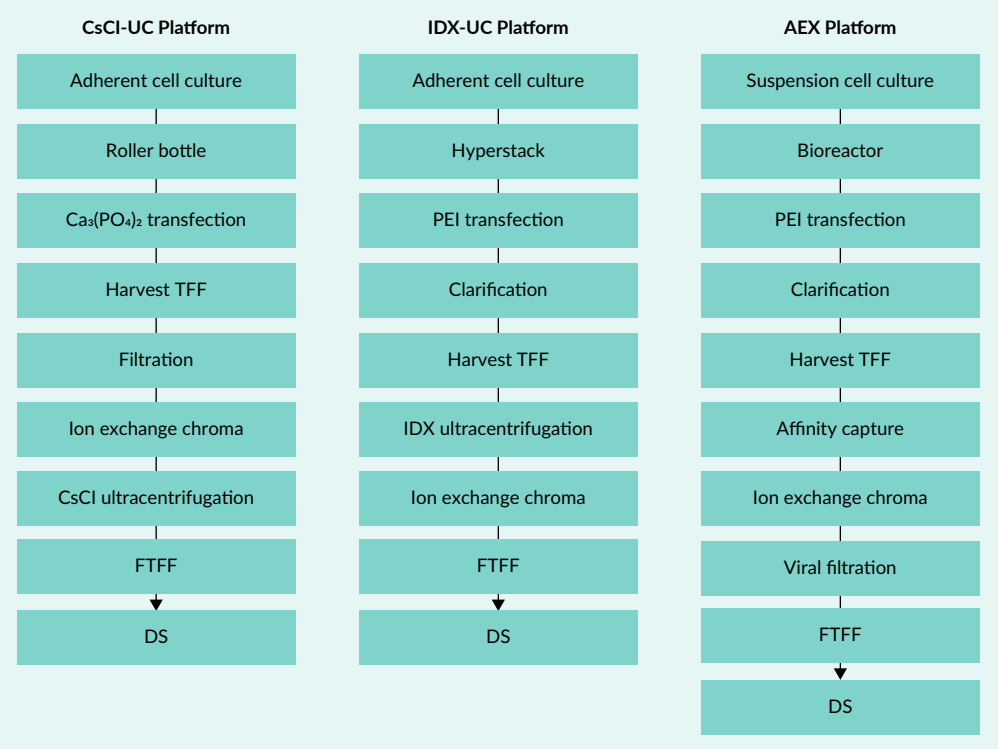
Each platform presents distinct strengths and limitations regarding scalability, GMP suitability, and process consistency. Both CsCl-UC and IDX-UC rely on density-based separation, exploiting the slight buoyant density difference between full and empty AAV capsids. Density gradient ultracentrifugation (DGU) methods typically yield 45–95% full particles, contributing to improved final product potency and reducing immunogenic risks associated with empty capsids. However, these methods are inherently batch-based, labor-intensive, and difficult to scale beyond 500–1000 L batches, limiting their suitability for commercial GMP manufacturing. Additionally, operational complexity and manual handling increase the risk of process variability.

By contrast, AEX leverages subtle charge differences between full and empty capsids to achieve separation. While AEX chromatography is more scalable, automatable, and better aligned with single-use, closed-system GMP operations, its resolution is generally lower, often resulting in 30–60% full particles without further process refinement. Recent developments in multi-step AEX peak-cutting strategies and optimized salt gradient elution profiles have improved separation efficiency, but achieving consistently high % full across different serotypes remains a challenge.

In summary, while density gradient ultracentrifugation remains the gold standard for small-scale, high-purity vector production, AEX chromatography represents the most promising platform for large-scale, GMP-compliant manufacturing, provided

FIGURE 1

Evolution of AAV manufacturing platforms.



that further optimization in separation fidelity is achieved.

ANALYTICAL DEVELOPMENT STRATEGY DURING AAV MANUFACTURING PLATFORM EVOLUTION

Developing a robust analytical strategy is essential to support process development for AAV gene therapy. While process teams focus on improving yield and product quality, the analytical team must provide sensitive, reliable assays to accurately evaluate these effects. Without such a framework, linking process changes to product attributes, ensuring comparability, and managing quality risks become challenging. Here are a few analytical strategies for AAV vector development, which includes raw materials quality control, drug substance (DS) and drug product (DP) release and characterization, and in-process analytics strategy. Other hot topics, include the gold standard method to measure full/empty and assay development for impurity, are highlighted as below.

RAW MATERIALS QUALITY CONTROL

Raw materials represent a major source of variability and must be characterized with the appropriate level of control, especially when they are classified as high risk. For AAV manufacturing, key raw materials, such as plasmid DNA and cell banks, fall into this category due to their direct and substantial impact on critical quality attributes (CQAs). Comprehensive testing strategies, including identity, sterility, endotoxin levels, and impurity profiles, should be developed for these materials to ensure consistency and functional integrity across batches. Understanding the material attributes at the source helps reduce variability and supports reproducibility

when evaluating changes like new plasmid configurations or cell substrates.

ANALYTICAL METHODS FOR DS/DP RELEASE

DS/DP analytical methods serve as the primary tools for assessing whether process changes result in product that is comparable in quality, potency, and safety. These assays include both molecular and biophysical tests that characterize key product features, such as vector genome titer, infectious titer, full-to-empty ratio, capsid integrity, and purity. Technologies such as quantitative PCR (qPCR), digital droplet PCR (ddPCR), analytical ultracentrifugation (AUC), Enzyme-Linked Immunosorbent Assay (ELISA) provide quantitative insights into these attributes and can be used to track performance trends as process changes are implemented. Importantly, qualified analytical methods at the DS/DP level serve as definitive readouts for determining whether a process modification—such as a new transfection reagent or an optimized plasmid backbone—has improved or compromised product quality attributes.

ANALYTICS FOR IN-PROCESS CONTROL

In-process analytics play a crucial role in supporting real-time process understanding and guiding optimization efforts during development. These methods include cell-based and biochemical assays that track parameters such as viable cell density, transfection efficiency, metabolite consumption, and viral particle formation throughout the production run. Tools such as automated cell counters, bioanalyzers, ELISAs for capsid protein detection, and qPCR for genome quantification enable the process development team to assess kinetics, harvest timing, and potential bottlenecks in production. These methods not only support immediate process adjustments but also contribute to the long-term goal of developing a scalable, reproducible, and controlled manufacturing platform. In parallel, manufacturers are increasingly adopting process analytical technologies (PAT) originally developed for monoclonal antibodies and small molecules—such as real-time metabolite monitoring, In-line or at-line glucose/lactate sensors, and In-line UV or fluorescence detectors—to enhance both upstream and downstream monitoring. Applying these tools to AAV production enables earlier detection of deviations, supports feedback control strategies, and accelerates the establishment of robust, compliant manufacturing processes.

GOLD STANDARD TO MEASURE FULL/EMPTY

One of the central challenges in AAV downstream processing is the quantification and separation of full versus empty capsids. Empty particles are considered non-functional and, at high levels, may contribute to immunogenicity or dilute therapeutic potency. Analytical techniques such as AUC, and transmission electron microscopy (TEM) are commonly used to assess the full-to-empty ratio. However, these methods often require sophisticated instrumentation, lengthy analysis times, and complex data interpretation. As a result, the quantification of full capsids remains one of the most urgent demands. To address these limitations, a range of orthogonal and alternative methods have been explored, including charge detection mass spectrometry (CDMS), infectivity/transduction bioassays, and optical density-based techniques [11]. These methods, while sometimes less precise than AUC or TEM, offer advantages in speed, scalability, and ease of integration into routine workflows. Employing multiple, orthogonal approaches can improve confidence in results and support method

robustness, which is critical for process comparability, lot release, and regulatory compliance.

ANALYTICAL ASSAY DEVELOPMENT FOR PROCESS & PRODUCT-RELATED IMPURITY

In parallel, downstream purification must achieve substantial removal of process- and host-related impurities, including host cell proteins (HCPs), residual host cell DNA (hcDNA), transfection reagents (e.g., PEI), plasmid DNA, and endotoxins. These impurities are introduced during upstream production and must be monitored throughout the downstream process using highly sensitive and specific assays. PCR is commonly employed to track residual DNA, while ELISA-based methods are used to detect HCPs. Residual plasmid DNA and endotoxin levels are also tightly controlled, often requiring a very low detection limit. These assays are not only technically challenging due to the low detection thresholds required but must also be validated for use in complex, changing matrix conditions across various purification stages.

To effectively support downstream process development, analytical methods can be integrated at multiple points throughout the purification process. In-process samples are collected after each major unit operation, and analyzed to evaluate product recovery, impurity clearance, and step consistency. For example, total viral genomes may be monitored by qPCR or ddPCR, while total capsid content is assessed by ELISA. Comparing these results across steps allows teams to calculate yield-to-purity ratios, identify losses, and evaluate the contribution of each purification step to overall product quality. Combined with DoE and QbD approaches, analytical data can guide the optimization of critical process parameters such as gradient elution profiles, buffer compositions, membrane selection, and shear-sensitive handling conditions.

