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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:
Viral vector bioprocessing

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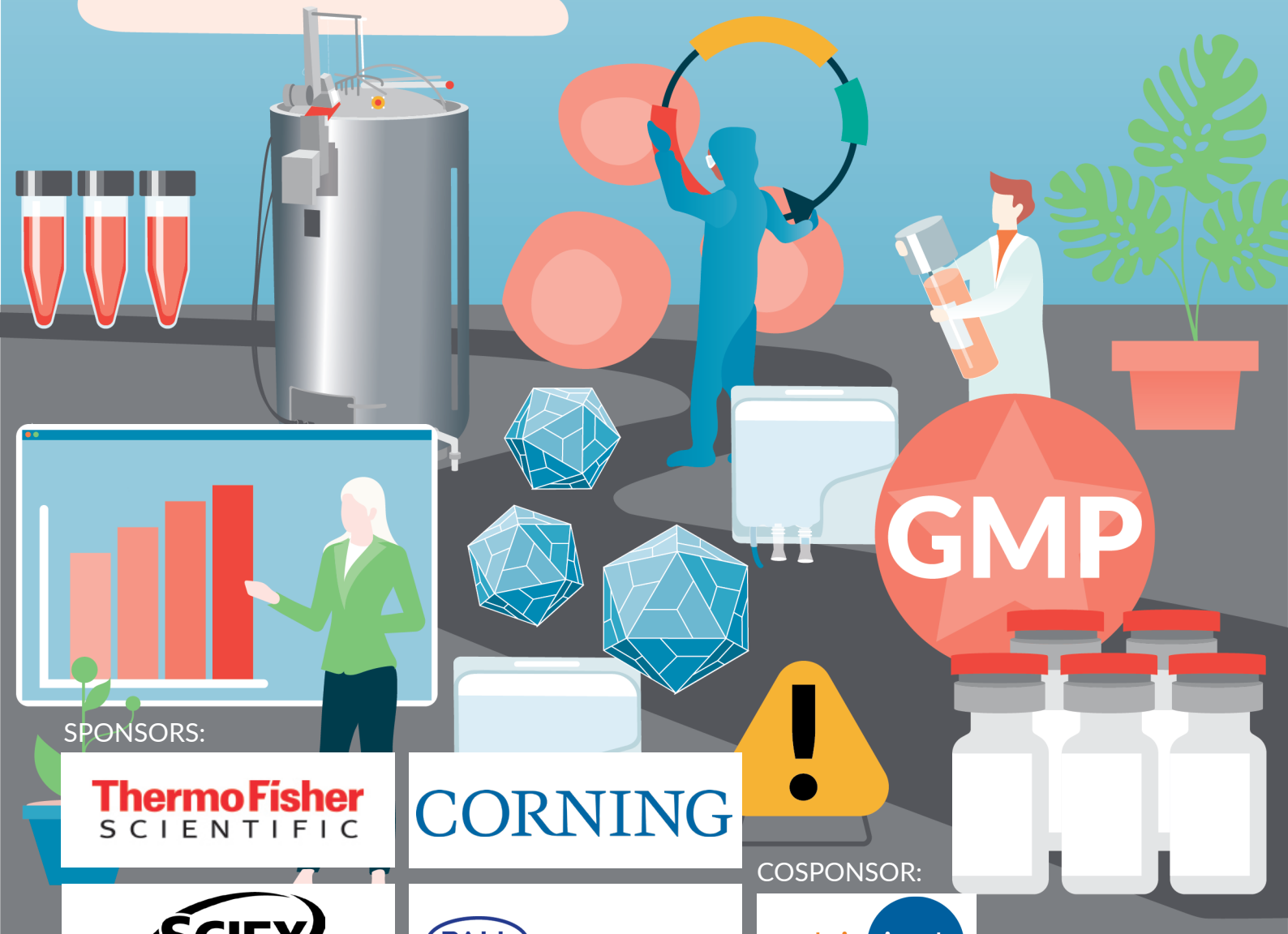

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COMMENTARY/OPINION

Yesterday, today and tomorrow: the evolving landscape of gene therapy manufacturing and process development

David R Knop

The first approved gene therapy procedure, which entailed reinfusion of a patient's cells that had been modified *ex vivo*, was performed more than 30 years ago. Over the past three decades, the field has seen tremendous progress, achieving a key milestone with the 2017 approval of the first gene therapy designed for *in vivo* administration. Today multiple *in vivo* gene therapies are in late-stage trials, and a diverse array of earlier-stage programs are targeting rare genetic diseases as well as large indications. Along the way, manufacturing of gene therapy vectors became an obstacle to advancing programs from proof-of-concept studies to late-stage clinical development and commercial use. With additional gene therapies moving toward approval, manufacturing continues to be a critical component of success with respect to regulatory approval and commercial viability. Realizing the full clinical and commercial value of gene therapy requires that we understand how the challenges of the past have informed and shaped the state of gene therapy manufacturing. To drive continued improvements in gene therapy manufacturing processes, we must employ lessons learned not only in the gene therapy space but also from the development of other therapeutic classes.

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The first approved gene therapy procedure, which entailed reinfusion of a patient's cells that had been modified *ex vivo*, was performed more than 30 years ago [1]. Over the past three decades, the field has seen tremendous progress, achieving a key milestone with the 2017 FDA approval of LUXTURNA® (voretigene neparvovec-rzyl), the first gene therapy designed for *in vivo* administration [2] (the *in vivo* gene therapy Glybera® was approved in Europe in 2012 but never received FDA approval and was withdrawn from the market in 2017 following commercial challenges) [3]. Today multiple *in vivo* gene therapies are in late-stage trials, and a diverse array of earlier-stage programs are targeting rare genetic diseases and larger indications. Along the way, manufacturing of gene therapy vectors became an obstacle to advancing programs from proof-of-concept studies to late-stage clinical development and commercial use.

With additional gene therapies moving toward approval, manufacturing continues to be a critical component of success with respect to regulatory approval and commercial viability. Realizing the full clinical and commercial value of gene therapy requires that we understand how the challenges of the past have informed and shaped the state of gene therapy manufacturing. To drive continued improvements in gene therapy manufacturing processes, we must employ lessons learned not only in the gene therapy space but also from the development of other therapeutic classes.

THE BENEFITS OF SUSPENSION VERSUS ADHERENT CELL MANUFACTURING PROCESSES

Consistent with many innovative therapeutic approaches, gene therapy technologies originated in academic laboratories where vector manufacturing was largely a means to the end – producing material for preclinical studies. As a result, initial manufacturing processes were largely designed to be time- and cost-effective, with less emphasis on yield, productivity or scalability. Adherent cell manufacturing processes (e.g., cells are grown in contact with the surface of the culture vessel) were more than adequate to generate material for *in vitro* and *in vivo* work that supported preclinical safety and efficacy studies. These processes allowed the nascent field of gene therapy to demonstrate its potential to address unmet need in a wide variety of disease indications.

However, as academic laboratories and the biopharmaceutical industry recognized the promise of gene therapy and began to pursue gene therapy product development activities, particularly large-dose or high-patient population indications, the need for larger quantities of vector to support clinical trials and meet potential market demand starkly revealed the limitations of adherent cell manufacturing approaches. Increasing production of vectors manufactured using adherent cells can only be achieved by scaling out – using an increasing number of fixed volume vessels (e.g., tissue culture plates, roller bottles or cell stacks) rather than scaling up through the use of increasingly larger volume production vessels. Scaling out increases reagent, personnel and facility costs as well as the potential for contamination that may occur while cells are being fed, passaged or harvested in an open system.

In contrast, suspension-based vector manufacturing processes (e.g., single cells or cell aggregates float in agitated media) offer economies of scale that have long been achieved in the manufacture of other biologic therapies, including therapeutic proteins and monoclonal antibodies. Suspension-based methods also enable streamlined scale-up through the use of larger bioreactor vessels that do not have significantly increased reagent, personnel or facility requirements. Additionally, the use of a small number of suspension bioreactors, which can be designed as closed or semi-closed systems, reduces the potential for operator error and contamination. Most importantly, suspension-based manufacturing enables processes with higher yields via process intensification with established

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approaches defined with other biologics. This approach is essential for meeting the needs of indications with large populations, such as age-related macular degeneration, or indications requiring systemic delivery to achieve therapeutic benefit, such as Duchenne muscular dystrophy. High density, fixed-bed, adherent cell growth bioreactors (e.g., iCeLLis® system) provide benefit over other adherent cell manufacturing processes, but are still constrained by a scale-out approach. The largest fixed bed bioreactor available (500 m^2) can reasonably accommodate 5×10^{11} – 1×10^{12} total viable cells. By contrast, this total viable cell population can be achieved in 25–50 L using a well-established, commercial suspension cell perfusion process that reaches 2×10^7 vc/mL and is executed at 1000 L scale [4]. Systems achieving in excess of 1×10^8 vc/mL have been reported, illustrating the potential for suspension process intensification to meet the needs of gene therapy when adapted from other biologic production paradigms [5], given 2000–4000 L single use bioreactors are currently in use. Fixed-bed, adherent bioreactors may be most cost-effective for gene therapy manufacturing processes that rely on transient transfection, but this benefit is lost when using stable producer cell line methods, such as herpes-assisted vector expansion approaches, adenovirus helper systems or baculovirus infection of insect cell technologies.

Finally, from a regulatory standpoint, there are clear paradigms and guidance for suspension-based manufacturing of other biologic therapies that can inform the development and implementation of robust and scalable methods for producing gene therapy vectors.

