EDITION
2024

USP and DSP for viral vectors

mabdesign
THE BIOTHERAPY EXPERT

ABOUT **MABDESIGN**

MabDesign, the French Association of the Biotherapy Industry

MabDesign, the French biotherapy industrial association, aims to support, federate and increase the visibility of the biopharmaceutical industry, foster exchanges, promote the development and competitiveness of companies, and stimulate innovation by encouraging the emergence of start-ups from academic research.

In order to carry out its development strategy and to adapt to changes in the industrial ecosystem, MabDesign's governance has evolved to meet the specific needs of the various companies working in the biotherapy industrial sector. Therefore, the Board of Directors of MabDesign already composed of DBV Technologies, Lyonbiopole, Pierre Fabre and Sanofi, has been strengthened with the arrival of ABL Europe, bioMérieux, Institut Pasteur, Thermo Fisher Scientific and TreeFrog Therapeutics as well as three Qualified Persons with Nicola Beltramineli (Innate Pharma), Hervé Broly (Merck), and Stéphane Legastelois (33 California). Their arrival to the Board of Directors reinforces MabDesign global vision of the current challenges and opportunities of the biopharmaceutical industry.

Moreover, to achieve its goals MabDesign sets up a coherent set of actions promoting exchanges, collaborations and skills development. In this dynamic MabDesign has developed a **national directory** that brings together industrial and academic players in biotherapy and allows to identify online the know-how available in France. MabDesign organizes high-level **international scientific events**, in collaboration with key ecosystem players, to highlight innovation and stimulate exchanges between companies in the sector. With the help of its Scientific Committee (**COSSF**), MabDesign writes summary reports (**ImmunoWatch and BioprocessWatch**) for the biotherapy industry. MabDesign offers specialized and **innovative continuous professional training** solutions to enable companies to adapt their skills to the market evolution and maintain their competitiveness. Finally, MabDesign offers its members a **wide range of services** to help companies of all sizes to optimize their positioning, protect and enhance their innovations, conquer new markets and raise public funds.

Operational since September 2015, MabDesign currently has over **280 member companies** and its diversity is its strength. MabDesign's dynamic network includes pharmaceutical and biotech companies, service providers (eg. CROs, CDMOs, etc), professional training actors, high-tech equipment suppliers and specialized consultants.

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INTRODUCTION

For several years now, MabDesign has been actively participating in national and regional programmes and organising scientific events and gatherings focusing on bioprocessing. In parallel, we have also been providing strategic consultancy services together with various training opportunities to key actors of this field, including academia, public bodies, SMEs and biotech and pharmaceutical companies, that are involved in the shaping of the bioprocessing industry in France through their R&D, innovation, technologies, services and products. In line with these past and current actions and to further our commitment and support to the French bioprocessing industry, MabDesign has launched in 2021 a second information-monitoring letter, the BioprocessWatch series.

Each edition of BioprocessWatch will focus on current challenges, a critical step or a recent innovation linked to the manufacturing of a specific biopharmaceutical product or affecting the whole field. BioprocessWatch will feature invited scientific contributions from academia and/or the industry, the most recent pipeline, economic and financial data (where applicable), insights into the intellectual property related to the theme and opinion articles and interviews from one or two experts working in the field.

Finally, we would like to acknowledge the continued support from the Centre-Val-de-Loire (CVL) region, through their Ambition Recherche et Developpement (ARD) CVL Biomédicaments programme, in making the launching of the BioprocessWatch series possible.

EDITORIAL

Jérôme BEDIER
CEO Flash Biosolutions

Navigating the Analytical Landscape in Modern Bioprocessing

Lentiviral vectors are rapidly emerging as indispensable tools in gene therapy and immunotherapy, thanks to their remarkable capacity to efficiently transduce a diverse array of cell types, including immune cells. This versatility positions them as key candidates for developing innovative therapeutic approaches across a range of diseases.
In the realm of immuno-oncology, lentiviral vectors have become instrumental in genetically modifying T cells to express chimeric antigen receptors (CAR-T), specifically targeting tumor cells. CAR-T therapies utilizing lentiviruses have already yielded impressive clinical outcomes, particularly in treating certain hematological malignancies like leukemia and lymphoma, leading to the approval of the first genetically modified cell therapies, marking a milestone in the fight against cancer. The potential of lentiviruses extends beyond cell therapies to include cancer vaccine development, where they are being used to transduce dendritic or tumor cells to provoke robust anti-tumor immune responses.
This successes opened doors to new strategies beyond oncology.

Expanding Beyond Oncology

The applications of lentiviral vectors are not limited to cancer. In autoimmune disease treatment, researchers are exploring their potential to deliver immunomodulatory genes or suppress pathogenic gene expression. Preclinical studies have shown encouraging results in models of multiple sclerosis, rheumatoid arthritis, and type 1 diabetes, opening new avenues for therapeutic interventions for chronic immune-related conditions.
Concurrently, lentivirus applications are expanding into other critical medical domains, including neurodegenerative diseases, primary immunodeficiencies, and hemoglobinopathies. Clinical trials employing lentivirus-modified hematopoietic stem cells have demonstrated efficacy in treating conditions such as X-linked adrenoleukodystrophy and β-thalassemia, diseases that previously had limited treatment options.

Clinical Impact and Innovation

By 2023, lentivirus technology has supported 64% of clinical trials involving genetically modified cells, with nearly 340 studies in progress, underscoring their pivotal role in shaping future medical treatments, with implications across a broad spectrum of genetic, immune, and degenerative diseases.
Flash BioSolutions continues to push the boundaries of innovation with advanced Lentiviral technologies: FlashRNA®. This cutting-edge technology, which marries the cell transduction capabilities of lentiviruses with the transient, multi-stranded RNA transport and delivery prowess of bacteriophages, has achieved unprecedented levels of purity and potency.
The inaugural clinical application of this groundbreaking technology is set for 2025, in collaboration with Inserm and Toulouse University Hospital, as part of the European TheraLymph program.
This ambitious initiative aims to cure secondary lymphedema, a common complication following breast cancer surgery, offering new hope for patients suffering from this debilitating condition.

Looking Forward: The Promise of Lentiviral Technology

As the landscape of gene therapy and immunotherapy continues to evolve, lentiviral vectors are set to play an increasingly prominent role. With daily advancements expanding the boundaries of possibility, lentivirus technology is poised to reshape the future of medicine, offering new and more effective treatments for a wide range of diseases.
This rapidly advancing field, exemplified by the innovations in lentiviral vectors and FlashRNA® technology, offers a glimpse of a future where genetic therapies are not just a last resort, but a frontline strategy for treating and curing some of the most challenging diseases of our time.

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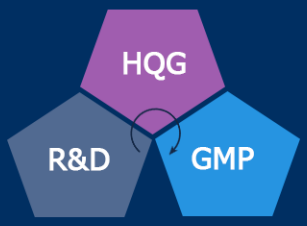

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Read the different inputs from the scientific community on viral vector bioprocessing

THE BIOPRODUCTION OF VIRAL VECTORS: ISSUES, INNOVATIONS AND MARKET PROSPECTS

By MabDesign

Viral vectors are essential therapeutic tools for gene therapy, genetically modified cell therapies and next-generation vaccines. They are used to deliver therapeutic genes or genetic material coding for antigens into host cells.

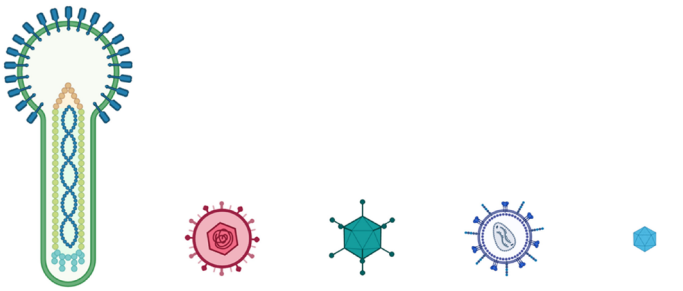
Gene therapy, an innovative class of biological products, has opened up new horizons making it possible to solve previously unmet medical needs, particularly in oncology and rare diseases. With more and more cell and gene therapy products coming to market, the FDA has forecast the approval of 10 to 20 new products a year between 2019 and 2025, based on an assessment of the pipeline and the clinical success rates of these products.

Against this backdrop, the bioprocessing of viral vectors, which is the most widespread modality, has become a strategic sector stimulating innovation and development to meet the technical, economic and logistical constraints of the field.

Here we provide an overview of the current challenges in viral vector biomanufacturing, as well as the emerging innovative technologies to meet the growing demand. We highlight the key figures for the viral vector biomanufacturing market and its major players.

I. The challenges of viral vector biomanufacturing

Viral vectors are used for a variety of applications, including 1) in vivo gene therapy targeting rare acquired or inherited disorders, 2) engineering of autologous/allogeneic cells for ex vivo cell therapies, 3) recombinant vector vaccines, and 4) engineering of plants and the animal gut microbiome. In the field of gene therapy, adeno-associated viruses (AAVs) are the vector of choice due to their tissue tropism, low immunogenicity and genotoxicity, and efficient transduction with sustained gene expression. Lentiviruses provide highly stable transgene expression, which is why they are used for ex vivo gene therapy, mainly to produce CAR-T cells or modify pluripotent and haematopoietic stem cells. Adenoviruses have become a vector of oncolytic agents and vaccines based on their innate immunostimulatory role and replication activity



	Baculovirus (BV)	HSV	Adenovirus (AdV)	Lentivirus (LV)	Adeno-Associated virus (AAV)
Size (nm)	length ~200 - 450 diameter ~30 - 100	~80 - 100	~70 - 100	~80 - 120	~20-25
Genome	dsDNA	dsDNA	dsDNA	ssRNA	ssDNA
Genome capacity (kB)	~100	~40	~8-38	~9	~4.8
Biosafety Level	BSL1	BSL1	BSL2	BSL2	BSL1
Advantages and Applications	▸ It can be easily modified to accommodate large insertions of foreign DNA. ▸ BacMan is a technique for the expression of proteins inside mammalian cells. ▸ BV produces high levels of properly folded recombinant proteins.	▸ Strong neural tropism ▸ Large transgene capacity	▸ Efficient transduction in most mammalian cell types and tissues ▸ Infects dividing and non-dividing cells	▸ Continuous transgene expression via host cell genome integration ▸ Infect dividing and non-dividing cells regardless of their proliferation status ▸ Low inflammatory potential	▸ Low Immunogenicity ▸ Safe transgene delivery ▸ Non-pathogenic ▸ Different serotypes have specific tropism
Limitations	Not fully characterized	High cytotoxicity	Transient transgene expression.	Possible oncogenesis	Low transgene capacity

Figure 1: Heterogeneity of viral vectors: size, genomic capacity and main applications. Source : R. Kilgore, 2023.

The development and production of viral vectors are more recent than those of other classes of biologics, such as monoclonal antibodies. There are currently no universal, standardised manufacturing processes as compared to the many gold-standards of monoclonal antibody bioprocessing. In addition, there are major differences between these two types of molecule that prevent methods from fluidly being transposed: viral vectors are larger and more complex molecules, requiring a higher level of control and biosafety (BSL-2 for most viral vectors compared with a BSL-1 classification for mAbs), which adds to production and installation costs.