DIRECTION FOR PLATFORM-BASED TECHNOLOGIES TO BE IMPLEMENTED IN AAV MANUFACTURING

Analytical technology platform for AAV have evolved from bulk, average measurements to high-resolution, single-particle or population-resolved techniques that more precisely define critical quality attributes. Empty-to-full ratios have progressed from indirect averages obtained by qPCR or ELISA to a suite of higher-resolution techniques. Methods such as AUC and SEC-MALS provide detailed population distributions of empty, partially filled and full capsids, while imaging approaches like transmission electron microscopy (TEM) can directly visualize individual particles. When combined with digital droplet PCR for precise genome quantification, these complementary tools give a much more accurate picture of capsid content, ranging from bulk trends to near single-particle resolution.

To further implement and establish platform-based technologies in AAV viral vector production, the industry should prioritize the adoption of stable suspension-adapted cell lines, which have demonstrated improved batch-to-batch consistency and scalability over traditional transient transfection methods. On the downstream side, transitioning from density gradient ultracentrifugation to AEX enables scalable, automated purification processes compatible with GMP standards, though ongoing optimization is required to consistently achieve >80% full capsid purity. Integration of these upstream and downstream improvements into closed, single-use bioreactor systems coupled with PAT facilitates real-time monitoring and control, thereby enhancing product quality and manufacturing

robustness. Finally, harmonizing regulatory frameworks and encouraging knowledge sharing through consortia such as the Alliance for Regenerative Medicine (ARM) and the Parenteral Drug Association (PDA) will be essential to streamline platform validation and accelerate the adoption of standardized manufacturing approaches.

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CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

VIRAL VECTOR MANUFACTURING

SPOTLIGHT

Unpacking the challenges of AAV gene therapy: exploring capsid engineering, immunogenicity management, and scalable manufacturing solutions

INTERVIEW

"Although it has been over 50 years since AAV was first described as a possible vector...we still lack a comprehensive understanding of its biology and mechanism of action."

Abi Pinchbeck (Editor, BioInsights) speaks to **Sherif Gabriel**, (CEO and Co-Founder primAAVera Therapeutics) about the persistent challenges facing AAV-based gene therapies, including tissue-specific targeting, immune responses at high vector doses, and the lack of standardized manufacturing protocols. They explore how progress in capsid engineering, producer cell line development, and human-relevant selection platforms could enhance specificity, reduce immunogenicity, and support scalable clinical translation. Sherif also emphasizes the importance of early-stage design for manufacturability, offers practical guidance for early-stage vector developers, and reflects on the potential of emerging platforms such as *Bocaparvovirus*.

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Q What are the current key challenges in AAV-based gene therapies in your view?

SG There are three main challenges that have remained fairly consistent since the early days of AAV gene therapy.

The first challenge is vector specificity. By that, I mean being able to selectively target the organ of interest, rather than delivering a large volume of vector intravenously, which will inevitably pass through the liver first, where the majority of the dose will be absorbed or metabolized. Ideally, we want to direct the vector to the intended target, whether it is a particularly challenging organ such as the kidney or brain, or something more accessible such as the heart or skeletal muscle, without losing significant amounts to systemic circulation and liver uptake. That is still one of the most critical issues that we have not resolved; except in rare locoregional delivery such as the subretinal, intravitreal and intracranial administrations.

The second major challenge is managing the immune response that naturally occurs when a large bolus of AAV vector is introduced into the circulation. Clinical trials often involve doses on the order of 10^{12} to 10^{14} vector genomes per kilogram body weight, which can trigger a significant even deadly acute and/or chronic immune reaction. In response, both academic groups and industry are actively working to develop strategies that mitigate this effect and enable more sustained vector expression. Addressing immunogenicity remains a central concern in the field.

The third key issue is manufacturing. Currently, there are no standardized protocols for either upstream or downstream manufacturing. A range of different cell lines and purification processes are in use, leading to significant variability and, ultimately, high costs of goods. This poses a challenge not only for therapeutic developers, but also for regulators and payers, who must evaluate and support these products. It remains a complex and pressing issue.

Although it has been over 50 years since AAV was first described as a possible vector, most notably in a seminal paper by Jude Samulski, we still lack a comprehensive understanding of its biology and mechanism of action [1]. Beyond what we know from cultured cells and basic vector production, the full range of host factors involved in supporting AAV requires additional understanding.

Many of the current manufacturing challenges are compounded by this limited biological insight, particularly with respect to understanding how AAV unfolds and which cellular compartments it interacts with. A deeper, more nuanced understanding of AAV biology would go a long way in helping to address the three key challenges discussed. A typical example is the poorly understood relationship between AAV capsid components and promoter activity.

Q How can the field address the remaining process-related challenges to ensure AAV-based gene therapy's future as a competitive modality?

SG AAV certainly does need to maintain its competitive edge, but it is worth noting that it is already the leading platform in the gene therapy space by a wide margin. It remains the predominant modality in the clinic today.

"There are many steps in the production workflow that must translate effectively across different environments, and often they do not."

To preserve that position, the field needs to make meaningful progress in improving vector targeting and selection, while simultaneously addressing the challenges associated with high dosing. At present, we are seeing doses on the order of 10^{14} vector genomes per kilogram body weight, which almost inevitably provoke a significant immune response. If we can reduce the volume required and deliver the vector more directly to the tissue of interest, that would not only mitigate immunogenicity, but also enhance specificity and, critically, reduce the cost of goods for manufacturing. That would be welcomed by regulators, payers, patients, and developers alike.

Several strategies can help us move in that direction. One is the development of consistent, stable producer cell lines that support efficient and scalable vector production. Jude Samulski and Josh Grieger played pivotal roles in developing the Pro10 cell line [2], which has been a foundational tool in the field. However, that was a decade ago, and it is still not widely adopted. While Pro10 remains one of my personal favorites, it is not a one-size-fits-all solution, and there are many other cell lines and manufacturing protocols in use. Establishing a more standardized, broadly adopted production process will be essential.

Another important factor is the design of the vector genome itself, especially when considering tissue-specific targeting. There are elements within the capsid and genome that influence tissue tropism, and some have been shown to reduce liver uptake or enhance delivery to other regions. Incorporating those insights into design is key, as is identifying elements that improve expression efficiency.

Selection strategies are also critical. Many groups begin their screening and selection in small animal models, such as rodents, before moving to non-human primates. However, those platforms are not always predictive of performance in humans, particularly with respect to immune evasion. Shifting toward human-based evaluation systems, such as *ex vivo* models or humanized platforms, could represent a significant leap forward. It might dramatically change how we approach vector development and make the process more translatable to the clinic.

Q The continuing lack of standardization in AAV manufacturing is often cited as a critical challenge in the commercialization of gene therapies. What steps are necessary (technically, operationally, or collaboratively) to drive greater harmonization across the field?

SG It would be ideal if we had a thoroughly characterized benchmark or standardized protocol that the field could rely on. However, at present, we simply do not. There are so many different systems used for vector production and purification, many of which originate in academic laboratories, and they often do not align with what is used in industry. Yet, it is the industrial sector that is largely responsible for translating these processes into the clinic.

This disconnect creates significant challenges. There are many steps in the production workflow that must translate effectively across different environments, and often

they do not. The systems differ, the equipment varies, and the protocols are not always compatible.

What we need is a standard approach, a common baseline that, even if it is not optimal for every vector, can serve as a reliable point of comparison. A platform that allows us to say, "This is a validated process that works in a general sense, and here is how your production compares to it." If the output meets or exceeds that benchmark, then that is a strong starting point from which to optimize. However, without that kind of standardization, it becomes extremely difficult to evaluate or compare production outcomes across different settings.

At present, we see many impressive papers from highly talented groups reporting very high vector yields. However, when those same processes are transferred to another laboratory, or more often, to an industrial setting, the results are rarely the same. That inconsistency remains a major barrier.

As a community, we need to move beyond these discrepancies and work toward a shared understanding of the true production capacity of each vector product. Only then will we be able to achieve the level of output and reliability required to support broad clinical use. This could happen by establishment of two AAV type 2 and 8 Reference Standard Materials at ATCC [3,4,5].

Q What are the most promising innovations in AAV capsid engineering that you believe will shape the next generation of gene therapies?

SG We have seen significant advances in AAV capsid engineering, particularly through the application of artificial intelligence and data mining, which can now generate capsid libraries containing 10^6 to 10^9 variants. That scale is remarkable, and the potential is enormous. However, the true key lies in the quality of the input. Designing those inputs thoughtfully is essential. They must be tailored to specifically target the organ or tissue of interest while minimizing immunogenic elements as much as possible.

If the inputs are designed effectively, the aim is to move away from enormous, unfocused libraries toward a more refined and deliberate approach. A diverse pool is still necessary. It is important to have a solid group of capsids to test, but ideally, all candidates should be directly relevant to the disease target in question. Key to success is also to maximize library vitality, defined by the proportion of viral particles in the pool that efficiently assemble and package DNA. To this end, along with my co-founder, Dirk Grimm, primAAVera Therapeutics aims to harness the power of machine learning to predict viral capsid sequences that concurrently fulfill both criteria, i.e., diversity and vitality.