THE CRITICAL IMPORTANCE OF ANALYTICS IN GENE THERAPY PROCESS DEVELOPMENT

In 2002, the US Food and Drug Administration (FDA) announced the Pharmaceutical Current Good Manufacturing Practices (CGMPs) for the 21st Century initiative [6] to modernize the regulation of pharmaceutical manufacturing and product quality. The five key goals of the initiative with respect to pharmaceutical manufacturing and regulation are:

- Ensure product quality and performance through the design of effective and efficient manufacturing processes;
- Develop product and process specifications based on a mechanistic understanding of how formulation and process factors affect product performance;
- Real-time quality assurance;
- Tailor relevant regulatory policies and procedures to accommodate the most current scientific knowledge;
- Develop and implement risk-based regulatory approaches that recognize scientific understanding of how formulation and manufacturing process factors affect product quality and performance, and the capability of process control strategies to prevent or mitigate the risk of producing a poor-quality product.

In 2004, the FDA issued Guidance for Industry on PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance [7], which outlined how process analytical technology (PAT) could be incorporated into pharmaceutical manufacturing to achieve the goals of the 2002 CGMP initiative. A key objective of PAT is to move from assessing quality at the end of the manufacturing process to providing real-time quality assessments throughout the entire manufacturing process and controlling critical quality attributes and critical process parameters. The latter approach is especially important for the manufacture of gene therapies because gene therapy vectors are highly complex, and diverse components can individually and collectively contribute to the safety and efficacy of the finished material.

PAT should have the goal of real-time assessment of quality attributes. For example,

as separation of empty and full capsids during AAV manufacturing gets reduced to practice, at-line PAT for monitoring and controlling the purification step could be implemented.

More broadly, analytics for gene therapy manufacturing need to address multiple issues, including:

- The ratio of full to empty vectors (i.e., viral particles containing the therapeutic gene of interest versus particles without the gene), which plays a critical role in the total viral load administered to patients. As increased viral loads may contribute to adverse events, maximizing the number of full vectors is essential for delivering therapeutic doses while optimizing patient safety;
- The purity of the final material. This is especially important in gene therapy manufacturing processes that can use helper viruses or other process materials that need to be removed from the final product;
- The potency of the final material. The therapeutic effect of gene therapies is a function of multiple vector characteristics, including the ability to transduce target cells effectively, the expression level of the therapeutic gene of interest and the stability of the expressed protein. Analytic tests need to be developed, qualified and validated to adequately characterize such characteristics;
- In-process testing during upstream production, such as assessment of cell culture metabolites and cell viability, and downstream purification, such as extent of aggregation and ratio of full to empty vectors can further help refine processes.

Analytic methods for specific gene therapy vectors often need to be tailored to the manufacturing approach and unique characteristics of each candidate therapeutic.

Additionally, recent advances in analytics for characterization of other biologics should be evaluated and considered for incorporation into gene therapy manufacturing processes. These include the use of "smart" sensors, pumps, valves and motors that can monitor and provide feedback on cell culture conditions in real-time without the need for serial manual sample collection [8]. Quality by Design (QbD) approaches, which are systematic approaches predicated on achieving predefined endpoints and based on robust science [9], should also be considered. QbD approaches comprise real-time monitoring using physical sensors and advanced process monitoring and control that utilizes data from these sensors to model unit operations and predict process performance [8], and leverage technology to ensure that target metrics are maintained throughout the manufacturing process. These approaches may help to reduce lot-to-lot variability and lot rejection.

THE VALUE OF INVESTING EARLY IN LATE-STAGE MANUFACTURING METHODS

Manufacturing process development and analytics initiatives require investment of capital, personnel and resources. Therefore, companies developing gene therapies must consider the amount and timing of these investments in the context of their larger product portfolios and strategic objectives. While there is inherent risk to investing in later-stage needs early in development, recent events within the biopharmaceutical industry suggest that failing to invest early may actually pose a greater risk to value creation, especially if this failure leads to delays in clinical development and commercialization [10–13]. As more gene therapy programs advance to late-stage trials and approach commercialization, it is becoming clear that manufacturing issues have the potential to delay clinical advancement. In 2020, the FDA requested additional information related to gene therapy manufacturing from multiple companies [10]. In one of these cases, the FDA asked for a new potency assay to demonstrate consistency between

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the commercial manufacturing process and earlier processes used to produce material for clinical trials [11]. A similar issue regarding potency assays in 2017 led to a delay in initiating pivotal trials for a gene therapy for spinal muscular atrophy (SMA) [12]. More recently, a planned pivotal trial for hemophilia B was delayed by approximately two years in order to address CMC-related feedback from the FDA partially related to scaling [13].

These events underscore the importance of developing, implementing and optimizing commercial manufacturing processes prior to the initiation of pivotal trials to minimize clinical development delays, which can be costly in their own right. They also highlight the need to engage in frequent and productive dialog with the FDA and other regulatory agencies to ensure that appropriate measures are taken to adapt or amend gene therapy manufacturing processes to reflect an evolving regulatory landscape. This landscape is likely to undergo continued changes as a growing number of gene therapy programs move toward commercialization and regulatory agencies address tangible rather than theoretical manufacturing-related issues and concerns.

PROMISE & PERIL: WHAT'S ON THE HORIZON FOR GENE THERAPY MANUFACTURING

The potential for gene therapy to address critical unmet medical need in diverse indications is being validated by a growing number of approved products and an expanding base of clinical safety and efficacy data. For better or for worse, gene therapy manufacturing is now subject to greater regulatory scrutiny, and our industry's ability to improve patient outcomes demands that we continue to improve scalability, productivity, purity and potency and adapt to an evolving regulatory landscape. While our ability to achieve these important goals is in large part a function of manufacturing processes, continued innovation in vector development will also be important for enabling improved yields and reduced costs. Novel capsids continue to be an industry focus, and they hold great promise not only in the manufacturing realm but also with regard to enhancing efficacy and safety.

Additionally, the gene therapies approved to date, Zolgensma® (onasemnogene abeparvovec-xioi) for SMA and LUXTURNA® (voretigene neparvovec) for Leber congenital amaurosis, are currently the two most expensive drugs in the United States. Cost will play a significant role in enabling equitable access to the next gene therapies to reach the market. Here again, high productivity, cost-effective manufacturing will be a critical component to the overall price of these therapies.

Only by placing as much emphasis on manufacturing as we do on safety and efficacy can we truly provide the benefits of gene therapy to the many patients whose health and survival are in our collective hands.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING

SPOTLIGHT

INTERVIEW

Improving AAV vector manufacture: a question of scale





(Pictured from top left)
Silke Wissing,
Juliana Coronel,
Jens Wölfel,
David Mainwaring &
Amar Joshi

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Q Could you tell us about the chief drivers for improving AAV vector manufacturing, and what the key target areas in this regard are?

SW: AAV is the viral vector most commonly used for *in vivo* gene therapies. For these kinds of therapies, in particular when applied systemically, enormous amounts of vector of up to 1 x 10^{15} viral genomes (vg) per patient are needed.

If we want to produce these amounts of vector with transient transfection, this can correlate to batches of up to 50 L, just to produce enough material for one patient. The problem is that transient transfection is extremely difficult to scale, and very costly. If we want to bring these therapies to patients in need, there is a clear production gap – particularly if we want to target diseases with high incidence such as Parkinson's. Cevec's goal was to simplify AAV production, and ultimately make it as easy as monoclonal antibody (mAb) production.

The solution was to eliminate the transient transfection step. Nobody uses transient transfection for antibody production; instead they use stable cell lines. This is why we generated our stable AAV production platform, called Elevecta®, in which we stably integrated all components necessary to produce AAV in the genome of our suspension cells.

This allows us to scale-up the cell culture to the intended production volume, and then simply induce AAV production, with titers of 1 x 10^{14} vg/L for a normal batch, and so far 3 x 10^{15} vg/L for more intensified processes.

Q How would you sum up the pros and cons of transient of transfection versus stable producer cell line-based AAV upstream bioprocessing?

DM: The main advantage that transient transfection offers is speed, as it is very quick, and has also got a lot of flexibility. It is very easy to swap in new constructs, such as modified genes of interest, or even looking at different capsids. It is typically done in adherent culture, but we are now seeing this come through to suspension as well.

> "...transient transfection is extremely difficult to scale, and very costly. If we want to bring these therapies to patients in need, there is a clear production gap."
>
> - Silke Wissing

As Silke mentioned, the reagents are incredibly expensive. For example the volumes of PEI, if you are using that as a transfection reagent, are quite high. The amounts of DNA that can be needed are also very high, and it is expensive to get good quality GMP grade DNA.

There are also issues around liquid handling. Doing something at the small scale is easy, but if you come to scale-up to large-scale, it becomes difficult to add the DNA and your transfection reagents in a timely manner. The timing for some of these things

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> "Stable cell lines have a key advantage in that you only need to make them once. Obviously, that brings with it better cost of goods, because you are not having to add transfection reagents every time, or have DNA made every time.
>
> - David Mainwaring

is critical. For example, it can be between 5 and 20 minutes, but adding large volumes into a reactor isn't straightforward when you scale-up. The other disadvantage is the consistency of batches. If you are always having to make a transient transfection, the quality of that product could be different with each transfection if it is not well controlled.

Stable cell lines have a key advantage in that you only need to make them once. Obviously, that brings with it better cost of goods, because you are not having to add transfection reagents every time, or have DNA made every time. Additionally, these things can be difficult to get hold of, and there can be long lead times – especially with the current extended lead times we are seeing (due to the COVID-19 pandemic).

On top of that, it is fair to say it is easier to scale-up a stable cell line compared to an adherent one. There is also a lot of background knowledge within the industry from mAb production, so people are really familiar with this. A stable cell line is much more normal for people scaling up. However, stable cell lines do need some time to generate.