From small-scale to industrial production

The players involved use different upstream production systems and downstream processes, and face various challenges. In particular, scaling up is a difficulty encountered by many academic laboratories, which are developing production processes on a small (laboratory scale), with cell cultures adhering in multilayer flasks. These processes, which require manual handling, make scale-up difficult if not impractical. For time and cost issues, companies still often produce the first early batches using these 'laboratory' processes. They are then faced with major challenges in transferring production processes and scaling-up to manufacture larger batches while meeting regulatory requirements.

The production of viral vectors is preceded by the design and manufacture of plasmids. Plasmid requirements are high and can be a critical step. Unlike CHO, the gold-standard cell line used for monoclonal antibody production, HEK293 cells, used for viral vector production, have fewer stable clones. Most of the time, viral vectors are produced using transient transfection. In other words, each time a recombinant virus is produced, several plasmids are needed to transfect the cells. This puts pressure on the manufacture of plasmids, as demand increases and influences plasmid supply costs.

Meeting the need for strict regulatory compliance

The challenges of meeting regulatory requirements and quality control in the production of viral vectors are crucial, as these products are intended for sensitive medical applications such as gene therapies and vaccines. Regulatory authorities, such as the FDA, Food and Drug Administration (United States) and the EMA, European Medicines Agency (Europe), impose strict standards to guarantee the safety, efficacy and traceability of vectors used in clinical treatments.

Analytical methods for controlling product quality throughout the process are therefore developed right from the research & development of the therapeutic product at laboratory level. Several quality control attributes (QCAs) are commonly analysed, including safety, identity, functionality, purity and viability. Controlling these QCAs enables us to demonstrate that the final product does not vary significantly from one batch to the next.

Determining these critical attributes is part of the Quality by Design (QbD) approach, which aims to integrate product quality right from the development stages. The aim is to ensure upstream that the sources of variability in the process are identified and dealt with, so as to guarantee that the finished product downstream conforms to the predefined characteristics. The QbD approach is not a regulatory requirement but a quality assurance approach, strongly recommended by the health authorities: all the new ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use), FDA and EMA guidelines use QbD concepts and terminology.

Figure 2: Methodology overview using process and product knowledge to develop the QbD approach to serve safety, purity and efficacy of AAV drug products. QTPP: Quality Target Product Profile, CQA: Critical Quality Attributes, CPP: Critical Process Parameters. Source : Cytiva.

II. Innovations in the bioproduction of viral vectors

Evolution of culture methods

The HEK293 cell lines usually used for viral vector production are adherent cells. To overcome the limitations of traditional adherent cell culture methods, new technologies have been developed that offer considerable advantages for industrial production. These include suspension cell culture methods, which offer titers similar to those of adherent culture and support higher cell densities (Figure 3).Innovations in bioreactor suspension culture processes have led to production capacities of 1,000 to 2,000 litres.

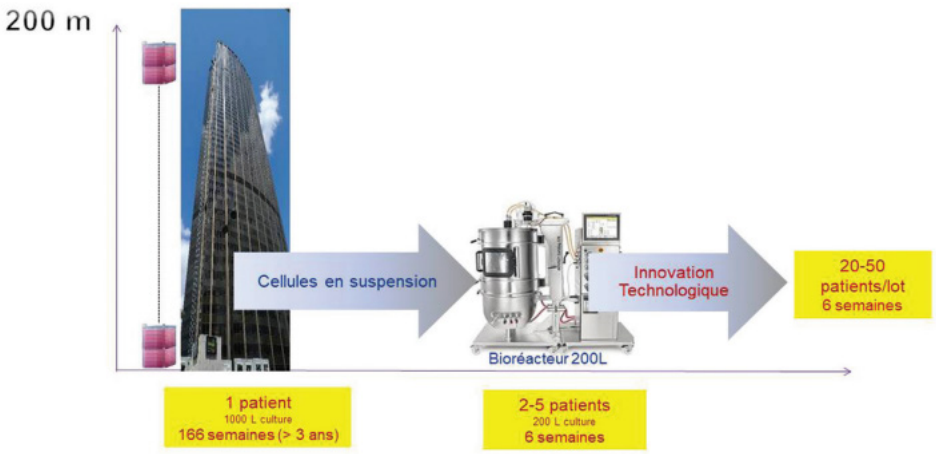


Figure 3: Evolution of viral vector production processes using bioreactors for suspension production. Source : A Lamproye, 2023.

Purification technologies

Downstreaming viral vectors involves a number of constraints, particularly in relation to the size of the molecules, the fragility of the viruses and the presence of a large number of impurities (plasmid DNA, cellular proteins, etc).

Immunoaffinity chromatography (IAC) is currently the most widely used method for purifying viral vectors. Dynamic research by academics and industry has identified antibodies and antibody fragments used as affinity ligands specifically for viral vectors. A major breakthrough in IAC technology has been achieved with Cytiva's AVB ligand, based on a single-domain camelid antibody (VHHs or Nanobodies®). This technology has stimulated the development of ligands designed for the purification of VVs (ThermoFisher's POROS Capture Select or Repligen's CaptureSelect).

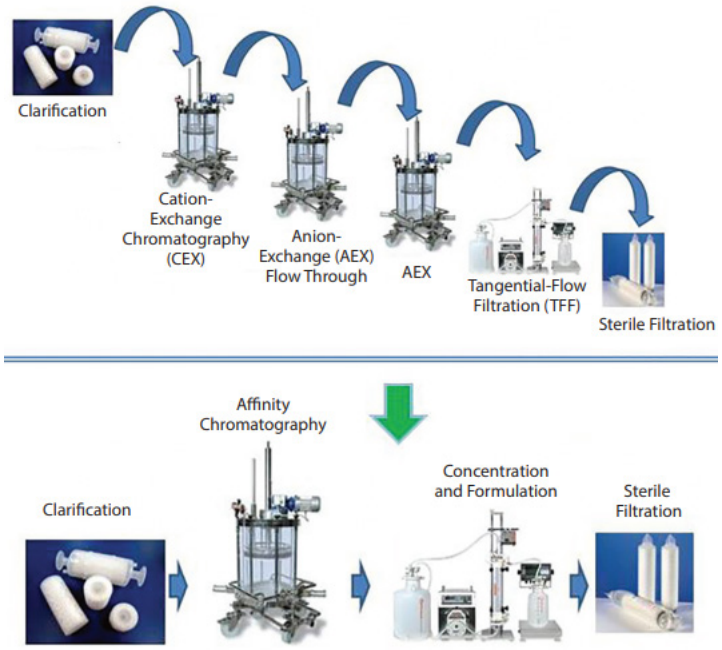


Figure 4: Parallel between standard downstream process techniques (top) requiring several chromatography, filtration and capture steps, and innovative techniques (bottom) with a single affinity chromatography step. Source : O. Terova, 2016.

Ion exchange chromatography (IEX) is the main alternative to affinity chromatography for the primary capture step and is the predominant technology used for viral vector polishing. IEX has a powerful ability to separate empty capsids from full capsids, which is a significant advantage over affinity chromatography. This separation is important because full capsids are the carriers of therapeutic genetic material in gene therapy applications and higher full-to-empty ratios can increase treatment efficacy.

Recent successes with new methods such as steric exclusion chromatography and supports such as monoliths and membranes can reduce processing times by taking advantage of the size properties of viral vectors. Further developments should be done on continuous chromatography, which could enable the process to be intensified and costs reduced, which is important to facilitate access to these therapies.

III. The viral vector biomanufacturing market

A growing market

In recent years, the number of viral-vector-based gene therapies approved and in development has increased, with 10 products having received marketing authorisation and 1,229 products in development in the active pipeline (Figures 5 & 6). This increased number of approvals and ongoing clinical trials related to viral vector-based gene therapy has led to a clear increase in demand for large-scale viral vector manufacturing.

Name	Brand	Company	Indication	Vector
Onasemnogene abeparvovec	Zolgensma	Novartis	SNC	AAV
Gendicine	Gendicine	Shenzen SiBiono GeneTech	Oncology	Adenovirus
Voretigene neparvovec	Luxturna	Novartis	Ophtalmology	AAV
Beremagene geperpavec	Vyjuvek	Krystal Biotech Inc	Dermatology	HSV
Delandistrogene moxeparvovec	Elevidys	Sarepta Therapeutics Inc	Genetic disorders	AAV
Eladocagene exuparvovec	Upstaza	PTC Therapeutics Inc	Genetic disorders	AAV
Etranacogene dezaparvovec	Hemgenix	CSL Ltd	Hematology	AAV
Nadofaragene firadenovec	Adstiladrin	Ferring Pharmaceuticals	Oncology	Adenovirus
Valoctocogene roxaparvovec	Roctavian	BioMarin Pharmaceutical	Hematology	AAV
Fidanacogene elaparvovec	Beqvez	Pfizer	Hematology	AAV

Figure 5: Approved in vivo gene therapies based on viral vectors. Source : GlobalData 2024, MabDesign.

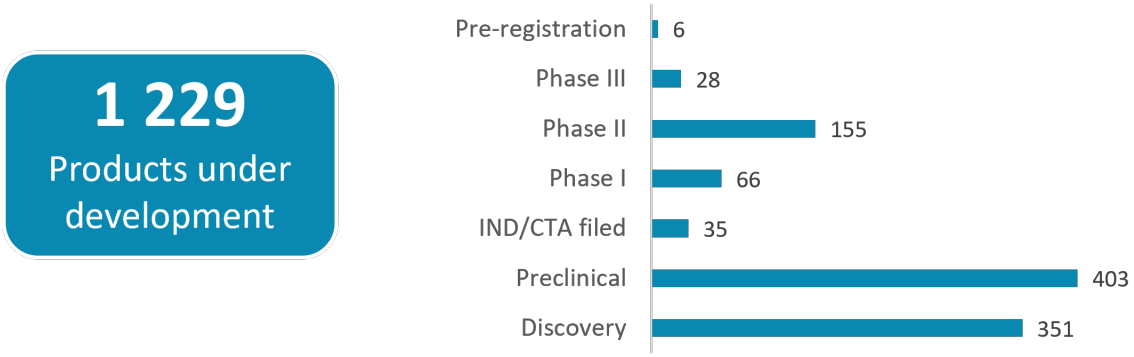


Figure 6: In vivo gene therapy products based on viral vectors in development. Repartition by development phase. Source : GlobalData 2024, MabDesign.

This dynamic has been reflected in the number of investments and deals by CDMOs and biotech and pharmaceutical companies, either to obtain new production capacity or to acquire existing capacity. In addition to CDMOs and in-house production sites, hospitals and university centres are also building capacity to meet Phase I/II production needs. The landscape of CDMO players has thus expanded, and by 2022 there will be more than 50 players in Europe and the USA (Figure 7). The market can be defined as concentrated, with 5 players holding 40% of the total available capacity: Catalent, FujiFilm Dyosinth, Lonza, Thermo Fisher Scientific and WuXi Advanced Therapies. These CDMOs have existed for many years and have often developed in the field of gene therapy following acquisitions, such as that of US company Brammer Bio by Thermo Fisher in 2019. With an estimated CAGR of between 10 and 20% between 2024 and 2030 in the gene therapy field, further mergers or acquisitions of companies in the near/medium term are to be expected.