Selection is of primary importance. The goal should be to develop capsids that are effective in humans, not merely those that perform well in cell culture, rodent models, or even non-human primates. Efficacy and/or specificity in those systems does not guarantee translation, and we must be more intentional about that from the beginning. Using a human-based selection platform, as we are developing, could represent a significant advance for the field.

Another promising area that has come up repeatedly in recent industry meetings is the potential of hybrid systems. These approaches combine the most effective elements

"Ultimately, if we can consistently achieve delivery to the intended target while minimizing the immune response, the solution may not be universal, but it could represent a major leap forward."

of AAVs with components from other delivery reagents such as polyethylene glycol, often referred to as a hybrid virus [6]. The goal is to leverage the strengths of each. Whether it involves phage-AAV chimeras or vectors incorporating elements from viruses such as *Bocaparvovirus*, these combinations may open up new possibilities.

That said, we should not lose sight of an important point. It has been more than five decades since AAV was first identified, and we still do not fully understand its biology. As we incorporate new components and engineer increasingly complex systems, we may also complicate the picture and introduce additional unknowns.

Ultimately, if we can consistently achieve delivery to the intended target while minimizing the immune response, the solution may not be universal, but it could represent a major leap forward. This is about creating tailored tools that address specific clinical needs, and I believe that kind of innovation is within reach.

Q What advice would you give to early-stage biotech companies or academic groups aiming to bring a novel vector platform from bench to clinic?

SG This advice is not specific to AAV, and in truth, it is not even specific to biotechnology. I believe it reflects a broader principle: a general lesson in life, to begin with the end in mind. If the ultimate goal is to bring a vector into the clinic, then one must consider from the outset all of the components required to reach that point. It is important to think carefully about how the vector will be produced, what the manufacturing process will entail, both upstream and downstream, and how it will ultimately be delivered to the target tissue.

I have been as guilty of this as anyone, but many researchers begin with a single interesting observation, or with evidence of efficacy in a particular indication. That is often where the process starts: with a compelling biological effect. However, translating that observation into something viable for clinical use involves a series of additional hurdles. If one instead asks, "What needs to be true from the beginning in order for this to succeed in the clinic?" the chances of success will be much greater.

This means embedding best-in-class practices early in the development process, especially with respect to manufacturing. It also requires taking a thoughtful and strategic approach to screening. Rather than relying on the most straightforward approaches, such as a simple mouse study, it is important to design screens that meaningfully inform downstream success, likely whole body human analysis. The immune response should always be a key consideration, as it remains one of the most significant limitations on long-term efficacy. That must be addressed from day one.

CMC, along with scalability and long-term storage, are all critical considerations. There is limited value in identifying a high-performing transducer if it cannot be manufactured efficiently or scaled for clinical deployment.

Ultimately, all of this connects to a single point: the focus should be on product development from the outset. That is where attention must be directed.

Q Are there any emerging vector systems or non-viral alternatives that you are particularly excited about, or do you see AAV maintaining its dominance in the near term?

SG **In the near term, AAV will continue to maintain its dominance.** The vast majority of clinical trials currently rely on AAV-based vectors. Even though the biology of AAV is still not fully understood, it is far better characterized than most alternatives.

That being said, there are a few emerging platforms that I find quite promising. *Bocaparvovirus* is one viral vector I consider particularly compelling. It has a very large DNA capacity, which makes it capable of delivering significantly larger payloads [7]. There are also several non-primate *Bocaparvoviruses*, such as bat virus, that may hold considerable potential.

Still, the field feels somewhat caught in between. We have a strong foundation with AAV, and we know that it performs reliably, but it is not without its limitations. This raises an important question: should we shift toward a potentially superior alternative, even if it is less well understood? There will be pioneers who choose to explore these newer systems, and once we begin to see robust efficacy data, we may be able to integrate them more broadly. However, in the immediate future, I expect the field will remain largely centered on AAV.

Q What are your key predictions (or what would you like to see) regarding the progression of the viral vector-based gene therapy field over the next 5–10 years?

SG **First and foremost, I would like to see improved safety and reduced mortality across all gene therapy products, particularly those using AAV.** One of the distinguishing features of gene therapy is that there is often a clear early signal indicating whether efficacy is achievable. When a therapeutic is designed against a specific gene of interest, there is a strong expectation that the intended biological effect will translate. Unlike pharmacotherapy, which can involve alternative or compensatory pathways, gene therapy typically allows for a more direct and predictable approach.

The challenges we face are those we have already discussed. Can the vector be manufactured consistently? Can the immune response be effectively mitigated? Can precise tissue targeting be achieved? I hope to see advances across all of these dimensions, particularly with regard to safety.

Alongside that, I would like to see a significant reduction in the Cost of Goods (COGs). That would help bring regulators, payers, and developers into closer alignment, particularly given that in many cases, this represents a highly effective and potentially curative treatment. Supporting the companies advancing this work will be critical to making that future a reality.

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BIOGRAPHY

Sherif Gabriel joined primAAVera in May 2025, bringing nearly two decades of expertise in the life sciences sector. He has successfully led R&D organizations, advancing both platform development and pipeline creation. Sherif has overseen the progression of gene therapy and pharmacotherapy products from R&D through to clinical-stage assets. His career also includes experience as a buy-side analyst and a term as CEO of a medical device company.

Prior to his role at primAAVera, Dr Gabriel served as Senior Vice President of Research and Development at Asklepios Biopharmaceutical, Inc. (AskBio), where he reported to the CSO, Jude Samulski, and was part of the executive leadership team under CEO Gustavo Pesquin. Previously, Sherif was CEO of BioMedInnovations LLC (BMI), guiding the company to receive FDA Emergency Use Authorization for a mobile ventilator during the onset of the COVID-19 pandemic. Earlier, at Nivalis Therapeutics (formerly N30 Pharmaceuticals), Dr Gabriel held various executive positions, contributing to its IPO, which raised over $88 million, and leading an R&D asset through Phase 2 clinical trials.

Dr. Gabriel obtained his undergraduate and doctoral degrees from the University of Saskatchewan, Saskatoon, SK, Canada, before joining the Department of Pediatrics at the University of North Carolina at Chapel Hill (UNC) as a professor for nearly 22 years. Throughout his academic tenure, he fostered collaborations that resulted in multiple molecular therapies for cystic fibrosis (CF), ultimately motivating his transition into the biotechnology industry to further advance disease-modifying treatments.

As CEO of primAAVera, Dr Gabriel leads a dynamic gene therapy organization dedicated to designing AAV platforms that optimize delivery, enhance immunogenicity profiles, and address manufacturing challenges.

Sherif Gabriel PhD, Chief Executive Officer and Co-Founder, primAAVera, Heidelberg, Germany

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

VEIN-TO-VEIN SUPPLY JOURNEY

Beyond the biologic: rethinking supply chain models for cell and gene therapy

INTERVIEW

"Breaking into neurology is a major priority. Society is currently bearing a heavy burden from neurological diseases."

Cell & Gene Therapy Insights 2025; 11(9), 1217–1222 · DOI: 10.18609/cgti.2025.140

As cell and gene therapies move from concept to clinic, the complexity of their supply chains demands new levels of coordination, innovation, and adaptability. In this interview, Abi Pinchbeck, Commissioning Editor, *Cell & Gene Therapy Insights*, speaks with Phillip Ramsey, Chief Technical Officer, Sangamo Therapeutics, who shares insights from Sangamo's evolving strategy, highlighting the critical role of technology, collaboration, and platform development in building a more resilient and patient-centric delivery model.

Q What are you working on right now?

PR At Sangamo, our current lead program is a traditional cDNA gene therapy for Fabry disease. We initially began this program with a Phase 1/2 trial, and last year we were pleased to receive US FDA agreement to apply for accelerated approval based on our current data. We are very excited about that and are working diligently to submit a Biologics License Application (BLA) as soon as the first quarter of next year.

Another major focus at Sangamo is our zinc finger platform, which we are applying to neurological diseases. We are using zinc finger repressors to epigenetically regulate in neurological indications. At present, we have programs for neuropathic pain targeting the sodium channel Nav1.7, and a program for prion disease that we are excited about.

We have integrated our editing technology with a novel AAV capsid delivery platform called SIFTER (Selecting *In vivo* For Transduction and Expression of RNA). This platform generates novel AAV capsids and has produced several novel capsids that have shown the ability to penetrate the blood brain barrier and deliver payloads directly to the central nervous system (CNS). This is a highly active area in the industry right now. We have demonstrated this in non-human primates and are working to couple our zinc finger repressors with our delivery platform using these novel capsids.

Q With your extensive background in biotech operations and quality, how have you seen the cell and gene therapy (CGT) supply chain evolve over the past decade?

PR The supply chain has certainly matured significantly with all the approvals we have seen, both on the cell therapy and gene therapy sides. Initially, it was very fragmented and reactive, but now there is much more infrastructure available to support personalized therapies, and certainly more integrated systems. I believe there is also good regulatory alignment.