SW: This is true – cell line development takes time. We all know time to market is very important for everyone. But it is also really important to produce enough material in order to secure supply, with consistent quality, and also at a reasonable price.

For the generation of our stable producer cells, we use our well-characterized alpha cell line in which we have already integrated the replicase and the helper genes as a starting point. These are the two components which are common for each and every AAV project.

We then start cell line development by stably integrating the project-specific capsid, and the transgene, followed by one single cell cloning. Therefore, the very time intensive single-cell cloning step is only done once. The timelines for cell line development are around eight months from DNA – so from the plasmid to identification of the top monoclonal producer single cell clone. Also keeping in mind what David just said, with this system, you save the generation of plasmid DNA or helper virus in GMP quality, which currently has very long lead times.

Q What are the capabilities and advantages of the latest stirred tank reactors (STRs) for AAV vector production?

DM: Modern STRs have a number of advantages compared to the traditional systems, where you would be looking at adherent culture. Primarily, those are ease of use and familiarity of systems compared with flatware.

Scale-up is also very straightforward with STRs. With flatware, it can be difficult to scale up enough, so you have to do multiples rather than just do one large bioreactor. The seed train of STRs is also simplified, as there is less manual handling needed. You don't have to handle lots of flasks, and instead you can just go from one stirred tank to another, which is very straightforward.

Recently, single-use bioreactors have come to the fore, and they offer rapid set-up and turn-around. That is an advantage in a busy facility as you have less downtime, so you can get more batches through. With single-use there are also reduced validation requirements. For example, you don't have to do cleaning validation, which can take a long time. That is key in a multi-product facility. There are a large range of volumes; recently larger scale has become available, up to several thousand liters in single-use. Historically, that just hasn't been available.

Within the bioreactor itself, you can now have representative sampling from an STR. Because your cells are constantly being mixed, the contents should be homogenous. Unlike an adherent culture where you can only sample the supernatants and get an idea of what nutrients are doing, you can sample the supernatant and the cells. This enables you to get a very good idea of how the cultures are behaving.

As well as that advantage, with modern aseptic connectors it is possible to put novel sensor technologies in as these become available. Historically we have looked at pH and dissolved oxygen, but more and more now people are looking at things like Raman spectroscopy, for example. This leads nicely into the PAT (Process Analytical Technology) initiatives that are ongoing.

Q Building on that, what are the chief considerations for upstream bioprocess development with an STR?

DM: The considerations are similar for both STR and adherent technologies. It is key to really understand what your target final scale is. You may not know that when you start, so that can present a challenge. It is important that any process you are developing can operate within the design space that is available at the large scale, when you start to do your scaling up.

When we worked with Cevec, we started at the 50-L scale on our Allegro™ STR bioreactor and scaled to 200 L. Because of how we do our development work and our scale-up, we know that we could scale that process further. We are not limited by any parameters within the scale-up strategy that we have. For example, we know we can supply sufficient oxygen within the systems in all the reactors that we use.

This comes around to what your strategy is for scaling up. There are lots of different ways that people apply scale-up strategies. They may try and maintain power input, or tip speed, or gas flow rates per unit volume. It is also important to look at what the CO_2 accumulation is doing. For example, at small scale some people may apply a headspace gassing to remove CO_2 from the system. But when you get to a large-scale bioreactor, that simply isn't going to have much of an effect. It is important to understand these things right at the start, before you start scaling up.

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Additionally, what you do easily at the small scale, you can't necessarily do at large scale. A key example here is addition volumes. If you are adding 200 mL to a 2 L bioreactor, that is really easy to do.

When you are at the 2,000-L scale and it is 200 L you have to add in, that is a whole different ball game, both in terms of the timing it takes, and the logistics of physically maneuvering this volume around a facility. It is important not just to think of the bioreactor, but the whole facility fit, and how this is going to operate within a bioreactor suite.

There are also things that often get missed. There are differences in heating and cooling times within large-scale and small-scale bioreactors. If you are employing a temperature shift, and the timing and rate of that shift is important, that is going to behave very differently at large-scale than it will at small-scale.

Q Can you tell us about your own experiences in scaling-up into an STR?

JC: Before the transfer to Pall, we did initial work at Cevec. We did characterization in the mL scale that included some screening in deep well plates, as well as shake flasks. At the end, we did some screening in the Ambr® 15 stirred tank bioreactor system. This was then directly scaled to 10 L at Cevec, and this 10-L process was then transferred to Pall. This worked out very efficiently and smoothly.

At Pall this was later scaled up to 200 L, and the biggest thing I learned was the parameters used for this work. In our case, it was the power input and the superficial gas velocity for the air flow rate calculation. David, can you comment on the choice of these strategies you used?

DM: We chose these parameters, and it was key to understand the small-scale model before we started scaling up. Within Pall we generated a lot of characterization data around our bioreactors, and we looked at different scaling strategies. The reason we settled on power input and superficial gas velocity was that we were able to maintain those constant across the entire range of bioreactors.

At the end of the day it is a compromise, because you can't keep everything the same, but we found that this gave us the best scale-up. Having that well-defined strategy really simplifies scale-up to the larger scale.

Another of the key things here is that open communication is important, both to ensure the process is transferred to us, and then that scaled-up process can be transferred back to either the customer or to manufacturing sites. This is something that works really well when people talk together.

JC: After this scale-up we were excited to learn that the CAP cells grew very

> "...CAP cells grew very well in the Allegro bioreactor system at the two scales of 50 and 200 L, and the cells achieved short doubling times."
>
> - Juliana Coronel

"Pall have some great offerings in downstream for gene therapy products. We introduced technologies into both the clarification and the ultrafiltration unit operations for the Cevec downstream process.

- Amar Joshi

well in the Allegro bioreactor system at the two scales of 50 and 200 L, and the cells achieved short doubling times. High viability was maintained in the cell growth phase before the induction of AAV production. The processes were very reproducible, and very importantly, the productivity was maintained throughout the scale-up process.

Q We have talked a lot about the upstream side of things – Amar, could you introduce us to the downstream bioprocess technologies involved in the recent work with Cevec, and the advantages they offer?

AJ: Pall have some great offerings in downstream for gene therapy products. We introduced technologies into both the clarification and the ultrafiltration unit operations for the Cevec downstream process.

For clarification, we introduced Seitz™ Depth filters. These step filters come in a wide selection of pore sizes that can be tailored to different feed streams. They are suitable for both suspension feed streams and adherent feed streams, and give you excellent capacity and product yield.

The depth filtration process was developed on small Supracap™ 50 modules, which have 22 cm^2 effective filtration area, and we know how these linearly scale up to the larger Stax™ capsules which come with 0.5–2 m^2 effective filtration area for double-layer construction depth filters and 0.25–1.0 m^2 effective filtration area for single-layer construction depth filters. The advantage to these Stax capsules is that they are disposable and easy to use. In a facility, the Stax capsules can easily combined to create the process area that you want with a small footprint, and easily disposed of after use.

Usually, a bioprocess specialist would visit in the field, but during the first quarter of 2020 it was unfeasible to do site visits due to COVID-19 being rampant in Europe. We worked with Cevec to identify the filters we wanted to assess, we did two or three studies with them, and we analyzed the data together. We decided upon a process that gave us a good yield, good operating time, had a compact footprint, and amplified the good cost of goods. We tested that using 1–2 L at the Cevec site, and that process was then transferred to Pall's Accelerator℠ process development services team at the Harbourgate site in Portsmouth, UK, and we tested that at

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50 L or 200 L. We had very good scale-up with that, with very similar pressure profiles, and good product quality and yield.

Another technology we introduced to the Cevec process was Pall's Omega™ T-series TFF cassettes, to replace existing TFF technology within the Cevec process. Our Cadence® single-use TFF modules are great – they work at small-scale, where you can do development or small-scale lab work, and they scale up to large devices. These Cadence single-use devices are already gamma irradiated, which makes it much easier for processing and manufacturing where you can eliminate pre-use sanitization, and pre-use processing time, as well as buffer usage. Another advantage is that these gamma irradiated units come in the same arrangement and size as the conventional cassettes, so they can be swapped in and out easily as scale dictates.

The TFF was sized at around 100 cm^2, and for the Cevec processes we used the 100 kDa molecular weight cutoff membrane. Again, this worked really well for the Cevec process, and scaled up to 200 L.

Q Jens, it would be great if you could tell us about your downstream bioprocessing experience with tangential flow filtration steps. How did they perform in terms of scalability, for instance?

JW: The initial problem with the downstream process we developed at Cevec was that the main chromatography step of our AAV downstream process had a rather slow binding kinetic. Therefore, the intention of the development of the TFF step in the downstream process was to reduce the process time for the sequent chromatography step by concentrating the cell culture sample. This was successfully achieved, and the process time was shortened by several hours.

We developed and transferred the process for up to 3 L cell culture sample to Pall, and the process was easily reproducible at Pall. As Amar already mentioned, a linear scaling to 10-, 50-, and 200-L scale was working out as expected without significant changes in any of the parameters for the TFF.

Q What are the do's and don'ts on the tech transfer on the downstream side for you, and are there any examples you can share from the Cevec partnership?

AJ: The tech transfer is essential to give the receiving lab all the process knowledge to run the process and to get the right productivity and product quality. It is the responsibility of both parties to get it right.

Do start early – we started the tech transfer in the week after the project kickoff, many months before the first batch was going to be run.