Changing business models in biomanufacturing

The biomanufacturing offer available for viral vectors remains a challenge. In vivo gene therapy, where the final product is the viral vector injected directly into the patient, mainly uses AAV, which makes this vector particularly sought-after. On the other hand, volumetric capacities do not always meet the need:

the batch sizes required for in vivo gene therapies are increasingly large as their application extends beyond the therapeutic area of rare diseases.

Strategic divergences are emerging, leaving the way open for larger companies with sufficient financial resources to acquire the capacity to internalise production (e.g. Cellectis). This option also enables them to limit the risk of supply shortages and retain their intellectual property. Smaller companies that want to bring their product to market but do not have enough resources to invest in production capacity will turn to outsourcing, sometimes facing long lead times. Nevertheless, speed to market is essential for gene therapy products, which can often benefit from fast-track designations (or orphan drug status, for example) that reduce drug development time, putting even more pressure on CDMOs.

Alternative structures offer a response to the growing challenges of industrialising gene therapies, such as Shared Manufacturing Organizations (SMOs) or modular 'plug-and-play' facilities. The SMO concept is based on the idea of pooling production capacity between several players. Rather than building dedicated plants, biopharmaceutical companies, including start-ups and biotech companies, can access shared facilities where production is tailor-made for different customers. Modular plug-and-play facilities represent another key innovation in the production of biomedicines. These facilities are designed to be rapidly deployed, flexible and modular, enabling companies to adapt to production needs in the shortest possible time.

In September of this year, Sanofi inaugurated Modulus, a plant capable of adapting to manufacture up to 4 vaccines or biopharmaceuticals simultaneously, and of reconfiguring itself in a matter of days or weeks to change technological platforms (live attenuated viral vaccines, recombinant protein vaccines or messenger RNA vaccines, as well as biotechnology-derived treatments such as enzymes or monoclonal antibodies).INITS has invested near Montpellier to create the INITS-SMO (Shared Manufacturing Organization) plant, which is designed to provide biotechnology companies with premises that meet GMP pharmaceutical standards, enabling them to produce their own batches of innovative drug candidates for preclinical and clinical trials.

IV. Outlook and conclusion

Approaches such as Artificial Intelligence (AI) and Industry 4.0 may offer an interesting potential for transformation to meet the challenges of viral vector production mentioned above.

In particular, AI-based algorithms could provide more predictive quality control, using machine learning models to reduce production failures. Industry 4.0, which integrates AI directly into its overall system, makes it possible to create digital twins providing simulations of the process with the aim of optimising it. In 2023, Généthon and Thalès have announced a collaboration to develop a digital model that will use Artificial Intelligence to model bioprocessing steps and optimise yields.

75% of in vivo gene therapies currently being developed use viral vectors, which include retroviruses, lentiviruses, adenoviruses and adeno-associated viruses. Thanks to their high infectivity, viral vectors are often very effective for gene transfection. However, there are concerns about their clinical safety due to their tendency to induce immune responses and mutations caused by transgene insertion. As an alternative, non-viral vectors are increasingly being developed in the wake of the COVID19 crisis and with the emergence of mRNA vaccines. In particular, lipid nanoparticles (LNPs) or cationic polymers, which have demonstrated robust gene loading capacity, high safety and practicality. They thus offer interesting potential for gene delivery.

With technological advances and a growing understanding of virus biology, the prospects for development and innovation in the production of viral vectors promise to improve their efficacy,

safety and personalisation, paving the way for increasingly targeted and accessible gene therapies and vaccines for a wide range of diseases.

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NanoLuc Based Neutralization Antibody (Nab) Assay

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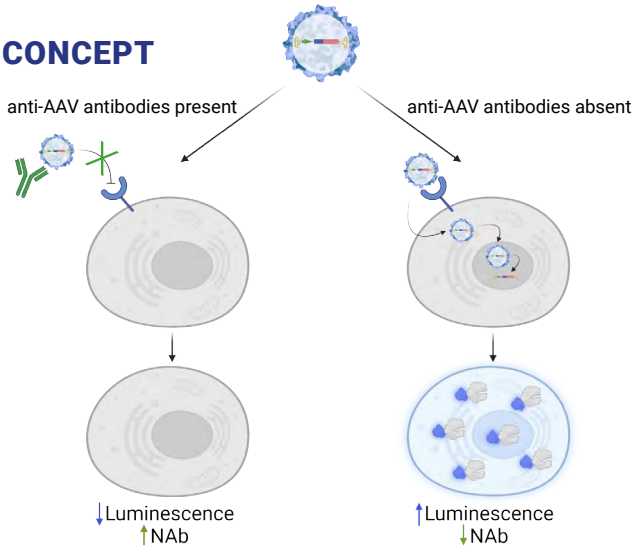
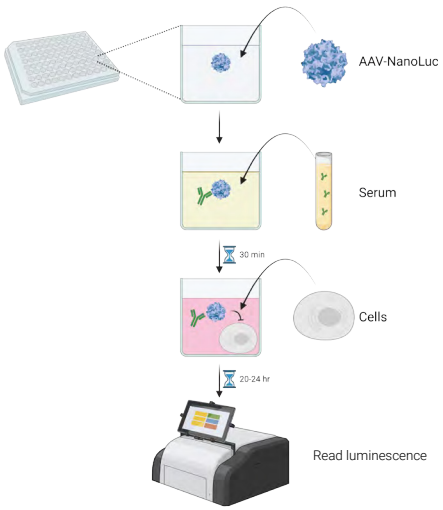
Reduction in luminescence would suggest the detection of anti-AAV antibodies in serum.

CONCEPT



WORKFLOW



APPLICATION

- Screen for AAV capsid variants that evade Nab
- Optimize proteases that efficiently digest NAb
- Screen for negative animals pre-clinical study enrollment
- Monitor NAb level for redosing opportunities
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INDUSTRIALIZING LENTIVIRAL VECTOR PRODUCTION: SCALE-UP STRATEGIES AND THEIR APPLICATION TO FLASHRNA SYSTEMS

Bruno Mateo, Christine Duthoit, Nicolas Martin, Charlotte Buzzolini
Flash BioSolutions

Introduction

Lentiviral vectors have emerged as powerful tools for gene delivery and modification in both research and clinical settings. Derived from the human immunodeficiency virus (HIV-1), these vectors have undergone extensive development and optimization over the past two decades. Their ability to efficiently transduce both dividing and non-dividing cells, coupled with a relatively large cargo capacity and stable integration into the host genome for stable long-term expression, has made them invaluable in the field of gene therapy.

Despite these advantages, early gene therapy vectors faced significant challenges. First-generation γ-retroviral vectors showed a high genotoxic potential, making them unsuitable for wide clinical application. Clinical studies on three primary immunodeficiencies, i.e., X-linked SCID, chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome (WAS), resulted in the occurrence of leukemias caused by vector-driven insertional oncogene activation[1]. Third-generation self-inactivating lentiviral vectors have demonstrated improved safety profiles, making them suitable for clinical gene therapy trials. These third-generation lentiviral vectors have been used for ex vivo modification of hematopoietic stem cells to treat primary immunodeficiencies and hemoglobinopathies, as well as for engineering T cells with engineered T-cell receptors (TCRs) or chimeric antigen receptors (CARs) for cancer immunotherapy[2]. CAR-T cell therapy, a groundbreaking cancer treatment, uses lentiviral vectors to engineer T cells to express CARs which makes them de novo efficient effectors against cancer cells. Several FDA-approved CAR-T therapies have utilized lentiviral vectors since 2017, including Kymriah and Yescarta treatments[3]. Lentiviral vectors have also been involved successfully in many other clinical fields such as hematological disorders[4], with the first commercially approved lentiviral vector-based gene therapy, Zynteglo, targeting beta-thalassemia in 2022.[5] They have also shown potential in treating central nervous system disorders, like adrenoleukodystrophy[6].

Recent developments in vector design have focused on enhancing tissue-specificity[7], regulating transgene expression, and improving manufacturing processes for clinical-grade vectors[8]. Non-integrating lentiviral vectors (NILVs) have received significant interest due to their improved safety profile and unique applications[9]. They allow transient expression that could be valuable for vaccines development, as well as for cancer immunotherapy, Induced Pluripotent Stem Cells (iPSC) generation and gene editing applications. FlashRNA®, a novel non-integrative lentiviral technology developed by Flash BioSolutions, combines the efficient cell transduction properties of lentiviruses with the multi-stranded RNA delivery mechanisms of bacteriophages. This allows for the encapsidation of several RNA copies per particle, ultimately achieving highly efficient RNA delivery and transient expression of multiple genes simultaneously [10].

This innovative approach has demonstrated exceptional potency in preclinical studies[11]. The technology's clinical launch is scheduled for 2025 as part of the European TheraLymph program, a collaborative effort with Inserm and Toulouse University Hospital (https://theralymph-europe.eu/). This initiative aims to address secondary lymphedema, a frequent post-operative complication in breast cancer patients, by leveraging the unique capabilities of FlashRNA® for multiple gene delivery and expression.

Flash Biosolutions is a French lentivirus-specialized contract development and manufacturing organization (CDMO) that offers customers dedicated project management from R&D grade lentiviral vector batches to GMP manufacturing of large-scale batches. In this context, we must pay close attention to the scalability and comparability of quality attributes (infectious titers, physical particles, impurity concentrations, and transduction efficiency of primary and stem cells) across all our different production scales.

Which scale up strategies are available for the production of lentiviral particles ?

1. **Upstream Process (USP):**

Cell culture systems are fundamental in the production of lentiviral vectors, with two predominant approaches: adherent culture and suspension culture. Each presents advantages and disadvantages that influence the quality and yield of the produced vectors.

In adherent culture, cells are grown attached to solid surfaces, such as flasks or plates, where they form monolayers. The advantages of this system include an easier control of environmental conditions: monitoring parameters such as pH, oxygenation, and nutrients is simpler in adherent cultures. Cellular characterization is also facilitated, as adherent cells maintain stable phenotypic and genetic characteristics, which is crucial for the homogeneous production of vectors. However, adherent culture also has limitations, particularly in terms of cell density. Scaling up can become complex and is often constrained by available space.

In contrast, suspension culture allows cells to grow freely in a liquid medium, facilitating higher cell densities and ease of scaling. This approach is often adopted in industrial bioprocesses. Advantages include easy transitions from research to large-scale development, particularly with the use of bioreactors, and the possibility of a continuous production, as suspension cultures can be easily fed with nutrients. Suspension culture also presents challenges, such as the need to optimize culture conditions to avoid cell aggregation and ensure homogeneity in vector production.

1.1. Adherent Cell culture

Lentiviral particles remain mainly produced by transient transfection of HEK293T cells in adherent systems[13]. The transition from small-scale to large-scale adherent systems' production is a significant hurdle.

While CellSTACK® and HYPERStack® technologies were widely used for LV production scale up, they still have limitations for truly large-scale production. The use of stacked systems for large-scale manufacturing can quickly become cost-prohibitive in terms of consumables, labor, and time.