Another important development is the emergence of a larger group of specialized vendors who can support this space. If I am a first-time CGT company trying to enter the field, I no longer have to reinvent the wheel, whereas in the beginning, everyone had to do that.

Q What, in your view, are the current most significant bottlenecks in the CGT therapy supply chain, and why?

PR The scalability of manufacturing itself affects the beginning of the supply chain. The ability to leverage previous data also acts as a bottleneck. Developing platforms relieves some of this pressure and expedites product development and approval. That's something where you see Sarepta's initial platform designation really helping future products. Several of us in the field are close to that designation. Many people have tried creative approaches, but so far, there has been no opportunity for platform development because everybody was starting something new. Now, we are beginning to see technologies being used for second or third applications, and that is where we can gain leverage and make improvements to scalability and leverage platform.

Cold chain logistics are still a significant problem. As long as we are trying to support ultra-low temperatures, that is going to remain a major challenge. I do not see some of the therapies, especially cell therapies, being able to overcome that for some time. It must be managed on the back end by finding better mechanisms for using shippers and developing more innovative systems in that area.

Scheduling is also still an issue. Some of the companies with approved therapies, who have been pioneers in this space, such as Kite and Novartis, have made improvements.

For new players, any kind of siloed approach simply will not work. People must adopt a completely different mindset.

However, aligning patient availability with manufacturing slots and coordinating the logistics around that remains challenging, and most others are still struggling with this.

Finally, regulatory viability is a major concern. In the US, the regulatory environment has previously been very proactive and supportive of sponsors. However, when considering the rest of the world, it becomes overwhelming for many, especially smaller companies that are entering the space. For them, expanding internationally can be an almost insurmountable challenge.

Q What role does collaboration between clinical, regulatory, manufacturing, and logistics teams play in ensuring a successful vein-to-vein supply chain?

PR Collaboration is essential. Getting everyone aligned is, in my view, the major challenge. Successful products that have made it through, including some of the partnerships we have had, such as with Kite, have demonstrated that it can be done in a seamless way. However, it was a real challenge.

For example, Kite had to manufacture multiple times for patients—probably hundreds—before they began to achieve consistent results. It was that repetition and the development of muscle memory across teams that made it work. Getting everyone to work together and reach alignment was what ultimately helped.

I believe that is true for everyone entering the space. For new players, any kind of siloed approach simply will not work. People must adopt a completely different mindset.

Q How do you see digital technologies, such as real-time tracking and AI transforming the future of vein-to-vein supply chain management in CGT?

PR These novel technologies can be game changers. People can become overwhelmed by AI and ask, 'What does AI mean, and what is it?' I remind people that drug modeling has been done for 20 years, and that is essentially a form of AI. The success of these technologies depends on how well people understand and use the tools. People must understand how they have already been using these technologies and then extend that understanding to take full advantage of where the technology is going. They should also help drive the development of the technology so that it fits their needs.

For AI groups in particular, and to some extent digital technologies more broadly, getting feedback from users will be critical. Whoever figures out how to do that most effectively will likely be the one who succeeds, because users will help shape technological development.

In the supply chain, the value is a no-brainer. Real-time feedback from digital technologies, the ability to track materials, and AI-assisted logistics can make a significant difference. Instead of making ten phone calls to four different countries, I can simply read the information the tool provides and troubleshoot and manage by exception. In supply chain operations, people know that it often feels like a fire drill. I believe these technologies can help eliminate that reactive mode and allow us to manage more proactively and efficiently.

AI is a basic tool, like every other tool that helps us think and work in new ways. Those who use it well will succeed, and those who do not will not. The key is not to be overwhelmed–we are already using these technologies in our day-to-day, with things like CoPilot and ChatGPT. There is also a variety of technologies, and they all work a little differently depending on the application. Some technologies are better suited for different needs, which we are beginning to understand.

Q What are some of the most common misconceptions you encounter about the CGT supply chain, and how would you clarify them for stakeholders new to the field?

PR CGTs are not traditional biologics and cannot be treated as such. Monoclonal antibodies have had 30 years to develop, including the supply chain.

The most important thing for those working in the CGT space to remember is that −80 °C is not considered standard for other modalities. I have worked on clinical trials where we shipped −80 °C freezers to sites across the world because they did not have that capability. It is critical to make sure stakeholders understand that many vendors cannot manage the ultra-low temperatures required for some cell therapy applications.

Another key point is with patient-specific cell therapies, the supply chain is more like surgery than traditional drug supply. You are preparing for a procedure, a single event, and you must understand how to scale that so it can be reproduced consistently. This is fundamentally different from having a large stock of product that you ship out. This may change with allogeneic therapies and induced pluripotent stem cell platforms, but right now, autologous therapies are much more like a surgical event than a standard drug delivery model.

Q Looking ahead, what do you believe are the key investments or shifts the industry must make to build a more resilient and patient-centric CGT supply chain?

PR Automation and digitization are critical. Both can enhance and streamline the current state of the field and make it significantly better for patients.

Decentralized manufacturing is another area that people have been working on for decades. What does that look like? Using models from Novartis and Kite, we see more production facilities distributed across the country. I have seen that work quite well, although there are still some hiccups. We are somewhere between that model and individualized treatment in a medical office. The question is whether we can push further toward individualized, in-office treatment.

Many of us have worked over the years on 'manufacturing-in-a-box'. Some groups are making excellent progress in that area, so I would not count it out. However, we are still somewhere between having three manufacturing sites across the US and individualized treatment in a clinical setting. That is where investment is happening and where it needs to continue.

Another key area is workforce development. Many professionals come from a biologics background and are learning how to adapt to this space. Continued investment in workforce training and skill development is essential.

The platform designation, as highlighted by the recent Sarepta news, is also important. Some cell therapy companies with commercialized products have done this well. It is all about scaling horizontally, and part of that involves standardization and platform development. I believe we will start to see more of this on the gene therapy side as well.

In traditional biologics, such as MAbs, once companies reached a platform stage, they were able to produce products more frequently and reduce barriers as the cost of goods came down. Right now, in CGTs, those costs are still quite high. Once those costs decrease, it will change the availability of these therapies for patients, especially when considering global access.

Q Finally, what are your key goals and priorities for Sangamo over the next few years?

PR For Sangamo, we are focused on treating debilitating neurological diseases and getting treatments to patients suffering from severe diseases for which today's medicine can often only offer symptom management at best.

Breaking into neurology is a major priority. Society is currently bearing a heavy burden from neurological diseases. In my view, this is the next major frontier, comparable in impact to the development of weight control medications. If we can begin to access the brain and treat neurodegenerative diseases, which are placing an enormous cost on society, it would be a major breakthrough. These conditions profoundly impact families. Unlike a cancer event, which is devastating but often time-limited, neurodegenerative diseases persist for decades and place a long-term strain on families and healthcare systems.

We are committed to developing treatments for these conditions, both through our own programs, starting in pain and in prion disease, as well as through ongoing partnerships with companies such as Lilly, Astellas, and Genentech, targeting indications such as Alzheimer's. Our focus is to transform patients' lives by replacing today's symptom focused treatments with tomorrow's genomic cures.

BIOGRAPHY

Phillip Ramsey is Chief Technology Officer at Sangamo where he oversees manufacturing, technical development, supply chain, and quality. He was previously the Senior Vice President overseeing Technical Operations and prior to that the Vice President of Technical Development overseeing process and analytical development, vector delivery and CMC program management. Phillip has over 35 years of experience in product development, operations, and quality.

Prior to Sangamo, Phillip was with Emergent BioSolutions in increasing roles of responsibility most recently Senior Director in the Biodefense division and helped design and facilitate construction of the CIADM facility in collaboration with BARDA. The facility was awarded the runner-up 2018 FOYA award for innovative design. While at Emergent he was involved with multiple commercial products, M&A activities, and their CDMO business.

Prior to this he was at Leidos where he participated in the design and construction of a 130,000 square foot vaccine pilot plant for the NIH. He has contributed to multiple regulatory submissions for product approval and more than 50 investigational new drug submissions.

Phillip received a BS in Chemistry from the University of Nebraska, a MS in Biochemistry from John Hopkins University and an MBA from San Diego State University. He has been active in multiple therapeutic areas including oncology, vaccines, infectious diseases, CNS, and rare diseases. He has worked on a variety of technologies including proteins, monoclonal antibodies, cell and gene therapy, genome editing, LNPs, bispecific antibodies, and immunotherapies.

Phillip Ramsey, Chief Technology Officer, Sangamo Therapeutics, Inc., Brisbane, CA, USA

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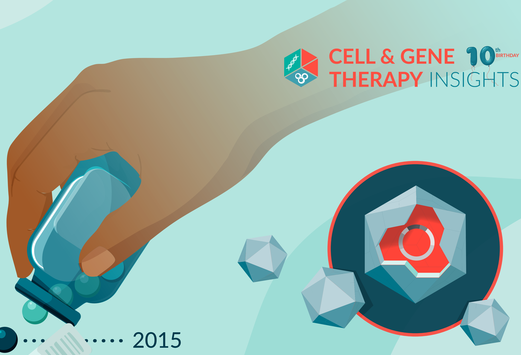
CELL & GENE THERAPY INSIGHTS 10 BIRTHDAY

A decade of breakthroughs

10 years of cell & gene therapy

Over the past ten years, cell and gene therapy has leapt from experimental trials to life-changing treatments. From the first CAR-T approvals to CRISPR's clinical debut, the field has redefined what's possible in medicine. In this infographic, we chart the milestones that transformed CGT from promise to practice.