Do structure information transfer – I prepared some process information templates for the different unit operations that gave details of the inputs and outputs, the filter areas, and the

> "In our experience...early communication during DSP development helps to avoid reliance on special devices or analytical measures during the development work that cannot be set up at larger scale or on different sides.
>
> - Jens Wölfel

flowrates, and that was the basis for the tech transfer. It is also helpful for scale-up.

Do maintain communication – during the tech transfer we communicated really often and had several side meetings as well as our usual group catchups. We identified early on that there was an issue with clarification, where the units come in discreet sizes and we had to deviate away from the linear scaling. Together we worked to identify the best solution that would help us get a bill of materials to proceed with the batch.

Finally, do review documentation and don't assume anything. It is very easy to make copy–paste errors, or to think something is so obvious as to not mention it. Detailing those things can really help in making tech transfer run smoothly. Overall, the transfer between Cevec and Pall went well.

JW: We experienced that some obstacles could be different equipment and capabilities of in-process analytics, which could result in transfer issues. In our experience, and this was also the case with Pall, early communication during DSP development helps to avoid reliance on special devices or analytical measures during the development work that cannot be set up at larger scale or on different sides. As Amar said, early communication is very important.

Q What are the key benefits in having a technology partner in vector bioprocess scale-up, and could you illustrate them with some examples from the partnership with Pall Corporation?

JC: At Cevec, we do not have large-scale capabilities in-house, so it is logical to collaborate with a very experienced partner who is able to do the scale-up for our processes. Now, we have a straightforward general process which can then be transferred to customers. This can be adapted if necessary, according to the project needs or adjustments for different single-cell clones can be done, for example.

Usually, Pall also offers customers to go on-site and chaperone in key steps during the production process. In our case this was unfortunately not possible due to the COVID-19 situation, but in the future if we work together and do more processes, we can learn more and see the actual scale-up happening.

JW: I also learned a lot in this cooperation with Pall. Although several processes have been successfully transferred to customers in the past, usually only minor information from the actual up-scaling is transferred back to Cevec once the project is in the customers' hands.

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Due to the close communication with Pall, we learned a lot about the possible obstacles and critical points in managing such a project when it comes to production scale. For example, lead times of materials at larger scale, which are typically not really relevant in research and development. This really helped Cevec to better oversee the demand of our customers during the research and development stage.

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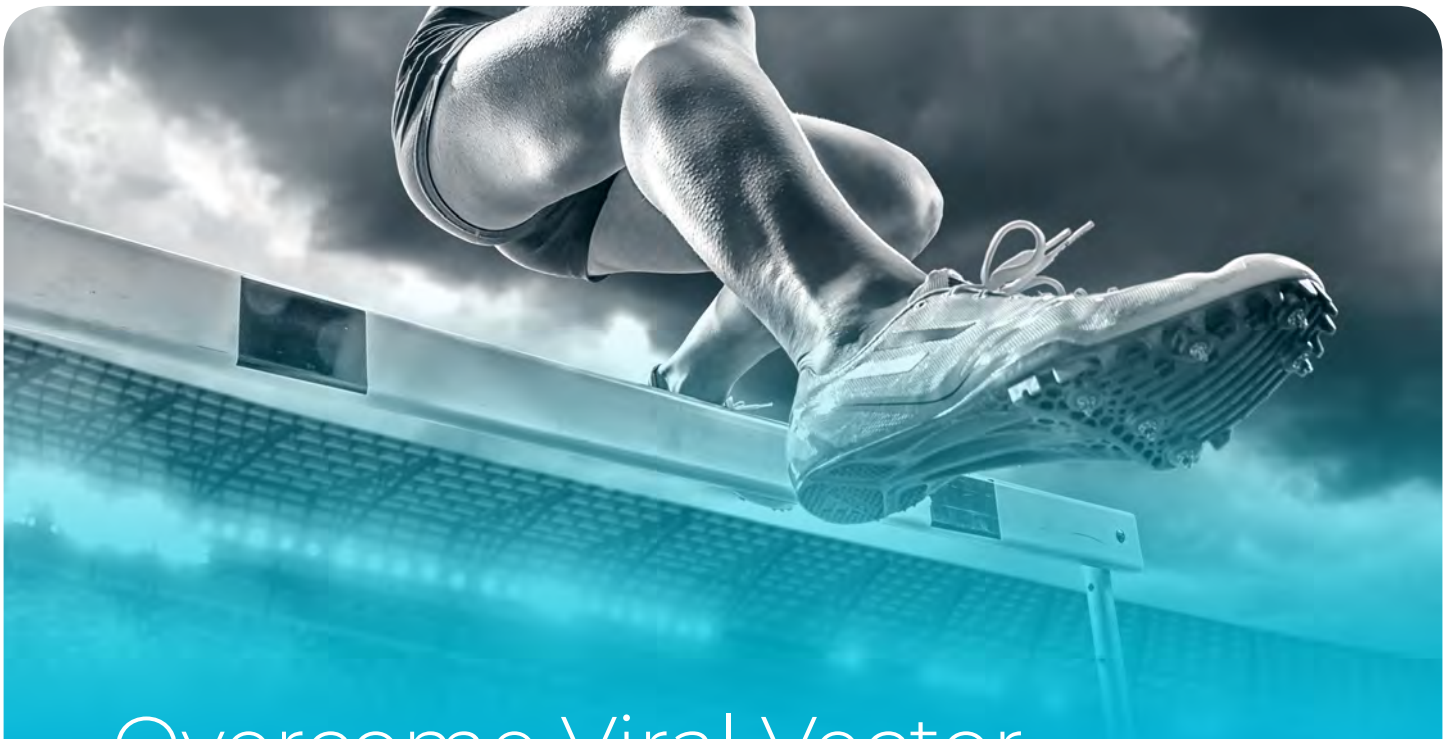
PALL CORPORATION



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FASTFACTS

Efficient and scalable purification of mRNA using affinity chromatography

Synthetic mRNA is a versatile modality with a wide array of applications, from stem cells and cell differentiation to vaccines. What all these platforms have in common is the need for a stable, reliable and scalable production and purification platform to enable production of high quantities of mRNA with the required purity and quality attributes. To support the development of mRNA-based therapies, Thermo Fisher Scientific developed the POROS™ Oligo (dT)25 affinity resin to enable efficient and simplified mRNA purification.

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SELECTIVE, SCALABLE AND CGMP COMPLIANT PURIFICATION

The POROS™ Oligo (dT)25 Affinity Resin was specifically designed for the purification and isolation of mRNA from *in vitro* transcription manufacturing processes, and offers a scalable and highly selective purification platform for any mRNA with a PolyA tail. The resin is fully cGMP compliant and animal-origin free, and Thermo Fisher Scientific can provide users with regulatory support.

RECOVERY AND IMPURITY REMOVAL

High recovery is seen independently of construct size and sample type. **Figure 1** shows percentage recovery for three different mRNA constructs. Consistent recovery well above 90% was seen in all cases.

PURIFICATION RUN

Figure 2 shows the typical output of a chromatographic purification run when using the Oligo (dT)25 resin. The sample was loaded at high conductivity, and upon loading the absorbance increases as the impurities flow through the column. An intermediate wash step was used to remove any loosely and non-specifically bound components, and elution was performed using pure water. A minor peak was seen during the alkaline cleaning step, showing that some residual components were still present and can be removed by alkaline conditions.

RESIN REUSE AND CLEANING

The effect of incubation of the resin in sodium hydroxide and hydrogen chloride was studied **(Figure 3)**. The ionic capacity, which has a direct correlation with ligand density, was well maintained after 48 hours of constant exposure to cleaning agents. A cycling study demonstrated consistent performance, with overlapping elution peaks, and no carry over seen in a blank run performed after 10 cycles.

HIGHER YIELDS WITH LOWER TIME AND COST

The POROS Oligo (dT)25 Affinity Resin offers a highly stable and reusable method for mRNA purification. With high affinity for the target molecule, it can deliver high yield and purity, and help to reduce the number of purification steps in the overall process. By reducing bioprocess development time, it can result in a decrease in overall cost of goods, and ultimately, a faster time to market for innovative mRNA-based therapeutics.

Figure 1. High recovery and purity is achieved with the Oligo (dT)25 resin, independent of construct size.

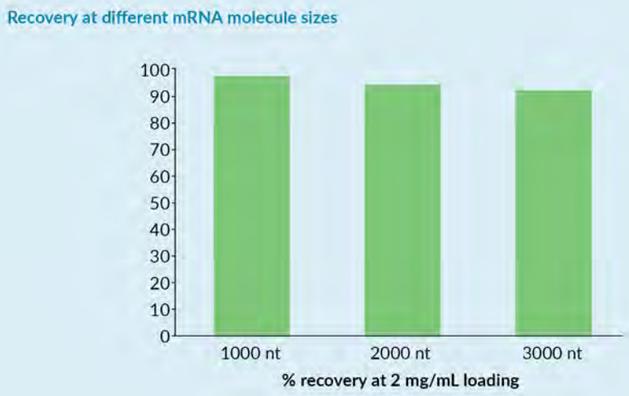


Figure 2. Output of a chromatographic purification run using the Oligo (dT)25 Affinity Resin.