Fixed-bed bioreactors, such as the iCELLis® system, have shown promise for large-scale LV production[14]. These systems provide a high surface area for cell growth while allowing for perfusion culture, resulting in increased vector yields. Optimized perfusion rates and pH control have been shown to significantly enhance LV productivity in fixed-bed bioreactors. These systems offer several advantages like increased surface area for adherent cell growth, improved nutrient and oxygen transfer and reduced shear stress on cells. They provide a more robust solution of scaling up the LV production in adherent system.

1.2. Suspension Cell Culture

Recent studies have demonstrated successful adaptation of HEK293T cells to suspension culture while maintaining high LV productivity[15]

This technology remains challenging concerning particular points:

- Minor adjustments in equipment or media chemistry can have major implications for scalability in particular concerning differences in sparger types and size of air bubbles that can affect dissolved oxygen levels but also impacting cell viability.
- Perfusion is not as easy has in an adherent system and can have a real impact on cell viability and viral particle quality.
- Finally the clarification step remains challenging for LV productions performed in suspension and can also impact functionality and purity of the particles.

The choice between adherent and suspension culture depends on various factors, including production goals, available resources, and the characteristics of the cells used. A thorough understanding of these systems is essential for optimizing the upstream process in lentiviral vector production, and ongoing research aims to enhance these techniques to maximize the efficiency and quality of the produced vectors.

1.3. Transfection Methods

Transfection method is essential for viral vector production but face significant challenges, including low efficiency, variability among cell types, and potential cytotoxicity of reagents. Scaling these processes also presents obstacles. Addressing these issues is crucial for optimizing viral vector production, ensuring consistent quality, and advancing applications in gene therapy and vaccines. Understanding these challenges will help improve the reliability of viral vector production.

One of the most challenging aspects of lentiviral production scalability is to find the best transfection conditions to reach high transfection efficiency and maintain consistent results from small scale to large scale batches.

Polyethyleneimine (PEI)-mediated transfection has emerged as a preferred method for large-scale LV production, offering improved scalability and reproducibility compared to calcium phosphate precipitation transfection method.

Several critical process parameters must be taken into consideration to optimize PEI-based transfection protocol, including cell density or concentration at the time of transfection, plasmid ratios, quantity of plasmid DNA per cell, PEI/DNA ratio, and transfection kinetic

1.4. Lentiviral production cell line development

Most current production methods rely on transient transfection which is costly. This entails high costs and long timelines to source plasmid DNA. Many lentiviral vectors providers are thus working on the establishment of stable producer cell lines[16], which remains an ongoing challenge due to the difficulty of generating good producer clones and potential cytotoxicity of vector components.

1.5 Production enhancement by avoiding auto transduction during USP

The unintended auto-transduction of viral vector-producing cells by newly synthesized lentiviral vector particicles during manufacturing processes constitutes an inefficiency which remains largely unaddressed. Recently it has been shown that over 60% of functional lentiviral vector particles produced during an upstream production process were lost due to auto-transduction. It was demonstrated that auto-transduction of cells by particles pseudotyped with the widely used VSV-G protein was inhibited by reducing extracellular pH during vector production, impairing the ability of the vector to interact with its target receptor. The implementation of post transfection pH reduction following transfection (pH 6.7-6.8) results in a seven-fold reduction in vector genome integration events[17]

2. Downstream Processing (DSP)

The DSP is a critical phase that involves the purification and concentration of the vectors after their initial production. This stage aims to isolate high-quality viral particles while removing impurities, such as cellular debris and unincorporated materials. Key steps include clarification, chromatography, and formulation, each requiring careful optimization to ensure yield and safety. Effective downstream processing is crucial not only for achieving the desired purity and potency of viral vectors but also for meeting regulatory requirements essential for clinical applications.

Advances in DSP have focused on developing scalable purification methods that maintain vector integrity. Purification processes must preserve the functional integrity of LV particles, including envelope proteins and enzymatic activities. The main obstacles in LV DSP have been handling of large volumes and loss of functionality during processing. Because of the versatile nature of LV, short process times and limited number of DSP steps are essential to maintain vectors activity.[18]. Current LV vector purification processes include several steps[19] such as clarification, DNase treatment, Tangential Flow Filtration (TFF), chromatography (mainly anion exchange and size exclusion), and sterile filtration.

TFF coupled with anion exchange chromatography has shown good results for large-scale LV purification[20]. Ion Exchange Chromatography is the most widely used approach for vectors capture in large-scale bioprocesses. The net negative charge of the LVs is used to separate them from Bovine Serum Albumin (BSA), residual host cell proteins, DNA and other impurities. However negatively charged molecules like DNA could be eluted with the LV vector, requiring definite conditions of elution with buffer ionic strengths between 0.5 and 1 M NaCl. However, these salt concentrations could lead to vector inactivation with moderate vector recovery. Achieving a balance between high purity and high yield is often difficult for chromatography step. For clinical applications the final product must be compliant with stringent residual impurities specifications and so low vector recoveries. Novel chromatography support and interaction like membrane specific vectors affinity[21] are also explored to improve purification efficiency and scalability.

The sterilizing filtration is also a critical step during the DSP phase. Due to their large size (about 120 nm), which is close to sterilizing membrane pores diameter, LV filterability is a major concern for this step. Aggregation of LV vectors in larger complexes during previous DSP steps can increase their size, further hindering their filtration. This often results in overall poor performance with LV recoveries.
The envelope protein can affect the size and aggregation tendency of the vector particles and so their stability. Finally, the formulation buffer is crucial and must be carefully taken into consideration for maintaining a high LV functionality from frozen storage up to its use[22].

The key DSP steps regarding the process performance are certainly the chromatography steps and the sterile filtration. These stages require careful optimization to increase vector particles yields but also high vector purity and transduction efficiency[12].

3.Quality controls (QC)

The increasing number of gene therapies using lentiviral vectors needs the implementation of robust and more automated QC methods able to quantify complete and functional particles and residuals impurities. Functional titer, expressed in IG/volume, is mainly determined by qPCR-based methods, allowing quantification of viral particles able to integrate the genomic DNA of transduced cells.
Enzyme-Linked Immunosorbent Assay (ELISA) targeting the p24 capsid protein is a common method to estimate total particles content. The concentration is thereby expressed in terms of Physical Particles (PP) per volume. Calculating the ratio PP/IG provides valuable insight into the proportion of functional particles throughout the process. Quantification of encapsidated RNA copies using Reverse Transcription quantitative PCR (RT-qPCR) is also of great interest.

The use of automated methods with optic-based technology, like Dynamic light scattering (DLS), and Nanoparticle tracking analysis (NTA), can provide valuable insights into the diffusion behavior of macromolecules in solution. This information can be utilized to quantify particle concentration and determine the size distribution of vector particles throughout the DSP steps, enabling lived optimization of process parameters, including concentration factors during TFF. Recent automated ELISA platform designed to deliver accurate, reproducible data with no manual steps could also permit to treat numerous samples during process development at lower costs. This kind of automated platform can perform p24, BSA, HCP quantitation giving precious information for process development.

4. Scale up considerations

Ensuring consistent quality across different batch sizes is essential but challenging.
The process of scaling up in biopharmaceutical industries presents several significant challenges, therefore several aspects should be considered at the earliest stages of process development:

- The quality and composition of raw materials can vary greatly when moving from small-scale to large-scale production. This variability can lead to significant impacts on manufacturing processes.
- Navigating regulatory requirements and product specifications that need to be met for the final application.
- Technical setpoints during scale up: only setpoints on large scale should be evaluated on small scale, to ensure process continuity in subsequent phases
- Nonlinear process parameters, like environmental process parameters or duration, must be considered according to vector stability, following risk analysis.
- Implementing robust Quality Control (QC) measurement is necessary to maintain compliance with Good Manufacturing Practices (GMP) throughout the scale-up process.

5. Influence of the vector's purity on transduction efficiency

The quality and titer of the lentiviral vector preparation are critical. The Theralymph project has yielded valuable insights into the correlation between residual vector impurities and transduction efficiency for FlashRNA® particles produced as a drug product. In vitro and preclinical data clearly demonstrate that GMP-grade FlashRNA® suspension exhibits enhanced functional output at comparable doses. This ultimately enabled the selection of a lower administered dose for human trials, compared to the dose initially selected from in vivo experiments using research-grade batches.

Conclusion

The complexity of LV production and purification contributes to high manufacturing costs, which need to be addressed for commercial viability. This includes optimizing the use of expensive reagents and developing more efficient processes. During the production and purification process, stability of particles must be carefully taken into consideration.

At FlashBiosolutions, we pay attention to key process parameters to enhance process performance with the highest possible vector yields to achieve cost-effective production. But our priority is to be absolutely focused on critical process parameters affecting critical quality attributes which leads to exceptionally high transduction efficiency. We stand at the cutting edge, unparalleled in our ability to deliver the most efficient vectors to our clients.

Lentiviral vector-based gene therapies are the most expensive medical treatments available today, costing hundreds of thousands of dollars per patient. To make this treatment accessible to more patients, manufacturing and QC expenses must be as low as possible. This calls for highly efficient processes on a larger scale.

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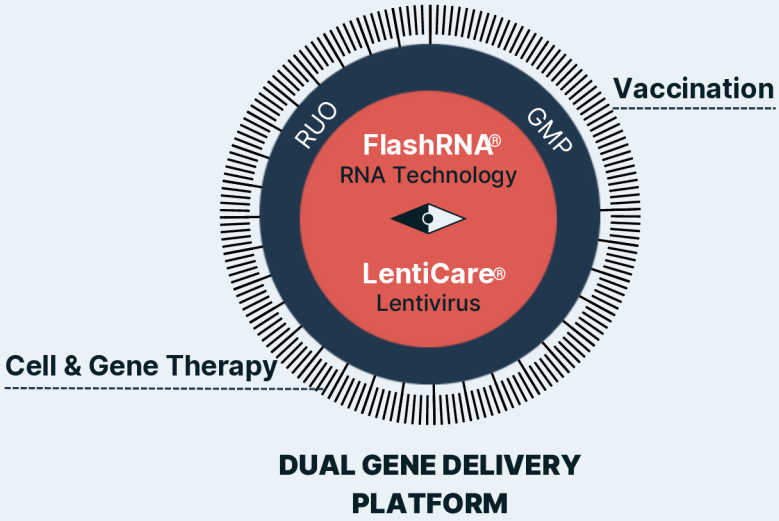

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ADENO-ASSOCIATED VIRAL VECTOR PRODUCTION PLATFORMS AND WHERE TO USE THEM

Chris Williams, Principal
DeSci Applied Research

Introduction

Gene therapies are generating far-reaching excitement for a genuine breakthrough in treating or even curing debilitating medical conditions. Novel genetic medicines have shown the ability to correct faults in the source code of biology, our DNA. These breakthroughs offer families and patients with many untreatable diseases renewed hope that modern medicine will improve their lives. Healthcare providers are also considering the potential to offset the cost of years, or even decades, of medical care with a single curative therapy.