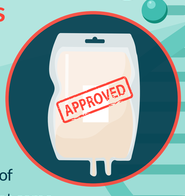
2015

Laying the groundwork

The field of cell and gene therapy (CGT) began to shift from promise to practice. Early clinical successes in CAR-T and AAV-based therapies were setting the stage for a wave of transformative approvals.

2017

First FDA CAR-T approvals

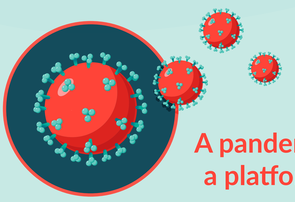


A landmark year for the field. The US FDA approved the first two CAR-T cell therapies: Kymriah (Novartis) for pediatric acute lymphoblastic leukemia, and Yescarta (Kite Pharma, later Gilead) for adult large B-cell lymphoma. These approvals marked the arrival of living drugs in mainstream oncology care. That same year, Gilead Sciences acquired Kite Pharma for $11.9 billion; the first major acquisition of a CGT-focused biotech, signaling growing commercial confidence in the space. Luxturna (Spark Therapeutics) also became the first FDA-approved directly administered gene therapy for a genetic disease, targeting inherited retinal dystrophy.

2018

Expansion into Europe

Kymriah and Yescarta received European Commission approval, expanding access to CAR-T therapies across the EU. Manufacturing and regulatory frameworks began to evolve rapidly to accommodate the unique demands of autologous cell therapies. The year also saw a surge in CGT-focused investment and infrastructure development globally.



2020

A pandemic and a platform shift

While the COVID-19 pandemic disrupted clinical trials and supply chains, it also accelerated innovation in viral vector and mRNA platforms. CGT trials surpassed 1,000 globally by the end of the year, and the industry began to explore decentralized manufacturing and digital solutions to support complex therapy delivery.

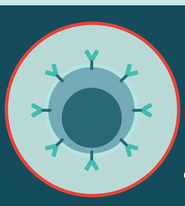
2019

Gene therapy goes systemic

The FDA approved Zolgensma (Novartis), a one-time gene therapy for spinal muscular atrophy delivered via systemic AAV9, and at $2.1 million, it became the most expensive drug ever approved. In Europe, Zynteglo (bluebird bio) was granted conditional approval for transfusion-dependent β-thalassemia, marking another step forward for lentiviral gene therapy.

2021

CAR-T diversifies



The CAR-T landscape expanded with the approval of Abecma (Bristol Myers Squibb/bluebird bio), the first CAR-T therapy for multiple myeloma, and Breyanzi (BMS) for large B-cell lymphoma. These approvals demonstrated the potential of CAR-T beyond initial indications and highlighted the growing role of dual partnerships in CGT commercialization.

2022

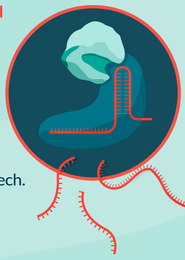
Hemophilia and beyond

Roctavian (BioMarin) received conditional EMA approval for hemophilia A, becoming the first gene therapy for this bleeding disorder. Meanwhile, Carvykti (Janssen/Legend Biotech) joined the CAR-T arsenal for multiple myeloma, and the field began to see increasing interest in allogeneic and off-the-shelf approaches. Upstaza (PTC Therapeutics) became the first approved gene therapy delivered directly into the brain, treating aromatic L-amino acid decarboxylase (AADC) deficiency.

2023

A CRISPR debut

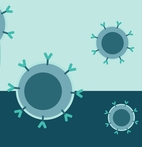
In a historic moment for gene editing, the FDA approved the first CRISPR-based therapies: Casgevy (Vertex/CRISPR Therapeutics) and Lyfgenia (bluebird bio) for sickle cell disease. These approvals validated years of research and opened the door to a new class of precision therapies. Blood cancer therapy NexCAR19 (ImmunoACT) became the first CAR T-cell therapy designed and approved in India, by a small Mumbai-based biotech.



2024

Decentralized CAR-T advances

Two new anti-BCMA CAR-T therapies for multiple myeloma were approved, each representing a milestone in regional innovation. In Spain, ARI0002h (Cesnicabtagene Autoleucel), developed by Hospital Clínic of Barcelona, became the first point-of-care CAR-T therapy approved in Europe. In China, Zevorcabtagene Autoleucel (CARsgen Therapeutics) received approval. Tecelra (Adaptimmune) also became the first engineered TCR therapy approved for a solid tumor indication, while Ryoncil (Mesoblast) was approved for steroid-refractory graft-versus-host disease – the first mesenchymal stem cell therapy of its kind.



2025

Individualized gene editing

The field of cell and gene therapy continued to expand both geographically and technologically. China approved Ruiboshen, its first MSC therapy, reflecting the field's growing global footprint. In a landmark for personalized medicine, clinicians at CHOP successfully treated an infant with CPS1 deficiency using a bespoke CRISPR base-editing therapy—the first case of personalized *in vivo* CRISPR gene editing. With over 4,000 CGT and RNA therapies in development, the field continues to evolve toward broader access and greater precision.

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

A decade of innovation

Perspectives from the *Cell & Gene Therapy Insights* Editorial Board

To celebrate the 10th anniversary of *Cell & Gene Therapy Insights*, members of our expert Editorial Advisory Board share their reflections on the remarkable progress of the past decade, the innovations shaping the field today, and their predictions for the future. From CRISPR-based therapies to *in vivo* engineering and decentralized manufacturing, their perspectives offer a snapshot of where the field stands and where it is heading next.

Cell & Gene Therapy Insights 2025; 11(9), 1187–1190 · DOI: 10.18609/cgti.2025.137

Q What has been the single most pivotal development in CGT over the past decade?

Across responses, two milestones stood out: the regulatory approval of CAR-T therapies, which proved that highly personalized treatments could be scaled and commercialized, and the rise of CRISPR/Cas9-based editing, which has revolutionized therapeutic design and enabled the first genome-editing approvals. Several editorial board members also noted advances in *in vivo* engineering and regulatory collaboration as key drivers of progress.

Clare Hague For me, as a health economist, the regulators' decision to fast-track CAR-T approvals, even with single-arm data, was pivotal. They struck a careful balance between unmet need and robust evidence, particularly for pediatric patients, and their willingness to work alongside manufacturers throughout the process set a strong precedent.

Philip D Gregory Two milestones stand out to me. On the technology side, CRISPR-based gene editing not only enabled the approval of Casgevy™ for sickle cell disease but also transformed our therapeutic toolbox for future therapies. On the commercial side, the approval of Kymriah™, the first CAR-T in the US, proved these treatments could become viable products and built confidence in their potential beyond B-cell malignancies.

Gary C du Moulin The broader adoption of rapid microbiological methods has transformed manufacturing. It has done so by enabling real-time contamination monitoring, strengthening quality control, streamlining regulatory review, and raising standards across the industry.

PANEL

(Left to right) **Jason Acker MBA PhD FCAHS**, Professor at the University of Alberta, Senior Scientist at Canadian Blood Services, and Senior Director at PanTHERA CryoSolutions; **Lee Buckler MBA LLB**, Managing Director of Cell Therapy Group, Partner at HighMont Advisors, and Senior Vice President of Advanced Therapies, Blood Centers of America; **Gary C du Moulin MS MPH PhD**, Executive Director of Product Development, Quality, and Regulatory Affairs at EASCRA Biotech; **Philip D Gregory DPhil**, Senior Vice President and Head of Regeneron Cell Medicines; **Clare Hague PhD LLM**, Founder, Oncology Access Solutions Ltd.

Q What are the most exciting recent advances in CGT?

Many contributors pointed to the rapid momentum behind *in vivo* therapies, new delivery technologies (viral and non-viral), and the expansion of CGTs into solid tumors and autoimmune diseases. Progress in automation, decentralized manufacturing, and quality standards is making therapies more accessible globally, while landmark case studies highlight the transformative potential of precision medicine.

Claudio Mussolino In a medical first, clinicians at CHOP and Penn treated an infant with a fatal urea cycle disorder using CRISPR base editors delivered via lipid nanoparticles. After three doses, the child showed significant health improvements, providing powerful proof-of-concept for *in vivo* therapies in rare genetic diseases.

Nate Manley Gene editing and *in vivo* therapies are advancing rapidly, with increasingly precise viral and non-viral delivery systems and new approaches for larger genetic payloads. Point-of-care manufacturing, bridging *ex vivo* and *in vivo* approaches, could ease cost and safety challenges while raising new regulatory considerations. Meanwhile, cellular immunotherapies are pushing into solid tumors, often in combination with checkpoint inhibitors and other modalities.