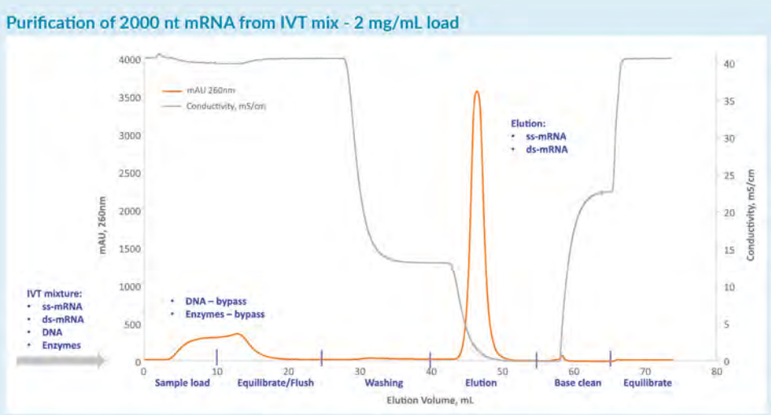
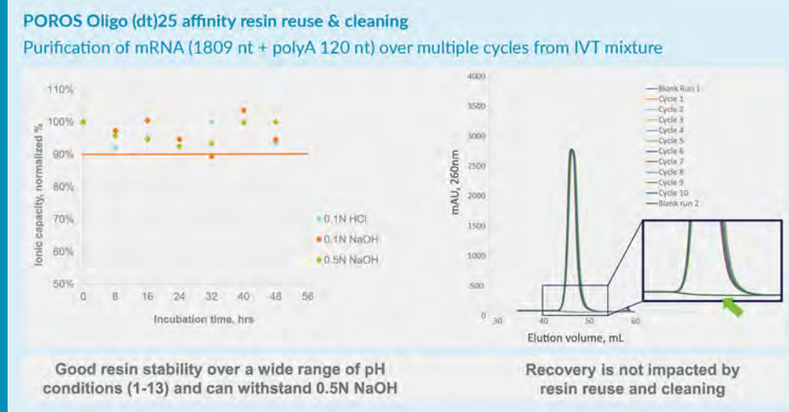


Figure 3. Resin stability over a range of pH conditions (right) and overlay of elution peaks over 10 cycles (left).



CELL & GENE THERAPY INSIGHTS

In partnership with: ThermoFisher SCIENTIFIC

FASTFACTS

Simplifying AAV protein analytics with Maurice

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SIMPLIFYING AAV PROTEIN ANALYTICS WITH MAURICE

In recent years, the pharmaceutical industry has seen a great increase in biotherapeutics, and companies have begun to leverage newer therapeutic modalities, including viruses and nanoparticles. Adeno-associated viral (AAV) vectors in particular have emerged as an attractive gene therapy delivery tool. However, they are also complex, and challenging to manufacture – the process is long and costly, and hard to scale. These challenges require manufacturers to carefully design and implement tests and control strategies to address the various attributes of their viral product. ProteinSimple, a Bio-Techne brand, have built on and evolved existing viral vector analytical techniques to provide faster, better analysis of AAV products through products like the Maurice CE-SDS PLUS system for AAV analytics.

CE-SDS ANALYSIS OF AAV PROTEIN PURITY

AAV protein purity is a critical quality attribute that Maurice can address. At a run time of only 35 minutes per sample, with up to 48 samples per batch, Maurice CE-SDS provides a rapid approach to gathering data from AAV samples. It is reproducible, with a relative standard deviation (RSD) of typically under 10%. Maurice provides a widely accepted platform for biopharmaceutical purity analysis, and is also easy to use, making it suitable for a broad range of users in applications ranging from research and development to quality control (QC).

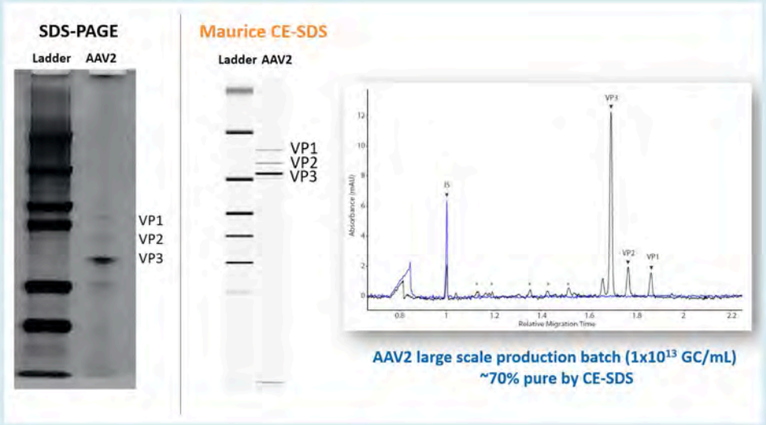
SDS-PAGE has traditionally been used for viral vector identity and purity analysis but is now a dated technique which presents several challenges. In contrast, Maurice CE-SDS can provide clearer results while using only a few microliters of sample **(Figure 1)**.

ICIEF ANALYSIS OF AAV CHARGE HETEROGENEITY

Maurice also provides the gold standard platform for charge heterogeneity analysis of biopharmaceuticals, using imaged capillary isoelectric focusing (icIEF) to characterize AAV charge variants. AAV protein charge heterogeneity information is crucial to understanding changes to individual viral proteins. Unlike ion exchange chromatography, iCIEF is a fast and high-throughput technique, at a run time of under 15 minutes per sample, with the ability to analyze up to 96 samples per batch. Maurice also provides detection flexibility, as it uses 280 nanometer light to provide absorbance data, but is also equipped with both native fluorescence and optional blue fluorescence (458 nm) in order to leverage multiple detection capabilities for AAV analysis.

For AAV analysis using the icIEF mode of Maurice, two methods were developed to characterize AAV capsid proteins or intact AAV particles. The capsid protein method breaks the capsid into individual proteins using a denatured approach, while the particle characterization method is much gentler, and maintains the AAV particle while providing sufficient solubility for analysis. Both methods can be run in under 12 minutes per sample, using only a few microliters of product **(Figure 2)**. These icIEF methods can be used for AAV stability testing, and for examining empty/full capsids.

Figure 1. CE-SDS analysis of AAVs.

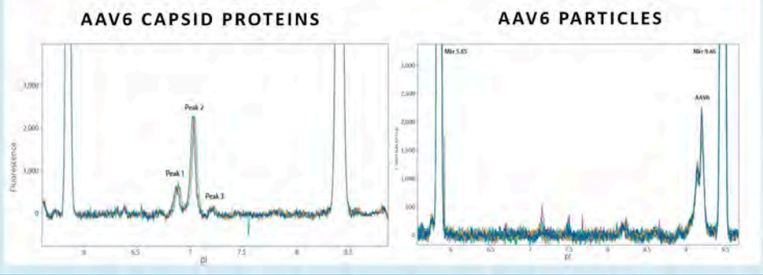


MAURICE: A FULLY INTEGRATED AAV ANALYTICAL SOLUTION

The demand for techniques for AAV protein analytics continues to grow, and Maurice addresses those needs by combining two AAV characterization tools in one instrument: CE-SDS to measure capsid ratio and AAV sample purity, and icIEF to allow both capsid protein and intact capsid analysis. Maurice is a QC-friendly system that is CFR 21-part 11 complaint, with optional Empower integration, and provides a fast, easy-to-use tool to assess a variety of viral vector quality attributes.

Figure 2. iCIEF analysis of AAVs.

	Capsid Protein Characterization	AAV Particle Characterization
Method Approach/Goal	Denatured/Break Capsid	Mild/Maintain solubility
Run time (per sample)	12min	7min
Sample Requirement	2-5uL at 1x 10^{13} GC/mL	
Detection Method	Absorbance and Native fluorescence	



CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING

SPOTLIGHT

COMMENTARY/OPINION

Making the move from antibody therapeutics to gene therapy: applicability of monoclonal antibody learnings to adeno-associated virus vector bioprocessing

Andrew D Tustian

The surge of interest in gene therapy in the past few years has led many companies to build process development groups to drive these therapies into the clinic. It can be hard to recruit bioprocess scientists with gene therapy backgrounds, therefore many bioprocess scientists from the monoclonal antibody field have moved to gene therapy development. Here we discuss, with specific focus on adeno-associated virus (AAV) based viral vectors, how knowledge of bioprocess manufacturing steps, regulatory expectations, disposable technologies, viral clearance and quality by design approaches make these scientists invaluable for the gene therapy industry. However, the larger size and mass of the AAV product, lower process productivity, and lack of molecular biology and virology knowledge can be pitfalls for those making the transfer.

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After decades of development and learning, gene therapies are a field of explosive interest for biopharmaceutical development. The US Food and Drug Administration (FDA) has predicted, based on current clinical pipelines, that by 2025 ten to twenty new cell and gene therapies will be approved annually [1]. One popular vector, due to its low immunogenicity and lack of integration into the host cell genome, is adeno-associated virus (AAV). AAV treatments first gained regulatory approval in the United States in 2017 with voretigene neparvovec (Luxturna®, Spark Therapeutics) for treatment of Leber's congenital amaurosis, followed by onasemnogene abeparvovec (Zolgensma®, Novartis and AveXis) to treat spinal muscular atrophy in 2019. Use of AAV has shown enormous potential in recent years for its use in gene therapy, with more than 150 ongoing clinical trials specific to AAV over a range of therapeutic applications [2,3]. Producing AAV for clinical or commercial use is a developing field with varied approaches taken including: transient transfection, baculovirus infection in Sf9 cells, the development of stable producer cell lines etc. [2].

In contrast, the first monoclonal antibody (mAb) therapeutic, muromonab-CD3, was approved for treating steroid-resistant acute allograft rejection in renal transplant patients in 1986: 35 years ago in a previous century [4]. The FDA has since approved more than 100 antibody-based therapies from more than 40 companies [5]. In total, 61 mAb products were approved in the past seven years (January 2014 – November 2020), and over 830 mAbs are in clinical trials, with 88 in Phase 3 trials [6]. For over a decade, commercial antibody bioprocessing typically conforms to an established production platform, with most antibodies expressed in producer CHO cell lines and purified via Protein A affinity chromatography and one or two polishing chromatographic steps to remove aggregated forms and process-related impurities. Although newer types of bispecific antibodies [7,8] can require modifications to traditional platforms, the commoditization of the mAb field and the clinical promise of gene therapy has recently led many bioprocess scientists to move across to take the plunge into gene therapy vector bioprocessing. What vital insights can such adventurers bring to the AAV field, and what extra knowledge is required for successful AAV vector bioprocessing?