A primary contributor to the effectiveness of gene therapies is their capability to target a therapeutic correction to the root cause of a disease. Most disease manifestations treated by gene therapies are within a specific organ or system of the body. Targeting a therapeutic to specific organs or systems, such as the eye, brain, or skeletal muscle, is critical to maximizing therapeutic effect while minimizing undesirable side effects.

Adeno-associated viral vectors (AAVv) have emerged as the most prevalent solution for achieving the targeting specificity needed for gene therapies. Several naturally discovered and synthetically engineered serotypes of AAVv are being used in clinical and commercial gene therapies because of their intrinsic tropisms for different tissues of the body. The structure of an AAVv, as illustrated in **Figure 1**, is a quite simple for a virus but complicated as a therapeutic modality.

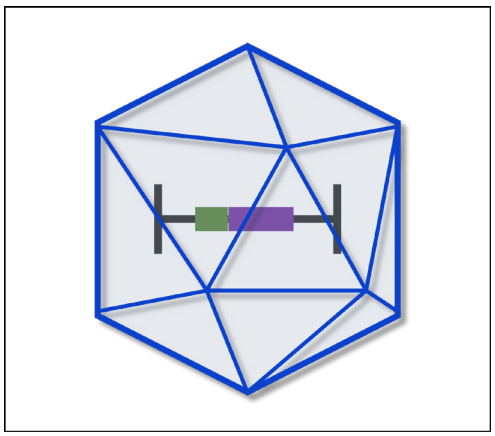

Figure 1: The structure of an adeno-associated viral vector consists of a protein capsid encasing a single-stranded DNA that encodes a promoter and a therapeutic gene sequence.

Historically, and presently, a major drawback to AAVv is the high cost of manufacturing sufficient quantities of high-quality AAVv required for clinical use. These high costs present significant barriers at all phases of a product's lifecycle, from a lack of translational investment in many promising therapies to restricted access due to high commercial pricing.

Perseverance in overcoming cost limitations is fueling a rapid acceleration in technical developments for gene therapies across the field. Compared to more common medicinal modalities, the science and engineering for manufacturing AAVv gene therapies are still very immature. The past decade has seen significant advancements in the industrialization of AAVv production. This progress is poised to continue as the industrial capacity to supply AAVv gene therapies has yet to catch up with the projected demand for these medicines.

The dynamic effect of the rapidly evolving technical landscape is that the industrial state-of-the-art will inevitably improve over the lifecycle of a novel gene therapy. In the context of manufacturing technologies, the trajectory of AAVv production is moving toward higher productivity, greater purity, and lower Cost of Goods (COGs). Nonetheless, clinical demand for these therapeutics, along with the financial advantages of being first to market with a curative therapy, incentivizes investment now despite the superior benefits that future technologies will undoubtedly provide.

When investing in a gene therapy, one must anticipate riding the wave of future innovations while developing a product for the commercial market. A few key decisions in process design, made early in a product's development, can help to de-risk future barriers to successful commercialization. Additionally, there is no single solution that best suits all situations given the broad applicability of gene therapies.

The following will examine major themes in the production platforms of AAVv gene therapies. By comparing the benefits and limitations of competing production technology platforms, a more informed selection of production strategy for a particular asset can be made.

Early Process Evolution

For most of the history of AAVv science, the production of vectors was carried out through protocols commonly employed in academic settings. Main unit operations originating in academic settings have persisted in AAVv manufacturing protocols even today. The simplest and most cost-effective method for producing small quantities of AAV is through adherent cell culture using transient transfection (TT). Small quantities of vector produced in T-flasks or roller bottles can be harvested and purified by iodixanol gradient centrifugation[1].

The industrialization of this academic process has been successful in delivering sufficient quantities and quality of AAVv to serve the purposes of several rare disease indications and clinical trials. Adherent cultures can be manually scaled out using multiple HyperFlasks[2] in parallel, achieving a maximum of 18,000 cm^2 of surface area per unit. Integrated systems, such as the Cytiva iCELLisTM bioreactor[3] shown in **Figure 2**, bring automation to this technology, providing up to 500 m^2 in a highly controlled environment.

The financial advantages of an adherent culture start with the simplicity of early process development. Cost-conscious researchers can utilize simple setups for optimizing constructs, identifying therapeutic leads, and producing materials for in vitro and in vivo proof-of-concept studies. The upfront capital costs for scaling out the production of manual adherent processes can also be significantly less than those for more technical production schemes. Operationally, 2D adherent cultures with high cell-specific productivities can efficiently use costly raw materials, such as media and plasmid.

The main disadvantage of the adherent system, which is not universally an issue, is the inherent upper limit to operational practicality. Scaling cell growth by adherent surface area is inferior to suspension-based systems that scale by vessel volume. While production scales above 10,000 L have yet to be demonstrated in AAVv production, they are common in the production of other biologics such as monoclonal antibodies (mAbs).

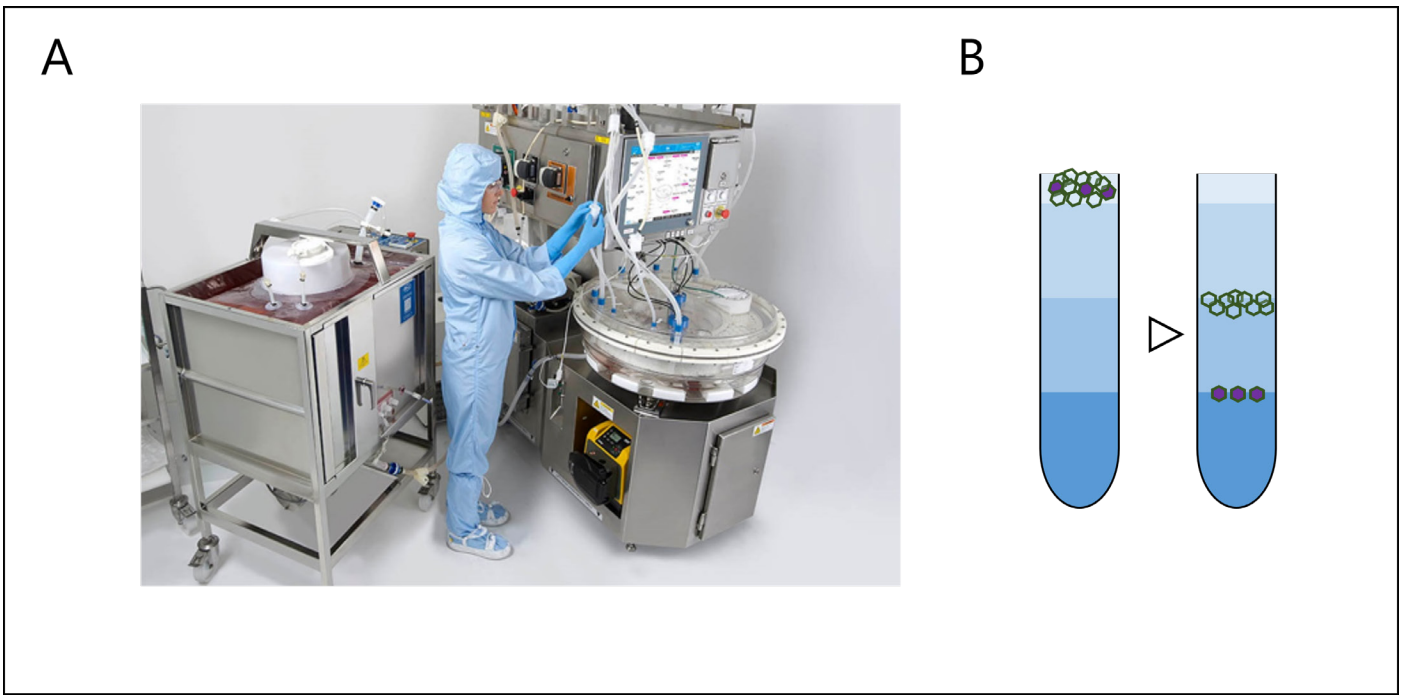

Figure 2: A) Industrialization of legacy production platforms has led to sophisticated instrumentation such as the iCELLisTM 500 Adherent Bioreactor. B) The use of gradient ultracentrifugation to separate less dense empty capsids (green) from denser full capsids containing DNA (purple) is simple and effective.

The use of gradient centrifugation for the enrichment of full capsids, as depicted in **Figure 2**, has also proven to be an effective purification option in industrial settings. Because the separation relies on relative density, it is the most direct way of discriminating between the therapeutic-containing full capsids and potentially harmful empty capsids. The added advantage of being serotype agnostic makes this operation more resilient to the growing variety of AAVv used in clinical settings. The primary drawback to gradient centrifugation is that it can be difficult to execute robustly, making it risky for high-demand commercial use.

The industrialization of gradient centrifugation has been demonstrated to be effective for producing AAVv batches enriched to well above 90% full capsids[4]. For larger batches, this purification can only be scaled out by running multiple centrifuges in parallel. Parallel manual centrifugation requires highly skilled technicians to reproducibly produce the gradient tubes and extrac

t the bands of full capsids. While preparative gradient centrifugation works well in many situations, it is intrinsically unrobust and has a low productivity ceiling at which it becomes impractical.

Lessons from Biologics

As the process and manufacturing technologies for AAVv continue to mature, many of the tools employed for the production of other biologics are being applied. To a casual observer, the GMP production suites for an AAVv and a mAbs would be equipped with indistinguishable single-use manufacturing equipment like those shown in **Figure 3**. The adaptation of commercially proven operations helps to break through the sensibility ceiling of manual operations while also contributing economics of scale to lower overall production costs. While the benefits of contemporary technologies have catapulted their popularity, these legacy operations are also not without drawbacks.

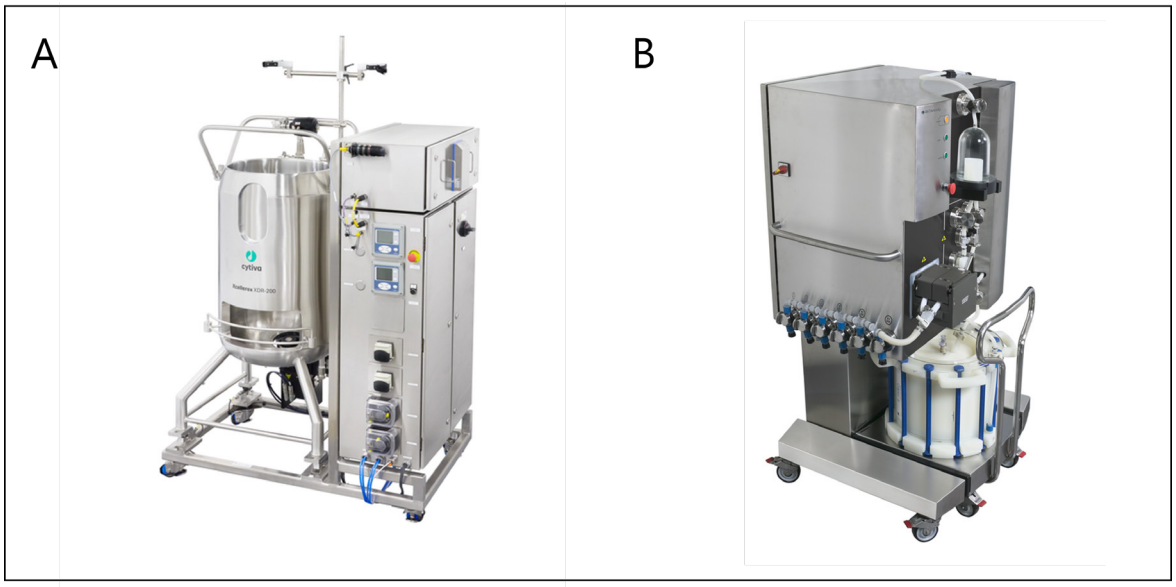

Figure 3: Single-use (A)Bioreactors and (B)Chromatography stations enable highly robust and efficient production processes while also minimizing risks of product cross contamination.