Jason Acker Closed-system, integrated bioprocessing platforms are making distributed manufacturing a reality, paving the way for advanced therapies to reach patients even in low- and middle-income countries.

Q What advice would you offer to researchers entering this field?

There was broad agreement that success in CGT demands early strategic planning, strong regulatory awareness, and cross-functional collaboration. Many emphasized the importance of mentorship and a focus on scalability and sustainability, alongside innovation.

PANEL

(Left to right) **Tamara Laskowski PhD**, Head, Biotech Partnerships & Strategy Head, Biotech Partnerships & Strategy, CTMC; **Nate Manley PhD**, Senior Principal and Head of Nonclinical at Dark Horse Consulting Group; **Aby J Mathew PhD**, Executive Vice President and Chief Scientific Officer at BioLife Solutions; **Claudio Mussolino PhD**, Group Leader, Transfusion Medicine and Gene Therapy at the University Medical Center Freiburg; **Pilar Redondo**, Site Head at Takeda Madrid (Tigenix)

Gary C du Moulin Adopt GMP principles early and embed a robust CMC strategy from the start. Building a culture of quality early on accelerates development and better equips teams for the challenges of scale-up.

Pilar Redondo "Begin with the end in mind." Ensure your therapy meets a real unmet need and is designed for long-term sustainability in both manufacturing and delivery.

Aby J Mathew Learn from the challenges others have faced in this space and surround yourself with experienced colleagues who have successfully brought therapies to market. Their expertise is invaluable.

Clare Hague Develop your pricing and market access strategy as early as your clinical plan. Innovation has little impact if patients cannot access it.

Q How do you see CGT evolving over the next 5–10 years?

Experts predict a decisive shift toward *in vivo* therapies, greater automation, and AI-driven innovation. Therapies will move beyond rare diseases to common conditions, with decentralization and manufacturing advances making treatments more accessible. Regulatory strategies are also expected to evolve to support this growth.

Claudio Mussolino In the coming decade, AI and synthetic biology will enable the design of safer, smarter therapies, including programmable immune cells equipped with logic-gated circuits that can dynamically respond to disease signals.

Aby J Mathew We are approaching a time when CGTs will move into frontline use. Advances in biopreservation and local delivery will expand accessibility, but vigilance will be essential to protect patients from unproven interventions.

Lee Buckler Within five years, we will see multiple autologous therapies manufactured in

closed, automated systems. By the end of the decade, cell therapies will be more affordable, widely available, and delivered in outpatient or community settings rather than specialized centers.

Tamara Laskowski The future of CGT lies in expanding beyond rare diseases to common conditions, with purpose-built therapies tailored to individual patients, designed for durability, and increasingly capable of delivering cures rather than disease management.

CELEBRATING THE JOURNEY AND ENVISIONING THE FUTURE

Taken together, these reflections capture a field that has made extraordinary strides, from the first CAR-T approvals to the dawn of *in vivo* gene editing. As highlighted by our Editorial Advisory Board, the next era of progress will be shaped by AI, novel delivery platforms, and decentralized manufacturing, moving CGTs beyond rare diseases and into mainstream medicine. Marking the 10th anniversary of *Cell & Gene Therapy Insights*, these perspectives remind us that the challenge now is not only to innovate but to make these therapies more accessible, affordable, and impactful for patients worldwide.

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CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

Founder's perspective

On 10 years of *Cell & Gene Therapy Insights*

As *Cell & Gene Therapy Insights* celebrates its tenth anniversary, **Abi Pinchbeck** (Editor, BioInsights) speaks with **Elisa Manzotti** (Founder of BioInsights) about the journal's origins, challenges, and evolution as an open access, digital, and translationally focused publication. Elisa reflects on the lessons she has learned about collaboration and quality, the importance of adding value in an increasingly crowded publishing landscape, as well as the joy of putting on her "science-geek hat".

INTERVIEW

Elisa Manzotti, Founder, *Cell & Gene Therapy Insights*

Cell & Gene Therapy Insights 2025; 11(9), 1223–1227 · DOI: 10.18609/cgti.2025.141

Q When you look back to the launch of *Cell & Gene Therapy Insights* ten years ago, what were your initial hopes and visions for the journal?

During the early stage of my career in STEM publishing, the field was all about print subscriptions—and getting more out of diminishing academic librarian budgets every year. Great content was being published behind a paywall, and no one was actually able to read it.

I felt that there were so many better ways to approach publishing. I thought it was the right time to take advantage of an ever-increasing number of digital platforms and technology. The speed with which you can get new research and information online is remarkable, especially without having to worry about compiling a print edition. Additionally, exploring the open access business model was, for me, incredibly important to ensure we could provide access to the community for free while supporting it through alternative revenue streams.

Meanwhile, my absolute passion for the CGT space cemented the idea. It has always been an area that have I loved since I launched *Regenerative Medicine* at the start of my editorial career. It is such a welcoming, exciting community to work within. When I spoke

to a few of my friends and colleagues who are experts in the field, there was a resounding noise of support about our launch, especially as there had been the emergence of exciting CAR-T cell therapy clinical data showing remarkable efficacy in patients at that time. The convergence of cell therapy and gene therapy was truly rejuvenating the space—with a great deal of investor confidence, but also huge challenges and hurdles ahead regarding manufacturability and tackling larger disease groups, as well as the rare diseases where these therapies were seeing success.

It felt like we could add a lot of value by positioning ourselves as a translational-focused journal. Basic research was already well covered in other publications and had lots of exciting developments, but addressing the challenges the sector was facing to make these therapies clinically and commercially viable was just as important. That, for me, was where it made sense to establish ourselves and provide a valuable content and discussion platform for that space.

I have always tried to ensure that we are adding value, not just noise. That has always been my approach as an editor: add value with the content you provide because people are busy and need a good reason to stop in their day and read your content, watch your webinar, or listen to your podcast.

Q Can you share a memorable moment or a challenge from the early days of launching the journal?

There were quite a few memorable moments, but certainly more challenges at the outset. Even though I had a good network of people in the cell therapy space, BioInsights was still a brand-new, relatively unknown publishing company. Much of the early work was arranging calls and meeting with people who were active in the space to understand how to shape the content.

There is a real danger if you only do desk research and assume you know what the community needs. You must go out there, ask the right questions, and understand the key pain points and challenges from those who face them day-to-day. Much of those early months involved knocking on as many doors as possible and speaking to as many people as I could to help shape the editorial calendar, ensuring that we were providing content that truly resonated with the community.

Another big challenge at the outset was running very lean. You become a bit of a jack-of-all-trades—I was teaching myself HTML coding for our first website, commissioning content, editing, and being a marketing manager at the same time. The early days were all about getting as much content online as possible to build the audience as quickly as possible.

The early goals were to build a reputation, gain trust as quickly as possible, and be seen by the community. As for memorable moments, I will never forget the slightly egotistical process of getting some of the launch issue printed purely for myself to be able to hold something tangible and show it to my parents!

Q Do you still have it?

I do—my mum recently sent me a photo of me with the launch issue of *Cell & Gene Therapy Insights* in print. I absolutely love it and was so proud in that moment. It is harder with

"I felt so privileged to meet people who were utterly transforming the face of modern medicine, yet were so humble and collaborative."

online publishing to get that sense of achievement once you click 'publish', versus holding the print copy, smelling the paper—it is lovely. I may one day frame it and attribute it to all my gray hairs.

There were many memorable moments after that, especially when I started attending events in the sector and we launched initiatives such as the Translational Pioneer Award, which we awarded to Professor Carl June one year, and Professor Maria Roncarolo another. I felt so privileged to meet people who were utterly transforming the face of modern medicine, yet were so humble and collaborative. Those are the moments where I would feel genuinely giddy, which really captures those early days.

Q From a strategic and business perspective now, what were some of the key hurdles?

It was crucial to be as clear as possible about the niche approach we were taking. I wanted to make it obvious that we were differentiating ourselves by focusing on the translational piece of the puzzle. This meant that having the right editorial advisory board and contributors from industry was key.

Having come from a much more traditional academic STEM publishing background, it was the norm to generally not engage with the industry because it would not get you an impact factor, and that was the metric you were supposed to care about. But that wasn't our business model. If you exclude the industry from the discussion because it is too commercial, then you are missing a huge and essential part of the conversation. Early on, I wanted to foster those relationships, gain their input, and help them feel that this was a publication and a community for them, too.

Considering the CGT space was abuzz with excitement, investment, and possibility, it was a wonderful time to engage with it and move quickly. It was also critical to respond rapidly when new topics, challenges, or exciting data emerged, and to ensure our editorial calendar reflected that.

For me, it was also about ensuring sustainability—meeting the needs of our clients and, in addition to being a trusted scientific publisher, building our reputation as a trusted partner in content marketing. It was about attending events, visiting clients' offices, chatting about their pain points in reaching their target audience, and co-creating exciting, valuable content with them. This approach has helped cement many of our long-term partnerships with clients.

Q What do you think sets *Cell & Gene Therapy Insights* apart from other journals in the field?