This topic is one in which the author has specific insights, having recently made the jump within his current employer, Regeneron, from mAb and bispecific development to AAV process design. Although Regeneron Pharmaceuticals is known primarily as antibody technology biopharmaceutical company, recently it has been entering the gene therapy field. This move is being driven through both internal development and external collaborations with companies such as Intellia Therapeutics. To that end, in April 2020 in the midst of a pandemic, Regeneron opened a newly constructed pilot plant and development laboratory specifically designed to perform process and cell line development, as well as preclinical manufacturing of AAV.

What learnings from 35 years of mAb development can be used to speed AAV bioprocess development? First, the two processes utilize broadly similar unit operations including bioreactor production, normal and tangential flow filtration, and packed bed low pressure chromatography (Figure 1) [9]. Therefore, the unit operation knowledge of a scientist skilled in the art of mAb production can be applied to the equivalent manufacturing steps in AAV production. Furthermore, the monoclonal antibody manufacturing industry has extensive experience with production at large scale whilst conforming to all current Good Manufacturing Practices (cGMP) to fulfill regulatory expectations for the production of pharmaceuticals intended for human use. Best practices have also evolved for technology transfer and performance of comparability assessments required due to plant or process changes, many of which are transferable and can inform the gene therapy field.

Second, since the 1990s mAb production has led the way in single-use technologies (SUTs) for bioprocessing. The first single-use

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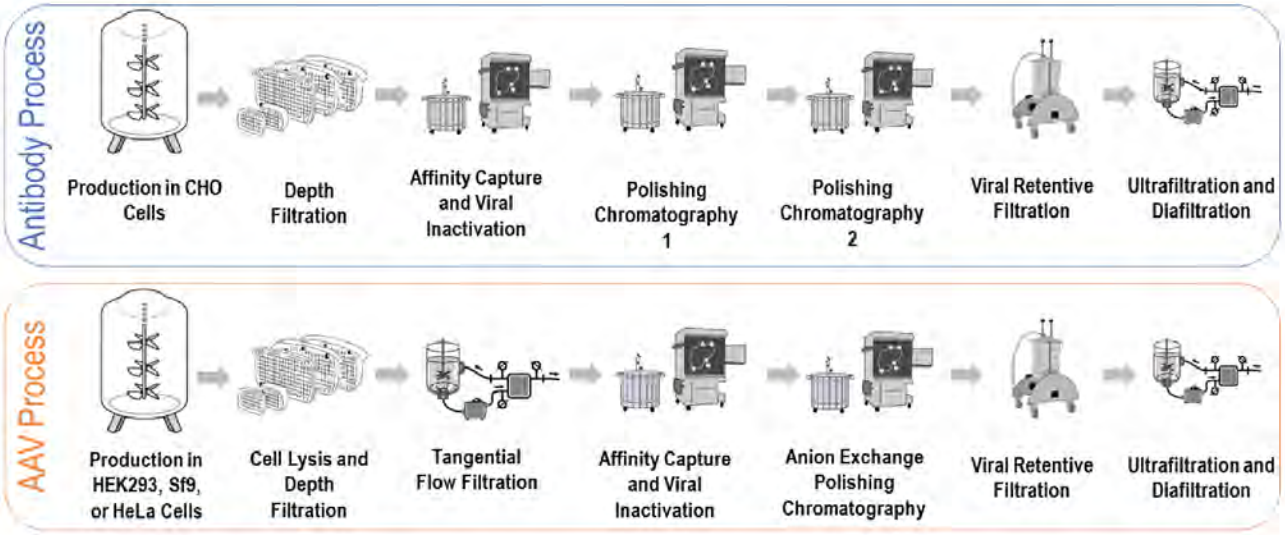
FIGURE 1

Comparison of mAb process platform to typical AAV production process.



Both processes are superficially similar with a batch or fed-batch bioreactor production followed by a series of chromatographic and filtration steps.

bioreactor was launched in 1996 by Wave Biotechnology (now Cytiva) [10] and the first single-use stirred tank bioreactor in 2004 by Hyclone (now Thermo Fisher Scientific) [11]. These new systems, relative to traditional stainless-steel equipment, have enabled fast-turnaround time between batches and products, with rapid setup, increased flexibility, and fewer resources required for cleaning, steaming, and validation activities. Speed to market authorization can be increased while capital cost is decreased. Disposable technologies are particularly applicable to AAV production due to smaller batch size, faster turnaround time between batches, and desire to isolate the process for sterility, cross-contamination, and operator safety concerns. Here mAb bioprocess knowledge of SUTs implementation, limitations and the need to control extractables and leachables is highly applicable [12].

The risk of viral contamination is a feature common to all biotechnological products derived from cell lines of human or animal origin [13]. As discussed in International Council on Harmonisation (ICH) Q5A (R1), three principal, complementary approaches have evolved to control the potential viral contamination of biotechnology products:

i. Cell lines, banks, and raw materials are screened for the absence of adventitious agents;

ii. The product is tested at appropriate stages of production for the absence of contaminating infectious virus; and

iii. As it is not possible to test for all viruses, manufacturing processes for biologics must be designed to inactivate or remove known infectious agents that could enter or propagate in the process stream when generating the product as well as unknown, adventitious viral contaminants [14].

Despite the product of AAV vector bioprocessing being itself a virus, appropriate clearance and viral safety controls must still be demonstrated [15]. Antibody bioprocesses bring in-depth knowledge of all three approaches for adventitious agents safety management. In terms of viral clearance, as with mAbs, the capacity of the AAV downstream process to remove viruses is assessed by

spiking studies, with knowledge of the design and performance of these studies easily translated from mAb to AAV bioprocesses.

Finally, while historically process development for biologics often emphasized the definition of setpoints and conditions for the process through well controlled single variable experiments, the principles of mAb bioprocess development have evolved. Now, the principles of quality by design (QbD) are routinely used ensuring a more systematic, goals focused approach leveraging both historical knowledge and results through multivariate experimental design and utilizing quality risk management during the entirety of the development cycle.

QbD is defined in the ICH Q8 (R2) guideline as 'a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management' [16]. Several reviews have been published that highlight the benefit of quality by design during development [17,18]. In short, a QbD approach can:

i. Increase process robustness through knowledge of both what quality attributes are critical, and what process parameters are the most relevant to those attributes;

ii. Enable process transfer across facilities and scale, and decrease regulatory burden upon so doing;

iii. Facilitate control strategies to enable more consistent products;

iv. Streamline lifecycle management;

v. Decrease the likelihood of failure across all stages of processes by reducing the likelihood that high-risk issues escape attention (although there is always the risk of novel unknowns surfacing).

Figure 2 shows a typical roadmap that can be used when developing processes within the QbD framework. These tools can be transferred from mAb development, coupling development based on pre-defined critical quality attribute goals, risk assessment, multivariate statistical design, process modelling and failure rate analysis, and knowledge-based process controls. Together, this approach can result in robust and reliable vector bioprocesses where the effect of process parameters and controls upon quality attributes is well-characterized.

However, a virus is not a monoclonal antibody and it is unrealistic to believe all aspects of mAb bioprocessing can be transferred to a new field. What knowledge gaps can those moving across to gene therapy anticipate? First, whilst a mAb is a large protein, being a tetramer of four polypeptide chains approximately 10 nm in size (Mw ≈ 150 kDa), an AAV is approximately twice as large at 20–25 nm in diameter and is composed of no less than sixty proteins. These proteins encapsidate a genome of 4.7 kilobases (kb) in length, for a total Mw of ≈ 4.8 mDa [19]. In other words, an AAV particle is over twice as large with 32 times the mass of a mAb, and is defined by the viral vector genome carried. Furthermore, whilst a typical mAb process may expect to produce 5–10 g/L titer in an industrial fed-batch bioreactor, AAV processes might produce 1e14 vector genomes per liter (vg/L) at harvest, equivalent to 0.8 mg/L of viral particles. Therefore, the volumes processed are correspondingly different: while a 2 kL fed-batch bioreactor, with a 70% process might be expected to produce 140 L of drug substance at 50 g/L, an equivalent scale AAV process with a 50% yield would only produce 5 L of drug substance at a typical concentration of 2e13 vg/mL (0.3 g/L). That is assuming the process allows scaling to 2 kL scale; not a given for processes involving transient transfection in GMP suites.

Practically, this means that best practices and concerns from mAb bioprocessing may not necessarily translate. For example, although the chromatographic steps in the two processes may look similar initially, the limitations are very different. Notably, the lower product concentration in the bioreactor means that capture column capacity is less of

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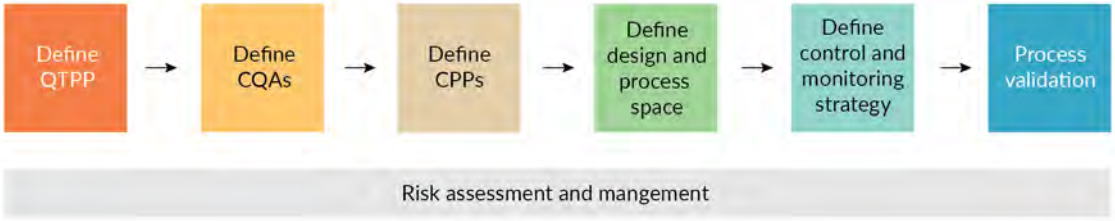
FIGURE 2

Schematic of quality by design-based process development.