The strongest trend in the cell culture production of AAVs is the transition to suspension-based bioreactors. Primarily single-use stirred tank bioreactors, with scales from 50 L up to 5000 L, are used for producing large GMP batches of AAVv. While the commonly employed HEK293 cell is naturally adherent, there are protocols for adapting adherent cell lines to suspension culture as well as commercially available suspension-adapted cell lines. As an alternative to fully adapting a cell line to suspension, microcarriers offer a compromise between a solid extracellular substrate and efficiencies in volumetric scaling[5].

Robustly executing the transient transfection with up to three plasmids adds to the challenges in scaling up from benchtop to 1000+ L manufacturing scales. The development of a triple-transfection process requires the identification of the optimum molar ratios between each of the three plasmids, the transfection reagent, and the number of cells in the bioreactor. Layered over this stoichiometry is the temporal dynamics of transfection complex formation and the homogeneous introduction of the transfection mix into the bioreactor. When executed well, titers up to E15 vg/L of cell culture and full capsid percentages between 20% and 50% can be achieved.

In AAVv downstream processing, the development of chromatography operations has led to the greatest overall process efficiency improvements. A two-chromatography step process, for capturing the product and polishing for full capsid enrichment, can be sufficient in most cases. In contrast to mAb downstream processing, AAVv operating volumes are proportionally small relative to the bioreactor due to limitations in capsid titers. Smaller volumes contribute to relatively lower material costs and water consumption. However, any volumetric losses, either necessary through sampling or unavoidable from material transfers, can contribute to measurable losses in overall production yield.

In recognition of the power and efficiency of affinity chromatography, several generic and serotype-specific affinity resins have been developed for AAVv. Albeit expensive, affinity chromatography media can provide superior process impurity removal compared to many alternative non-specific ion exchange resins. The level of purity achievable through affinity chromatography may in many cases contribute to cost avoidance by eliminating the need for a third chromatography step.

Anion-exchange chromatography is the most frequently employed polishing operation because of its capability to enrich the portion of full capsids. It is hypothesized that the DNA cargo within a full AAVv capsid contributes to a lower molecular pI, and thus presents a higher net surface charge at alkaline pH

than an empty capsid would present. This difference in surface charge allows for mobile phase matrices that promote the binding of full capsids while empty capsids flow freely through the stationary phase or are eluted. Relative to full capsid enrichment by ultracentrifugation, anion-exchange chromatography is more scalable and easier to automate for robustness. However, achieving highly enriched product, above 90% full, requires a relatively high percentage of full capsids in the starting feedstock, which may not have been developed in all upstream bioprocesses.

Irrespective of chosen unit operations, the careful selection of mobile phase components is critical to the successful purification of AAVv. Ionic strength, pH, the presence of metals and divalent cations, and other organic modifiers can affect a serotype-specific stability window for AAVv[6]. The mobile phase components also contribute synergistically to the binding behavior on chromatography media and potentially the surfaces of product-contacting materials. The complexity of these molecular interactions, combined with the numerous options for including various mobile phase components across robust operating ranges, creates perhaps the greatest challenge in developing an AAVv downstream process.

Emerging Production Trends

The projected growth in demand for AAV-vectored gene therapies is driving investments into more innovative approaches to achieving high productivities at an industrial scale. These technology platforms may sufficiently overcome the hindrances of high COGs in clinical settings and for highly debilitating rare diseases. However, the growth of gene therapies into more prevalent indications presents new bottlenecks in production capacity. The technical bar for producing AAV-vectored gene therapies at a reasonable cost is much lower than that for producing a sufficient quantity of AAV-vectored therapies to supply a prevalent indication with a high dose of product.

In upstream processing, cost-efficient alternatives are poised to break the productivity ceiling of HEK293 TT platforms. One of the greatest expenses in AAVv production, the plasmid starting materials, scales linearly with volume and therefore does not wholly benefit from economies of scale. Reducing or eliminating the cost and supply chain of plasmids has been the primary strategy for lowering production costs while potentially raising volumetric productivity and scale.

One alternative to TT production is to genetically engineer a cell line to specifically produce the desired AAVv gene therapy. This is achieved by integrating the genes transferred by the plasmids directly into the genome of the cell line. Expression of the genes necessary for AAVv production is controlled by inducible promoters. The engineered producer cells are cultured and expanded to the desired production scale, followed by the chemical induction of AAVv production. At an equivalent productivity to a TT culture, the stable producer platform benefits from a lower volumetric cost and potentially greater robustness at higher scales.

Another alternative to the HEK293 cultures is the less commonly used baculovirus production system. Production of therapeutic proteins with baculovirus is a more recent innovation than production with mammalian cells but not entirely novel. Like the stable producer cell lines, baculovirus production has the advantage of higher productivity at a lower cost without the need for plasmids. Baculovirus production has the additional advantage of producing a high percentage of full AAVv capsids. While studies have shown that the potency of AAVv produced in baculovirus systems and HEK293 systems can differ, the superiority of one over the other is mixed[7,8].

The most promising innovations in downstream processing are directed at overcoming many of the fundamental physics challenges when purifying AAV vectors. Relative to mAb feed streams, AAVv is a large molecule (20 nm vs. 5 nm) present in dilute concentrations. Both of these features reduce the efficiency of chromatography resin, which is restrictive of convective flow and relies on pore diffusion to

achieve maximum surface adsorption. Several innovations that have been previously developed for mAb production are actually better suited for these specific challenges with AAVv.

Presently, the most popular downstream innovation has been convective flow devices as an alternative to chromatography resin. Several of these devices rely on functionalized surfaces of membranes, fibers, and monoliths to achieve the comparable reversible binding behavior of traditional chromatography resin. The primary advantage of these devices is their ability to withstand much higher volumetric flow rates per equivalent binding capacity. This contributes to higher productivity and thus shorter operational times. One ancillary benefit is that many of these devices are inherently single-use, reducing the necessity to develop cleaning and re-use procedures required to offset the high cost of reusable chromatography resin.

API COGs

Irrespective of the production platforms, one of the key drivers in process development is the reduction of COGs. A significant proportion of COGs variability comes from the administered patient dose. Doses of AAVv in clinical trials can vary from as low as $1E^{11}$ vg/eye up to greater than $1E^{15}$ vg/intravenous infusion. While the execution of the production can influence the dose, for example by modulating potency, the dose is largely determined by clinical safety and efficacy.

From the manufacturing perspective, the strongest lever for reducing COGs is by increasing overall batch productivity and yield. Batch volumetric productivity across the industry varies by orders of magnitude. Comparatively, GMP batch costs may vary only from several hundred thousand to several million dollars/euros per batch.

A survey of various manufacturing metrics can assist in estimating an industry benchmark for the lowest COGs in commercial AAVv production. **Table 1** lists some published references for three key manufacturing metrics. As a hypothetical thought exercise, the combination of these metrics can

Cost Metric	State-of-the-Art Estimate	References
Production Scale	50 L, 200 L, 2000 L	Survey of 10 CDMO Websites
Volumetric Productivity	$1x10^{15}$ vg / L	9 10 11 12 13 14 15
Batch Cost	$1,500,000 - $7,000,000	16 17

Table 1: Publically Advertised AAVv Production Metrics

Using these metrics, one can calculate the cost to produce a single commercial dose of API for various therapeutic areas. The average dose-per-patient in Table 2 was taken from a clinical survey published by Burdett & Nuseibeh[1].

Therapeutic Area	Average Dose per Patient	50 L Batch	200 L Batch	2 kL Batch
Musculoskeletal (*n* = 12)	9.1 × 10^{13} vg/kg			
Child (15 kg)	1.4 × 10^{15} vg	$81,900	$40,950	$9,555
Adult (60 kg)	5.5 × 10^{15} vg	$327,600	$163,800	$38,220
Neurology (*n* = 24)	6.3 × 10^{13} vg/kg			
Child (15 kg)	9.5 × 10^{14} vg	$56,700	$28,350	$6,615
Adult (60 kg)	3.8 × 10^{15} vg	$226,800	$113,400	$26,460
Cardiovascular (*n* = 7)	3.3 × 10^{13} vg	$1,980	$990	$231
Haematology (*n* = 23)	1.9 × 10^{13} vg	$1,140	$570	$133
Metabolic (*n* = 16)	1.9 × 10^{13} vg/kg			
Child (15 kg)	2.9 × 10^{14} vg	$17,100	$8,550	$1,995
Adult (60 kg)	1.1 × 10^{15} vg	$68,400	$34,200	$7,980
Ophthalmology (*n* = 31)	2.9 × 10^{11} vg/eye	$17	$9	$2

Table 2: Average cost-per-dose at industry top benchmarks for productivity

While these costs-per-dose are encouraging for the financial viability and broad access to AAV-vectored gene therapies, some caveats need to be recognized. The illustrated costs assume that nearly the entirety of the batch is administered to patients. In a clinical setting or for rare indications, a single batch may produce more than is required, thus negating any advantages from economies of scale. Furthermore, especially in the clinical phases of production, a significant volume of a batch may need to be set aside for required development activities such as analytical method validation or product stability.

Finding the Right Tool for the Job

Across the breadth of gene therapy applications, there will be scenarios where each of the aforementioned production strategies is most suitable. Even between various phases of a gene therapy's lifecycle, differing technologies may seem advantageous. One common example is that while the simplicity of an adherent culture is attractive in an early research setting and can provide sufficient material for small early-phase clinical trials, a suspension-based platform may be more economical for commercial production.

Taking a holistic view of the lifecycle of a gene therapy for a specific indication can help guide the selection of the most appropriate production technologies to employ at each phase of development. While it is possible to change production platforms for a given asset, this comes at the significant cost of demonstrating biochemical comparability or even building in clinical bridging studies. A conservative approach to maximally de-risk comparability challenges through clinical phases is to develop even pre-clinical production on a commercially viable platform. A more measured approach, considering the statistically high risk of clinical failure, may be to defer any large investments into production technology until clinical proof of concept has been demonstrated.

High Demand Scenarios

Gene therapies with a high dose or a high-targeted patient population will benefit the most from at-risk investments into more efficient production platforms. Productivity ceilings of manual technologies that work well for research purposes can make commercial viability impractical or impossible. In these scenarios, a fixed at-risk investment into a product that may never reach the market needs to be weighed against the variable higher production costs for a marketed product in the future.