I wanted to carve out our niche in the translational space. Even though I love much of the basic research side of science, I love nothing more than seeing how it translates into actual changes in healthcare and outcomes for patients with really devastating diseases. That,

"...the key lesson I have learned about scientific publishing is that you must add value. There must be quality, and it must be obvious."

for me, was how we were going to differentiate ourselves from the rest of the publishing landscape and serve a genuine need.

It was also about ensuring as many people as possible could access that content—that it was not hidden behind a paywall and that we commissioned the content ourselves. By the time the open access movement had fully arrived, the landscape of journals had become oversaturated. It was difficult for researchers to differentiate between reputable journals and those that were simply trying to generate a new revenue stream with minimal quality underpinning their publication practices.

I did not want to run large calls for papers; instead, the focus was on ensuring the quality of our content so that our audience could trust it. That comes from conducting the research, finding the right people to speak to, and personally inviting them to contribute and implementing a rigourous peer review process. I think that is what truly sets us apart from other platforms and publications, in an era of somewhat crowded open access publishing.

Q What lessons have you learned about scientific publishing that you think are especially relevant in today's digital and open access era?

I learnt to not shy away from involving big pharma and biotech companies in the discussion. There is a huge number of amazing scientists and researchers within those industries who have valuable insights to share. Particularly at the outset of starting the journal, it was wonderful to see how collaborative people were. They shared real-world challenges, especially around the manufacturability of CGTs.

Beyond that, the key lesson I have learned about scientific publishing is that you must add value. There must be quality, and it must be obvious. In today's environment, where people are constantly bombarded with online content and are feeling more time poor than ever, it is crucial to find ways to demonstrate value and quality, even in shorter formats. It also is essential for us to keep abreast of trends in content consumption; we need to be aware of how people engage with content and innovate around that as well. We are focused on new product development within BioInsights, and a large part of that involves understanding how our audience wants to consume their content and making sure we meet those needs with innovative formats and channels.

Q What has been the most rewarding part of your journey with *Cell & Gene Therapy Insights*?

The fact that our publication is trusted and utlized by the cell and gene therapy community is incredibly rewarding. When I think about the early days, I can visualize myself in

my living room at midnight, coding on our WordPress website to get that first launch issue live. It feels like a lifetime ago, yet I can still picture it vividly. Seeing how far we have come, I occasionally allow myself moments of pride.

We receive a lot of incredible feedback—from our advisory board, webinar attendees, authors, and peer reviewers. People appreciate our personal touch, which, first and foremost, was very much ingrained in how I wanted to do this. From the outset, I wanted engagement with us to feel as personalised as possible. For instance, if someone has kindly agreed to speak on one of our webinars, they feel supported throughout the process, from preparing for the panel discussion to going live.

Much of our feedback revolves around how enjoyable it is to work with our team and how supported people feel, which, for me, is incredibly important. There have been so many rewarding moments. I have thoroughly enjoyed building up this business, and we have a wonderful group of people in the company with whom it is a pleasure to work. I feel very lucky to love what I do and so much of that is down to the great team I work with and our commitment to retain the mentality of a start-up even as we continue to scale. It makes for a very dynamic, fast paced environment!

I also feel very fortunate to work with so many people in the sector, to speak with them, and to learn from them, because it is such an exciting space. Being able to still put on my science-geek hat is very gratifying at this stage in my career.

The launch issue cover of *Cell & Gene Therapy Insights*, September 2015.

CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

Meet the *Cell & Gene Therapy Insights* Editorial Board

With 17 years of experience across academia, start-ups, pharma, and investment, Dr Nina G Bauer brings a unique, cross-sector perspective to the evolution of CGTs. As an Editorial Advisory Board member for *Cell & Gene Therapy Insights*, she reflects on the landmark approval of Kymriah, the rapid progress in *in vivo* CAR-T and non-viral delivery technologies, and the strategic and economic considerations shaping CGT's path toward accessibility and long-term sustainability.

INTERVIEW

Nina Bauer, Cell and Gene Therapy Consultant, Board Member, Strategic Advisor

Cell & Gene Therapy Insights 2025; 11(9), 1183–1185 · DOI: 10.18609/cgti.2025.136

Q What inspired your journey into CGT?

My journey began 17 years ago with an academic interest in tissue and organ regeneration. As the first cell therapies gained approval, I saw an opportunity to contribute to their commercialization and have been involved ever since.

Q What has been the most pivotal development in the past decade?

The approval of CAR-T therapies, particularly Kymriah in 2017, was a watershed moment. It validated the field and proved that complex, individualized therapies could be developed, approved, and delivered to patients.

Q What excites you most about the current landscape?

The first clinical data for *in vivo* CAR-T therapies is by far the most exciting development, alongside the expansion of CAR-T beyond blood cancers into autoimmune diseases and even early signs of success in solid tumors.

Equally transformative is the advancement of non-viral gene delivery in combination with gene editing. Together, these approaches signal a pathway toward greater accessibility and affordability; two things this field urgently needs to become a mainstay of modern medicine.

Q What is one piece of advice you would offer to researchers entering the field today?

In today's funding and geopolitical climate, focus is everything. I see three core areas that need attention:

1. Clinical development planning—Select indications and patient populations carefully, and plan how to manage them through clinical development into commercialization. Substantial preclinical data are essential to secure funding.
2. Health economics and manufacturing—Pricing and reimbursement depend on achieving sustainable COGs as well as well thought out health economic and competitive dynamics. Understand what price point is viable and ensure your manufacturing strategy can deliver.
3. The three Ps—Patients need education to drive demand, physicians need training to prescribe confidently, and payers need robust evidence of value to support reimbursement.

Q What developments do you expect to shape CGT in the next 5–10 years?

For autologous CAR-T, scaling to treat large autoimmune populations will require either making it a procedural product, like dialysis, or moving toward off-the-shelf solutions that combine small molecules, mRNA, or other modalities and potentially allogeneic approaches.

For gene therapy, viral approaches are likely to remain dominant for now, but innovations such as stable producer cell lines could help lower costs. Looking further ahead, I am optimistic about platform-based strategies, such as basket trials for gene editing, as demonstrated by initiatives like Danaher's Beacon program, which could streamline development and bring therapies to patients faster.

Q Outside of work, what are your hobbies?

I am currently learning a new language, which is proving to be a real challenge. I have also been traveling extensively, exploring regions both overseas and within the US. Seeing how people live, love, and think in different cultures is deeply humbling and provides an invaluable perspective.

Q What do you enjoy listening to?

I rely on the NPR app daily for news and insights, and I also enjoy the AltNPS account on BlueSky; make of that what you will.

Q What is your favorite snack?

Wasabi almonds and roasted seaweed snacks, particularly the salty or wasabi varieties, are my go-to favorites.

BIOGRAPHY

Nina Bauer is a trained neuroscientist whose career rapidly centered on the cell and gene therapy space, holding various technical and commercial roles at the Scottish Center for Regenerative Medicine (Edinburgh, UK) and the Cell and Gene Therapy Catapult (London, UK). She deepened her global commercial experience establishing Lonza's autologous Cell Therapy Business and strategic positioning by incorporating the Cocoon™ technology with a vision for near-patient manufacturing (Basel, Switzerland), as well as leading commercial teams at MilliporeSigma/Merck KGaA (Boston, MA, USA) focusing on the cell therapy product portfolio, gene therapy manufacturing services and CRISPR IP licensing. Aside from large corporate and public sector leadership roles, Nina established her start-up and C-suite acumen as the Chief Commercial Officer for FloDesign Sonics (Wilbraham, MA, USA), leading to the company's acquisition by MilliporeSigma, as well as a wide range of advisory board engagements.

Nina Bauer PhD MBA, Cell and Gene Therapy Consultant, Board Member, Strategic Advisor, Boston, MA, USA

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CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

Meet the *Cell & Gene Therapy Insights* Editorial Board

In celebration of *Cell & Gene Therapy Insights*' 10th anniversary, Editorial Advisory Board member Dr Alexis Cockroft reflects on 20 years in regulatory affairs and lessons learned from supporting the approval of Strimvelis, Europe's CGT innovation. They discuss the growing importance of decentralized manufacturing and global regulatory strategies, and why humility is essential to building safe, sustainable therapies with broad patient access.

INTERVIEW

Alexis Cockroft, Director and Regulatory Consultant, Lex Regulatory Ltd

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Q What inspired your journey into CGT?

I became the CMC regulatory lead on the Strimvelis marketing authorisation application in mid-2011. From that point, I was hooked and have stayed in the CGT field ever since.

Q What has been the most pivotal development in the past decade?

I consider the CGT field as a hive; a colony of specialists and generalists building on collective progress. While it is tempting for individuals to believe their contribution was pivotal, most advances are the result of many minds working together. For me, the most significant development has been the spirit of collaboration, sharing, and learning.

That said, while COVID-19 was a deeply challenging period, it also served as a catalyst for extraordinary innovation. The accelerated development and global rollout of adenoviral vector and mRNA-based products provided invaluable lessons in manufacturing, regulation, logistics, and distribution.

Q What excites you most about the current landscape?