Risk assessment and management overlays all activities of the QbD paradigm. The steps shown may not always be performed in the sequence shown.

a driver in process design for AAV, whereas the ability to capture at a fast residence time to allow processing of material in a reasonable timeframe is much more important. Furthermore, the larger hydrodynamic size of the AAV means that existing, diffusion limited, chromatography beads may not be appropriate. Both these considerations push ideal AAV chromatographic stationary phases away from packed bed toward membrane and monolith modalities. Considering the upstream process, a mAb developer may find that typical bioreactor medium and feed-based approaches to maximize protein production and product quality in terms of parameters such as protein folding, disulfide bond formation, and glycosylation may be of limited use when producing a typically non-glycosylated DNA virus without disulfide bonds, possibly expressed via transient transfection.

Scientist skilled in the art of mAbs may also find themselves limited in virology and molecular biology. The DNA innards of the AAV requires molecular biologic analysis techniques unfamiliar to a typical mAb bioprocess scientist. They must become familiar with molecular biology techniques, practices and nuances such as the impact of primer/probe design upon digital droplet PCR. It is essential for the upstream process to be optimized for maximal DNA- as well as protein-production: with correct packaging ratios. The material must be processed to maintain viral infectivity and potency. Although this is an enriching learning journey, when staffing a process development group, it is critical to recruit a broad mix of backgrounds such that these aspects of viral production are not neglected.

TRANSLATIONAL INSIGHT

The explosive interest in gene therapy as a transformational therapeutic modality has resulted in an increase in process development and manufacturing groups across a multitude of companies. Bioprocess scientists who have cut their teeth on mAb development have been attracted to this new frontier of bioprocessing. They can bring valuable skills, including:

i. Relevant manufacturing step knowledge;

ii. cGMP, tech transfer and comparability assessments expertise;

iii. Experience in the use and development of disposable technologies;

iv. Proficiency in viral clearance; and

v. A knowledge of QbD and related process development know-how.

However, due to the larger size and mass, the criticality of the DNA component of the AAV vector, the lower productivity of the bioprocesses, and the viral knowledge necessary, mAb bioprocess engineers alone may not be sufficient to guarantee success in this evolving and fascinating field.

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CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING

SPOTLIGHT

INTERVIEW

How fixed bed bioreactors are changing the game for adherent cell culture-based vector production

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Q Fixed bed bioreactors (FBRs) are proving to be a game-changer for adherent cell culture-based vector production. What benefits do they offer compared to other traditional adherent systems, and to suspension culture systems?

TU: Looking at the benefits of FBRs over traditional adherent cultures, what they typically do is take that 2D planar surface area and turn it into a more 3D surface area – even to a greater extent than things like cell stacks, which have many levels in a vertical dimension. They increase that surface area per volume ratio substantially, and that is one of the key benefits.

Another thing that they offer versus suspension systems is that they allow the cells to be immobilized. When you work with products such as viral vectors that are secreted into the liquid, it is much easier to harvest that the product when cells are immobile. This contrasts with suspension, where the particles of interest and cells are in suspension together, and you have to develop strategies to separate the two. These are probably the biggest differentiators between traditional 2D and suspension systems.

There are other benefits versus the traditional adherent system, in the way that you can monitor and control the system. For example, cell stacks are static culture systems in an incubator. With an FBR, you can monitor and control pH and oxygen, so you get better performance from the cells. That's one benefit FBRs have in common with suspension culture systems.

VG: At the current bioprocess and manufacturing scale, as it relates to suspension cell culture systems and microcarriers, most of the biology that has been developed in the research field is performed on a traditional 2D surface. As such, there is often a big gap between laboratory scale results on the bench, and how we implement this in a manufacturing setting.

Fixed bed reactors like ours are closing that gap, because we are beginning to take the same process that has been developed in the research laboratory for the standard 2D surface and transfer it into manufacturing scale production systems. This is the goal we had in mind with the development of the Corning Ascent FBR system.

Q Can you go deeper into the results that fixed bed bioreactors such as the Ascent system can generate in terms of metrics such as cell growth, transfection efficiency, and viral titer?

VG: As we have been developing the Ascent system, we did validation against existing FBR systems. For example, we used a HEK293T cell line for AAV2 GMP production for comparison, and we found with a comparable surface area the Ascent system demonstrated about four times higher genome yield per vessel. We also saw about three times higher viral genome per square centimeter and had higher titers of viral genome per cell.

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> "[Fixed bed reactors] increase that surface area per volume ratio substantially, and that is one of the key benefits. Another thing that they offer versus suspension systems is that they allow the cells to be immobilized."

In addition, due to the design of our system, we managed to reduce media consumption by about 30%. When all of those results are taken into consideration in the cost model, it amounts to a roughly 65% reduction of viral genome cost per dose in the future manufacturing setting.

TU: I would add that these are the improvements we see when we compare the Ascent system to other FBRs. The efficiency gains are far greater against traditional 2D platforms, which other FBRs exhibit as well.

Q Could you tell us more about the specific design features and considerations aimed at optimizing yield, and ensuring vector product consistency and quality?

TU: The key to optimizing performance is the uniformity of flow and cell distribution, as it results in a very homogenous culture setting. When you have this uniform flow and distribution of cells, you are also able to harvest the cells. The Ascent system is the only FBR designed to allow retrieval and recovery of the cells from the reactors themselves.

VG: The bioreactor design provides very good uniformity in both the cell distribution as well as perfusion of distributed cells within the media. We have very uniform delivery of nutrients and oxygen, as well as uniform removal of metabolites, which greatly improves the cell yields and functionality.

What we often see in alternative fixed bed reactors that are using the fixed bed to mimic 2D adherent culture is that many cells grow in 3D clumps rather than in 2D monolayers as intended. In our case, we get this high degree of uniformity of cell distribution in the packed bed, which ensures that cells attach to the packed bed in a 2D fashion. As a result, we have increased process stability. Product yield and consistency are improved through control of specific cell confluence during the production run, which allows us to avoid 3D aggregate formations. The transfection efficiency of cells in 2D monolayers is better, leading to higher titer and yield.

Q What else differentiates Ascent from other FBRs that are on the market at the moment?

VG: Ascent was developed as a single-use, closed system that is integrated with an automated bioreactor control platform. This full integration enables a high level of automation and thus minimizes handling and is less prone to user error.

Our system design demonstrates a predictable scalability from process development, to pilot, to production units. The unique reactor design provides high uniformity of cell distribution and media perfusion in culture conditions, through the whole packed bed. It enables our systems to be used as a cell producer because the design makes it possible to perform cell harvest from the fixed bed reactor, which is not possible in alternative fixed beds. This expands the use of the system and enables it to be used in a variety of bioprocessing applications, ranging from viral vector production and vaccine production, all the way to the production of cell therapies.

Q Could you share any results to date that you have achieved with the Ascent, and any specific considerations that are important across different vector types, cell systems, and other applications beyond viral vectors?

TU: From our AAV and lentiviral vector production we have a good understanding of how the Ascent system performs. We also have proof of concept in other cellular systems including mesenchymal stem cells, again coming from that ability to harvest cells.

There are other market opportunities, such as engineered meats. We have proof of concept of fish muscle cells growing in the Ascent reactor. This is quite an enabler for that industry, as they need enormous amounts of cells, and they need to recover those cells efficiently. We look at this as a great opportunity to apply our system at the scales necessary to support that type of market. We are also looking into cells that are common for vaccine production.

Additionally, we are looking into areas such as secreted product. The Ascent reactor has the ability to have very high density of cells per unit volume, and this is one of the critical things necessary to generate high concentrations of secreted product.

Q What is your vision for the evolution of FBRs moving forward? Are there any particular aspects or applications Corning is targeting for further development?

"...it was our intention from the beginning to design a system that is integrated with the controller."

VG: If you look into the state of the art of current FBR systems, they are functional, but they require a lot of specific knowledge and user handling to go through the whole process from beginning to end.

Users have a tendency to make errors. The vision for the future of fixed bed reactors is

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that it should ideally be a highly automated system that requires minimum user intervention – the user can provide cells to seed the reactor and receive cells or harvested product at the end of the process. The rest should be handled automatically by the system to minimize any user errors. Reactors should also be robust in their performance, which means stable under a variety of operation conditions during the production runs.

With the Ascent system, it was our intention from the beginning to design a system that is integrated with the controller, and the controller will make the system easy to use for the customers.

The Ascent technology has true scalability and is designed to provide a simplified workflow for customers. The principles of the technology are independent from the reactor size and can be scaled up efficiently from process development to production scale units.

TU: One of the things we want to develop is expanding the surface area intensification that FBRs have increasing the amount of surface area per unit volume. We feel confident that we have the capability to drive that to an even greater scale than our current soon to be launched product. We plan to have a scalable platform of FBRs starting as low as one square meter and reaching as high as a 1,000 square meters of surface area in a single FBR vessel.

Being able to harvest cells from the Ascent reactor makes it very scalable, and by combining that scalability with the automation previously mentioned, it can be translated to large-scale manufacturing rapidly, without many changes in the process. That should speed things up for customers looking to scale quickly, which is something a lot of customers are trying to do with these unique therapeutic modalities. Achieving speed to market is one of the most critical things customers are aiming for.