Overinvestment in optimizing technologies that cannot be scaled to meet future commercial demand can lead to dead ends that might be costly to overcome. For example, purification with ultracentrifugation can produce a product with highly enriched nearly 100% full capsids for pre-clinical and early clinical studies. Adapting these processes to a more scalable anion-exchange chromatography enrichment step may not be able to achieve such a high purity standard. Such a situation may raise questions from regulators on the representativeness of pre-change safety and efficacy data for the post-change process version.

The timing of process development investments can also be critical to maximizing the value of an asset. Thorough process development takes time and, when on the critical path, can delay the clinical advancement of an asset. Evolving from an adherent cell culture with gradient ultracentrifugation purification to a suspension process with chromatographic purification cannot be done entirely in parallel. Locking in a newly developed cell culture process can take several months. An additional several months with representative feedstock is required for locking in a purification process and confirming product comparability. The earlier in a product's lifecycle that significant production changes are introduced, the greater the flexibility will be to work those changes into the process.

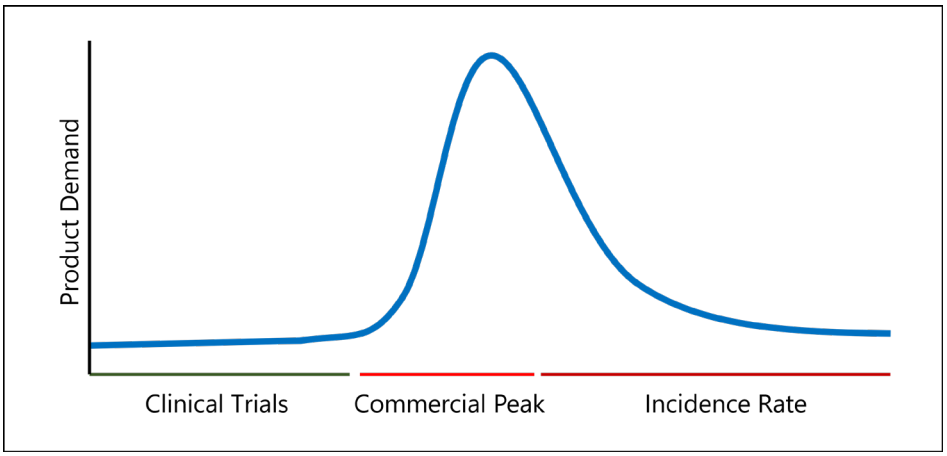

Figure 4: The demand for a single-administration gene therapy will be low during clinical trials and will rise rapidly to supply diagnosed patients after regulatory approval. Once the known patients with access are treated, the demand will gradually fall to the incidence rate of the indication.

A clinically successful high-dose gene therapy or one in a high-prevalence indication will also present challenges in itting supply capacity to the demand curve over the product's lifecycle. Demand curves, such as the one illustrated in **Figure 4**, are characterized by relatively low levels in clinical trials, followed by sharp growth at product launch, and a tapering after the initial bolus of patients is treated. Meeting this demand requires a rapid scaling-up or scaling-out of production capacity timed to the expected launch of the product. The necessity of such high production capacity is transitory, so capital assets would need to be reallocated to other products on similar production platforms. The decision to build such production capacity or to leverage a contract manufacturer will depend on the depth of a product pipeline for making efficient use of the production assets.

Ultra Rare Indications

On the opposite end of the demand spectrum are gene therapies for ultra-rare indications, which may be diagnosed in several hundred patients or less. In these cases, extensive at-risk investments into process technologies are often not economically sustainable to burden across a small number of patients. In extreme cases, there may not be a sufficient number of patients to justify commercializing a clinically efficacious product. Identifying the most appropriate business model may be more challenging than selecting an adequate production platform.

Technically, most platforms are sufficient for supplying adequate materials for the entirety of an ultra-rare patient population. However, the upfront, at-risk development costs contribute to a negative net present value in the early phases of development. Additionally, there is an inverted opportunity cost to investing capital into niche, small-demand products while larger unmet medical needs are still on the table.

A unique and underappreciated advantage to developing gene therapies for ultra-rare indications is the number of unique diseases that can benefit from pre-competitive technical collaboration. **Figure 5** gives an impression for the scope and scale of unmet medical need by illustrating only a subset of the known genetic diseases listed in the Ophranet Database[19]. While the therapeutic transgene of each gene therapy needs to be tailored to each individual disease, a common AAV vector and production platform can be used across a variety of related diseases. With this in mind, selecting production platforms that compromise on productivity in favor of flexibility can offer an advantage.

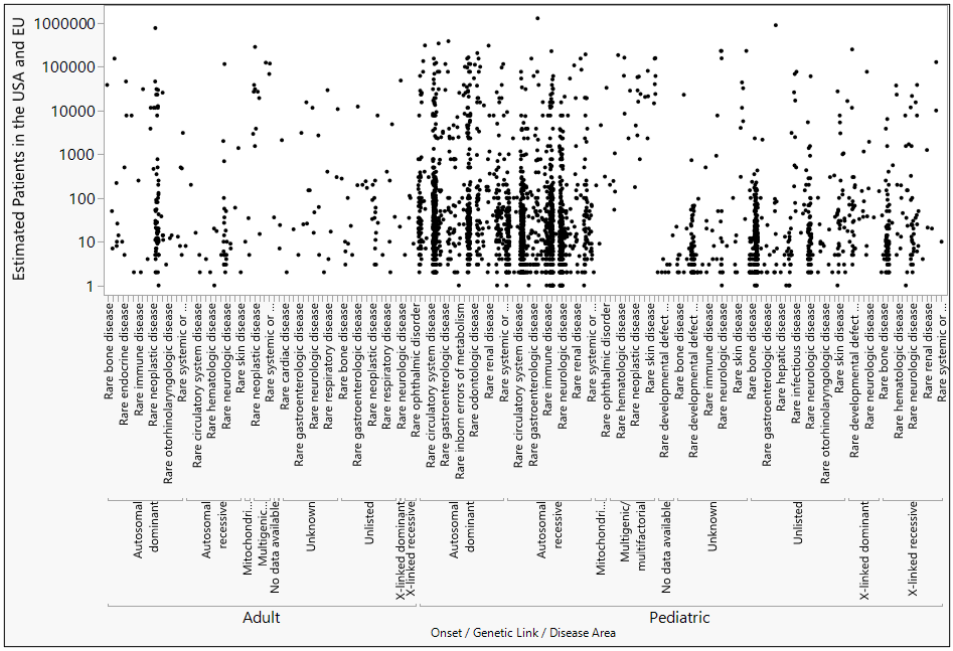

Figure 5: A subset of known genetic diseases plotted by an estimated total number of patients in the USA and European Union and clustered by disease area, genetic linkage, and age of onset.

A HEK293 transfection platform may be perfectly suited to providing flexibility to adapt a platform process to multiple related gene therapies. The minimum necessary process adaptation could be to simply switch out the transgene plasmid encoding the desired therapeutic, while keeping everything else the same. Although this strategy is often discussed, there has yet to be any tangible demonstration of this level of efficiency in process development.

Across ultra-rare indications, an open standard production platform could be utilized to lower development costs and thereby the threshold for financial sustainability in treating these diseases.

Last Remarks

While the future cannot be entirely predicted, informed decisions can help to improve the overall net value of gene therapies. Starting development with the end in mind can avoid unnecessary rework later in the lifecycle of a product. Demonstrating clinical efficacy with a commercially viable manufacturing process will increase the valuation of an asset and its attractiveness for future acquisition. A clearer path to commercialization is also more valuable to patients who may not have the luxury of waiting for delays due to redevelopment.

Locking into the right production technology is a significant commitment that does not alone guarantee the desired manufacturing outcome. Within each production technology is a complex set of parameters that must be developed and optimized to suit the uniqueness of each product. Early manufacturability assessments of novel constructs can forewarn future challenges in developing a robust and efficient process. If needed, finding partners with experience can help navigate the complexities of developing a GMP-ready process.

For the millions of patients who will someday benefit from gene therapy, investing in today's technology is a crucial stride toward groundbreaking treatments and cures. Each new investigational AAVv holds the promise of unlocking insights that lead to safer and more effective gene therapies. By sharing these discoveries within the scientific community, we can accelerate the creation of life-changing medicines, bringing hope and healing to patients and transforming lives around the world.

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VIDEO DROP SK

Real-time monitoring of transfection mix complexation

Myriade has developed a device, **Videodrop**, designed to **provide lentiviral vector concentration and size distribution**, suitable for both **in-process controls** and final product characterization. The strength of this method lies in its simplicity: the measurement is real-time (40 seconds), label-free, non-destructive, reproducible, and the cleaning process takes only a few seconds.

Therefore, Videodrop is particularly well-suited for laboratories where a large number of samples need to be characterized. It requires only a small drop of sample (5-10 µL) and **provides highly consistent results between operators**, making it ideal for the industry. By rapidly measuring the size and physical titer in solution, Videodrop can be integrated into lentiviral quality control strategies.

After demonstrating its suitability for GMP environments as a quality control method for monitoring the physical titer of lentiviral vectors, Myriade recently introduced a new feature, **Size Kinetics (SK)** focusing on the **temporal evolution of particle size**. This new metric offers a quantitative tool for monitoring the complexation of plasmid and polymer during transfection.

Reagent-based transfection is the primary method for producing viral vectors (LV or AAV). However, despite its significance, this critical step often **lacks real-time monitoring** and **quality controls.**

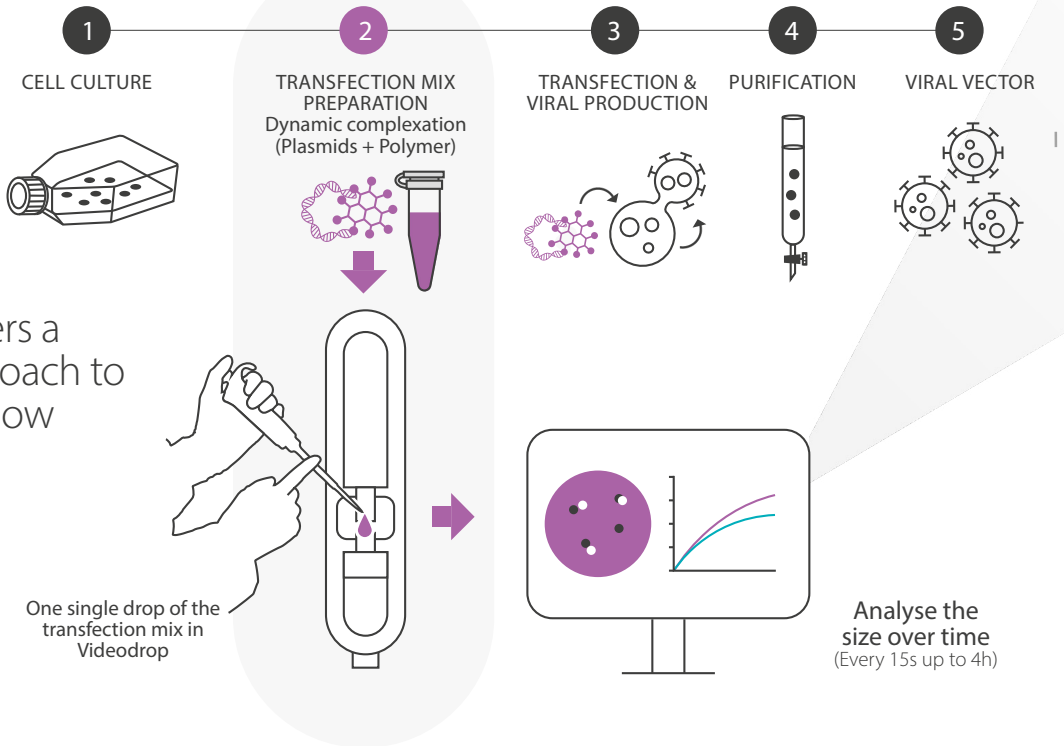
Automated Kinetics Monitoring

- Size measurement every 15s
- From 80 nm to 2 µm
- On the same sample droplet
- Over a defined time period up to 4 h