Decentralized manufacturing has enormous potential to improve patient access to advanced therapies, and I am encouraged by the close collaboration between experts across disciplines. I am also excited about approaches that increase precision and safety, such as *in situ* gene editing methods with minimal off-target effects, multifactorial strategies to reduce risks from T-cell therapies, and co-developed medical devices that protect products and standardize administration.

Equally inspiring is the expansion of regulatory approvals into more regions worldwide, which is critical for broadening patient access.

Q What is one piece of advice you would offer to researchers entering the field today?

Humility is essential. It helps us recognize what we do and do not know, how to identify who to consult, and how to focus on delivering therapies that are needed, reliable, and sustainable.

'Needed' refers to prioritizing unmet medical needs; 'reliable' ensures consistent efficacy and safety for every patient and batch; 'sustainable' considers the cost, resource use, and environmental impact.

Q What developments do you expect to shape CGT in the next 5–10 years?

Historically, many companies launched products in the US first, followed by other markets. Over the next 5–10 years, I anticipate a more comprehensive and balanced global regulatory strategy. I have seen remarkable dedication and collaboration from agencies such as the EMA, MHRA, FDA, and WHO. Widening regulatory pathways and working closely with these agencies will be key to positive progress.

Q Outside of work, what are your hobbies?

I enjoy horse riding, beekeeping, yoga, and walking my dog, and I have recently started breeding roses. Exploring new foods and drinks is another favorite pastime.

Q What do you enjoy listening to?

I particularly enjoy podcasts such as *In Our Time* and *You're Dead to Me*, which offer both educational and entertaining content.

Q What is your favorite snack?

If I had to choose, I would say nuts, peanuts, and dried figs, so perhaps I was a squirrel in a past life!

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BIOGRAPHY

Alexis Cockroft is an independent regulatory affairs consultant, specializing in CMC regulatory affairs (CMC RA) for cell and gene therapies (advanced therapy medicinal products). She has worked in CMC RA for more than 19 years and has focused solely on advanced therapies for the last 13 years. She has also worked in various science-based roles.

Alexis Cockroft PhD, Director and Regulatory Consultant, Lex Regulatory Ltd, Royston, Cambridgeshire, UK

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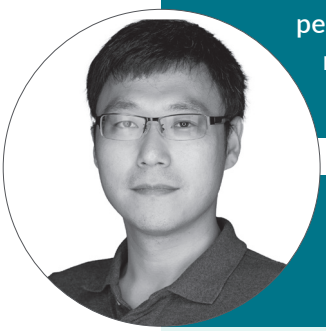
CELL & GENE THERAPY INSIGHTS 10th BIRTHDAY

Meet the

Cell & Gene Therapy Insights Editorial Board

To celebrate *Cell & Gene Therapy Insights*' 10th anniversary, Editorial Advisory Board member Dr Yu (Clay) Cao reflects on a decade in cellular immunotherapy, viral and non-viral vectors, gene editing, and stem cell therapy. He highlights the emergence of in vivo CAR-T therapies, transformative regulatory approvals in 2025, and the expanding reach of RNA- and CRISPR-based approaches. Dr Cao shares his perspective on translating cutting-edge science into scalable, patient-ready therapies, offering advice for researchers navigating this rapidly evolving field.



INTERVIEW

Yu Cao, Vice President and Global Head of Drug Discovery, GenEditBio

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Q What inspired your journey into CGT?

The past decade has been a transformative era for cellular immunotherapies and gene editing. I was drawn to this field by its potential to fundamentally reshape the way we treat diseases ranging from cancer to autoimmune and neurological disorders. Over time, my work has expanded to encompass viral and non-viral vectors, *in vivo* gene editing, and stem cell–based approaches, all with the goal of advancing therapies that are not only more effective and safer but also more broadly accessible to patients.

Q What has been the most pivotal development in the past decade?

The emergence of *in vivo* CAR-T therapy over the past two years represents one of the most exciting and potentially disruptive breakthroughs in the field of cell therapy. Unlike traditional CAR-T approaches, which rely on extracting, engineering, and reinfusing patient T cells in an *ex vivo* process, *in vivo* strategies deliver gene-editing and reprogramming components directly into the patient. By enabling T cells to be modified within the body,

this paradigm shift has the potential to redefine T-cell engineering, simplify manufacturing, and expand clinical access. If successful, *in vivo* CAR-T could make transformative immunotherapies faster, more affordable, and scalable to broader patient populations.

Q What excites you most about the current landscape?

2025 is shaping up to be a landmark year for CGT. We are witnessing rapid progress in *in vivo* gene editing and immune cell reprogramming, alongside the rise of novel RNA- and mRNA-based therapeutics expanding into rare diseases, autoimmune disorders, and cancer. Several *in vivo* CAR-T and CAR-M therapies have now advanced into clinical trials, showing early signs of efficacy and significant market potential. In parallel, highly effective *in vivo* gene therapies for sickle cell disease are demonstrating preclinical proof of concept, while precision CRISPR treatments are being designed and manufactured for individual patients within mere months. Beyond these milestones, RNA editing and exon-skipping strategies enabling durable dystrophin restoration in Duchenne muscular dystrophy, offering new hope to patients who previously had few options. While the long-term efficacy and safety of these approaches will require continued validation, the leap from concept to first-in-human applications represents the most exciting phase of innovation for me. I am confident that with iterative optimization, we will further mitigate risks and unlock the full potential of these transformative therapies.

Q What is one piece of advice you would offer to researchers entering the field today?

As a standalone motto: Master the biology but stay grounded in translational reality.

Exciting science alone is not enough; therapies succeed only when they are effective, safe, regulatory-ready, and manufacturable at scale. To truly advance the field, researchers should:

1. Understand the full translational journey from study design and platform development to clinical trials and patient access.
2. Collaborate across disciplines, working hand-in-hand with immunologists, bioengineers, regulatory experts, clinicians, and manufacturing teams throughout the process.
3. Gain early insight into CMC and regulatory science and build expertise in GMP manufacturing, potency assays, release criteria, and durability requirements upfront.
4. Think beyond the regulator. Patient safety and benefit are developer responsibilities. A strong drug developer goes deeper than regulatory minimums, not just 'checking the box'.
5. Stay agile. Recognizing that animal data may not translate to humans, and today's breakthrough may be obsolete tomorrow. Agility and innovation are essential.

Q What developments do you expect to shape CGT in the next 5–10 years?

The coming years will be transformative for cell and gene therapy—not only through scientific breakthroughs, but also through regulatory evolution and improved clinical accessibility. Therapies are set to become better, safer, faster, and cheaper. Key developments include:

1. Broadening indications
- Expanding from oncology and rare genetic disorders to autoimmune, cardiovascular, neurodegenerative, and metabolic diseases.
- Transitioning from high-mortality conditions to prevalent, chronic disorders affecting larger patient populations.

2. Rise of *in vivo* editing
- Rapid advancement in delivery modalities and editing platforms.
- Emergence of single-dose, outpatient 'needle-to-cure' approaches, supported by growing clinical evidence.

Requires strong frameworks for safety monitoring and off-target risk management.

3. Precision and control technologies
- Introduction of tunable expression systems, safety switches, and reversible editing.
- Enhances therapeutic specificity while mitigating long-term risks.

4. Smarter, decentralized manufacturing
- Leveraging AI, automation, and point-of-care systems.
- Improves scalability, consistency, and affordability of advanced therapies.

5. Evolving clinical and regulatory paradigms
- Ongoing updates to regulatory guidance for ATMPs, gene editing, and cell therapies.
- Long-term safety monitoring embedded as a requirement for equitable and accelerated patient access.

It is worth noting that Asian biotech companies are emerging as a major force reshaping the global cell and gene therapy landscape. Based on the surge of East-to-West licensing deals in 2025, this trend is expected to accelerate, expanding both the diversity of technologies and the range of therapeutic applications.

China, in particular, has introduced a more flexible clinical investigation framework, enabling local innovations to gain clinical proof-of-concept more rapidly. As a result, an increasing number of Chinese-developed platforms are becoming attractive to global partners and investors. Over the next few years, this dynamic is likely to broaden the global CGT pipeline and intensify cross-border collaboration.

BIOGRAPHY

Yu Cao, is an accomplished biotech team builder and scientific leader with over 15 years of experience in drug discovery and translational development. Currently serving as Vice President and Global Head of Drug Discovery at GenEditBio, Dr Cao leads cross-functional teams advancing cell and gene therapies across rare diseases, oncology, and neurodegenerative disorders. He has deep expertise in preclinical development, IND-enabling studies, and *in vivo* delivery technologies, with a strong track record of progressing novel therapeutics from discovery to clinical stage. Prior roles include team leadership at Tessera Therapeutics and Agios Pharmaceuticals, where he drove innovative programs in immuno-oncology and gene editing. Trained as an immunologist and molecular biologist, Cao holds a PhD in Immunology, with additional credentials from Harvard Business School and Johns Hopkins University. He actively contributes to the field as an advisor or leadership member of different non-profit organizations.

Yu Cao PhD, Vice President and Global Head of Drug Discovery, GenEditBio, Boston, MA, USA

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