Real-Time Visualisation Transfection reagent/DNA complexes formation

- Microscopic imaging
- Real-time kinetic curves construction

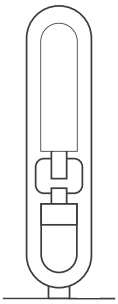
Videodrop SK in Viral vector production UPSTREAM process



Videodrop **SK** offers a revolutionary approach to transfection workflow optimization.

myriade

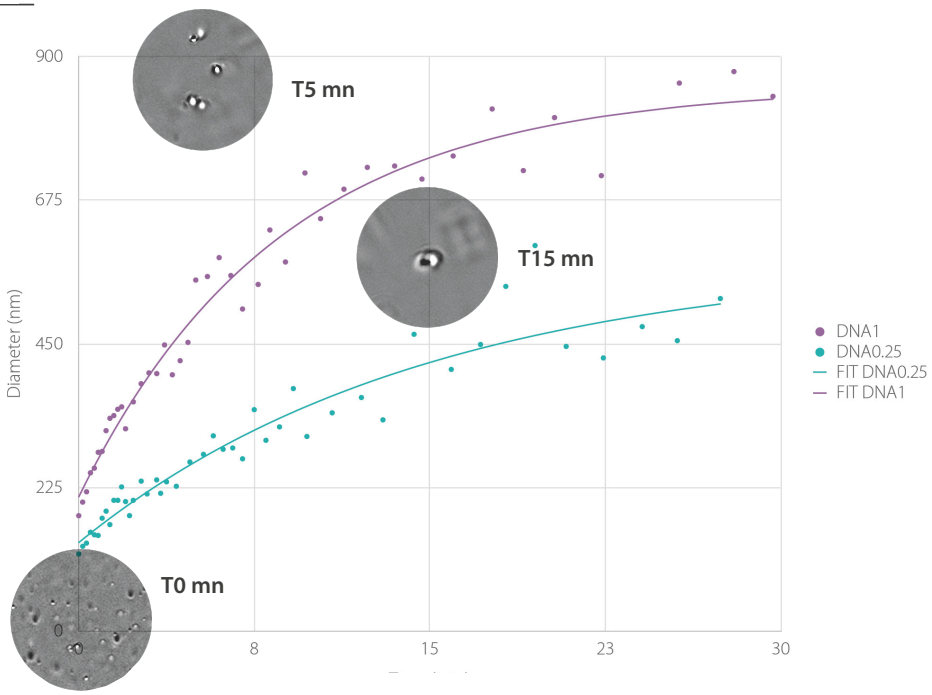
VIDEO DROP SK



Screening of transfection mix conditions

Videodrop enables in-depth real-time kinetic studies to monitor the impact of various conditions (DNA concentration, ratios, media type, pH levels) on the **size evolution of transfection complexes over time**. Studies show a clear correlation between size of the formed transfection reagent/DNA complex and the transfection efficiency.[1]

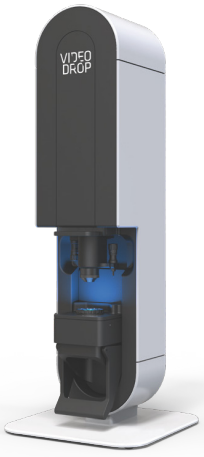
1. Hu, Y. et al. Size-Controlled and Shelf-Stable DNA Particles for Production of Lentiviral Vectors. Nano Lett. 21, 5697–5705 (2021).



In this case, we tested 2 concentrations of DNA (3 plasmids : 1 and 0.25 µg/10^6 cells) mixed with the same proportion of PEIPro® (Polyplus).

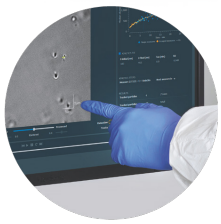
Videodrop allows to follow in real-time the DNA/PEIPro® complexation and highlight the kinetics difference between two conditions.

By incorporating Videodrop into their workflows, scientists can **achieve a new level of control and optimize transfection efficiency,** ultimately leading to faster and more cost-effective upstream processes.

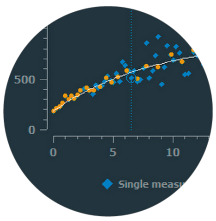


The Simplest Analytical Tool for Real-Time Transfection Mix monitoring

In a single drop
(5-10 µL)



Real time visualisation

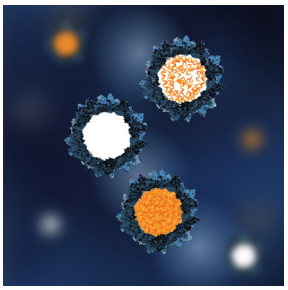


Size kinetics

myriadelab.com

ENSURE GMP COMPLIANCE WITH CONSISTENT AAV DATA: RELIABLE EMPTY, FULL, AND PARTIAL AAV CHARACTERIZATION ACROSS ALL SEROTYPES WITH MASS PHOTOMETRY

by Refeyn



In the field of AAV vector development, ensuring that only high-quality, fully packaged AAVs make it through to the final product is a significant challenge. Mass photometry is an emerging technology that addresses these challenges by providing rapid, in-house critical quality attribute (CQA) and process analytical technology (PAT) analytics (see link 1). These capabilities can be utilized from R&D to GMP environments, streamlining AAV development workflows.

The key advantages of mass photometry in AAV development include:

- Precise and reproducible analysis of capsid fill levels, distinguishing between empty, partially filled, and full populations, as well as assessing sample purity and capsid titer for any AAV serotype.
- Significantly reduced sample analysis times, from hours to just five minutes.
- Enhanced efficiency with minimal sample requirements (5-20 µL at ~10^{11} capsids/mL) and a low running cost.

This innovative approach empowers researchers and manufacturers to accelerate their AAV development processes with greater precision and efficiency, making it an essential tool for advancing AAV therapies.

AAV analytics in GMP-regulated environments

AAV manufacturing environments are GMP-regulated and subject to a series of rules established by agencies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). These regulations require that any software used in AAV manufacturing workflows has features that ensure data traceability and reproducibility. In addition, instruments to be used within AAV manufacturing processes need to be qualified at different stages of production and implementation. These qualifications include design, installation and operational qualifications (DQ, IQ, OQ).

Streamlining AAV workflows using mass photometry

Mass photometry can be used in GMP-regulated environments (see link 2) as it has a compliant software that fulfills the FDA and EU requirements for AAV manufacturing including:

Refeyn's software package for AAV manufacturing environments includes automated workflows to streamline data analysis, as well as standardize it between operators. In the automated mode, mass ranges are automatically defined to classify detected AAVs into empty, full and partially filled populations.

These ranges are automatically defined based on user inputs for the expected mass of the empty capsids and the size of the genomic payload. Manual and semiautomated modes are also available for users with advanced permissions.

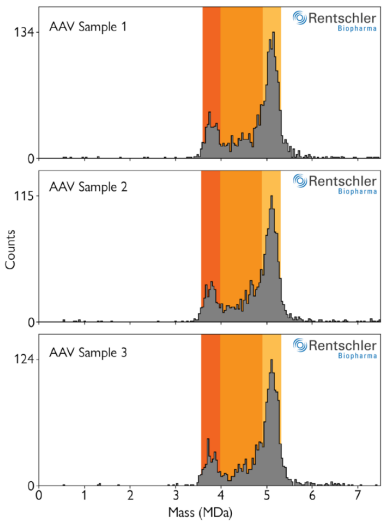
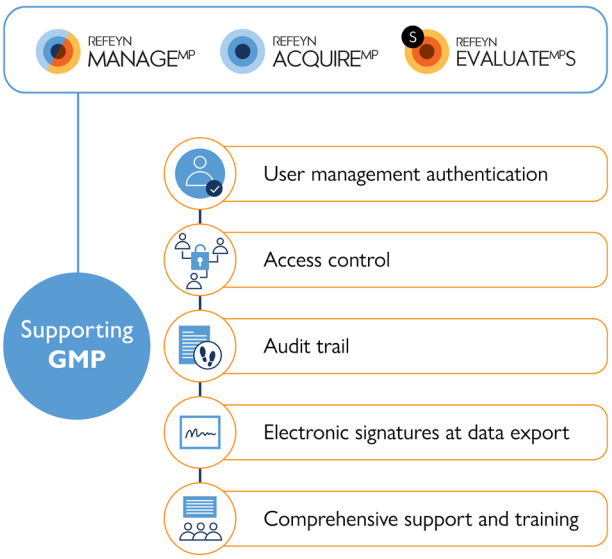


Image: Three measurements of an AAV sample using an automated workflow that detects empty, full and partially filled AAV capsids

The key advantages of mass photometry in AAV development include:



The Samux software package for GMP-regulated environments complies with GMP manufacturing regulations. It uses an automated workflow that detects empty, full and partially filled AAV capsids in a sample based on basic user input. The software also provides user management and access, audit trails, electronic signatures and individually traces every dataset. Details about the components and capabilities of the Samux software package for GMP and example data can be found in this whitepaper (see link 3).

For more information, please contact our Technical Sales Manager, Frederique.galetto@refeyn.com

About Refeyn

Refeyn pioneers analytical instruments that put molecular mass measurement capabilities within easy reach for scientists. Refeyn's unique products measure the mass of individual proteins, nucleic acids, complexes and viruses directly in solution – providing vital insights for scientific discovery, R&D and therapeutics production. Our instruments feature mass photometry technology, which uses light to quantify the mass of single particles in solution without labels, and macro mass photometry technology, which uses light to characterize large viral vectors. Providing intuitive data in minutes, mass photometry technologies help scientists solve their research questions, optimize R&D processes and focus on innovation.

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Link 2 https://www.refeyn.com/aav-analytics-in-gmp-regulated-environments

Link 3 https://info.refeyn.com/gmp-for-samux-whitepaper

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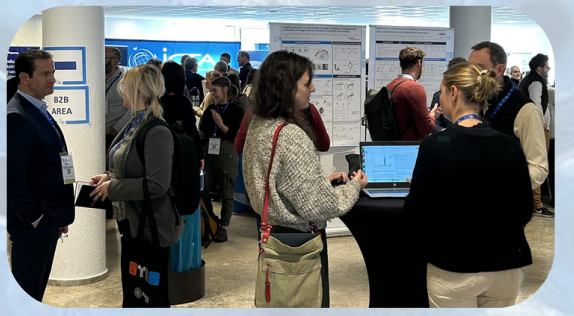

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