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CORNING

FASTFACTS

Cell and gene manufacturing: a case study approach to overcoming challenges

As cell therapy manufacturing moves from translational research to industrial scale-out, resolving the challenges associated with borrowed tools from the blood, biopharma, and academic sectors has become critical. Sexton Biotechnologies is working to understand these challenges, and to solve problems, rather than simply create new tools that may not move the industry forward. To highlight the importance of solving these problems together, Sexton asked competitors, customers, and collaborators some key questions in order to highlight both the current challenges, and importantly, the downstream implications of solving them.

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WHAT ARE THE BIGGEST HURDLES FOR CELL AND GENE THERAPIES?

"The challenges at scale haven't yet been addressed or proven. When designing for scale, the challenges need a different design philosophy and mindset."

- Dan Strange, TTP

"The need for cell therapy-specific equipment. This is not as easy as it sounds, because every customer has different needs. It is crucial to get to the heart of what those true needs are, and ensure that the equipment produced is meeting them."

- David Smith, Ori Biotech

"Our vernacular. What one person might mean by manufacturing is not what another means. When it comes to closed systems, how closed? With automation, how automated? Even for the tissue supply side, we find fresh often means 'fresh-ish'. Speaking the same language across the industry is a huge challenge, and hopefully we can bring further standardization to it."

- Priya Baraniak, OrganaBio

"One of the challenges is not the processes that we use, but how you harness all of that to produce a batch manufacturing record. We can manufacture large quantities of cells, and produce lots of doses, but the biggest problem is the huge amount of paperwork that goes with that."

- Alasdair Fraser, NHS Scotland

WHAT ARE THE CHALLENGES WITH CURRENT TECHNOLOGIES AND MANUFACTURING METHODS?

"Standardized flexibility seems like an oxymoron, but there is a real need to start standardizing the lower processing that we do, as well as the manufacturing, but maintain flexibility within that. We need processes that are standardized, but at the same time flexible enough to manage the inconsistency and the variation that we are putting into them."

- David Smith, Ori Biotech

"Most processes have been developed either using manual processes or equipment that wasn't designed for the purpose in mind. They either require a lot of manual transfer steps to integrate with analytics, or they have a very complicated set-up procedure."

- Dan Strange, TTP

"The concept of the pre-competitive space is really interesting, and one that as an industry we need to embrace more. To really be successful, we are going to need to open up a lot of our propriety concepts, at least to an extent."

- Priya Baraniak, OrganaBio

"Connectivity: we often use individual pieces of equipment, each of which have some sort of read out which feeds into our process, but it is not coordinated. There have been moves in the industry to try and coordinate this more effectively, and this is definitely a step in the right direction."

- Alasdair Fraser, NHS Scotland

WHAT ARE THE BIGGEST PAYOFFS OF SOLVING THESE PROBLEMS?

"What we really want is for people who are suffering to benefit from these products. If we succeed in the way that we culture, manipulate, scale and store cells, we will succeed in using them to improve health."

- Joaquim Vives Armengol, Vall d'Hebron Research Institute

"Everything we discussed – automating, standardizing, integrating – is helping to reduce the cost of these therapies. The real challenge is to make them affordable, while maintaining quality."

- David Smith, Ori Biotech

"It comes down to patient access and making these therapies available to more patients, for different indications, at lower costs, and with lower failure rates."

- Dan Strange, TTP

"If we can work together and figure it out, we are going to save lives. What could be more important than that?"

- Priya Baraniak, OrganaBio

This video is the first installment of a three-part series that explores the challenges and opportunities for cell and gene therapy manufacturing. The next two installments will feature case-based solutions focused on the implementation of tools that allow for flexible automation and standardization to improve downstream outcomes.

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SEXTON BIOTECHNOLOGIES

FASTFACTS

Scalable, single-use technologies for purification of lentiviral vectors

Todd Sanderson, Senior RnD manager, Pall Corporation

Although viral vector manufacturing is a relatively new field, there are a number of technologies currently used for large-scale manufacture of other therapeutics, such as monoclonal antibodies, that can be leveraged to provide scalable vector manufacturing solutions. As an end-to-end supplier for lentiviral vector (LV) manufacturing, Pall Corporation conducted a series of small-scale feasibility studies in collaboration with the Institute of Experiment Biology and Technology, Portugal, to evaluate a downstream purification process using Pall consumables.

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EVALUATION OF PALL CONSUMABLES FOR LV MANUFACTURING

Evaluation of the Pall process platform for LV manufacture was performed using a series of feasibility studies. LV was produced using standard adherent cell culture methods, and transient transfection was performed with PEIPro™ (Polyplus Transfection) using a third generation lentivirus packaging system, with green fluorescent protein as a transgene. The first step in the workflow was to clarify the harvest material. Various bioburden reduction filters were tested, both alone and in combination. Based on the results of the study, either a Supor® EAV membrane or a combination of a PreFlow™ UB and Fluorodyne® II DBL membrane was recommended.

The next step in the process was to evaluate the use of the Mustang® Q membrane for bind and elute purification. Overall, excellent global recovery of LV was observed with the Mustang Q membrane at the 0.86 mL capsule scale **(Figure 1)**. The membrane also resulted in significantly lower functional LV in the flow-through fraction, indicating higher capture efficiency compared to a competitor product. In addition, slightly higher functional recovery was observed in each of the three elution fractions. This resulted in an almost 20% increase in global total LV recovery with the Mustang Q membrane.

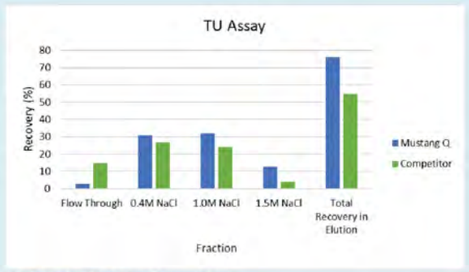
After purification by membrane chromatography, the next step is to perform a buffer exchange and concentrate to obtain the virus at the desired titer. This can be accomplished using ultrafiltration rated tangential flow filtration (TFF) cassettes. A series of trials were performed to evaluate the scale up to and performance of the 5mL mustang Q membrane device: Recoveries of greater than 80% were typically achieved. Finally, manual sterile filtration was studied using the Supor EKV membrane. Excellent transmission of virus through the Supor membrane was observed in two of three trials.

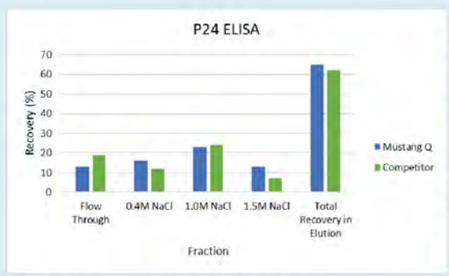
The final purified product was evaluated for LV purity, and showed 2.1 x 10^4 functional transducing units per nanogram of P24, indicating good functional activity. The high purity LV and low contamination profile obtained during this process demonstrate it is likely to be GMP compliant.

AIMING FOR HIGH RECOVERY, HIGH SPEED MANUFACTURING

A notable aspect of LV manufacture is the particularly unstable nature of these viruses. They are large and have fragile, shear-sensitive envelope layers, with half-lives generally reported at 8–12 hours. Potency of LV can drop rapidly upon harvest, necessitating fast downstream processing times and minimization of hold steps. This work established the feasibility of a full manufacturing process for clinical-grade lentiviral vectors, with over 80% recovery possible for all unit operations **(Figure 2)**. The process is also time efficient and can be completed in under 5 hours. Further optimization efforts could result in improved process robustness, vector recoveries, and contaminant removal. Further work will include more challenging higher titer feeds, and suspension-generated cultures.

Figure 1. Lentivirus Purification: Mustang Q Membrane.





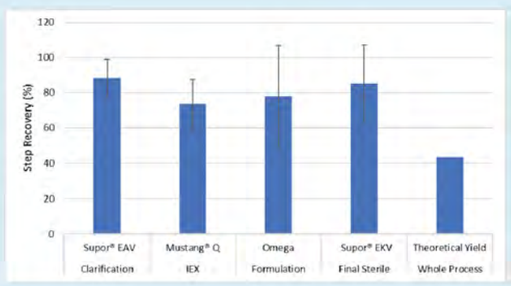
Mustang Q capsule shows high global lentivirus recovery (up to 93%)

Mustang Q membrane shows slightly higher functional recovery at each step, total recovery ~20% higher

- 0.86 mL Mustang Q capsules
- Washed with equilibration buffer and eluted with 3 step salt elution containing 3% sucrose
- High conductivity elution fractions eluted directly into low conductivity buffer

- Clarified supernatant adjusted to pH 7.0
- 10 MV/min loading
- Functional LV titer determined by TU assay
- Total LV particles determined by P24 ELISA

Figure 2. Full process summary.



Unit Operation	Trials Averaged
Clarification (20 cm²)	2
Mustang (5 mL)	3
Formulation	6
Sterile	6

- Feasibility established for a Pall lentivirus downstream process platform with >80% recovery possible for all unit operations. Theoretical process yield of ~43%
- Complete process can be completed in <5 hours.
- Optimal clarification found with Supor EAV Capsules
- Good global recovery with Mustang Q membrane - (>90% recovery observed)
- Good performance of Omega 300 kD membrane for concentration/formulation in all 3 trials further optimization could improve performance
- Excellent final sterile filtration transmission with Supor EKV membrane at ~107 TU/mL

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VIRAL VECTOR BIOPROCESSING

SPOTLIGHT

EXPERT INSIGHT

Gammaretroviral and lentiviral vector manufacture: brief overview

Ranjita Sengupta

Viral vectors are used as efficient gene delivery vehicles in gene and cell therapy. Viral vectors can be broadly divided into integrating and non-integrating vector based on their ability to integrate the gene of interest (GOI) into a host cell genome. Appropriate selection of vector system is based on the application. Manufacture of viral vectors requires vector backbone components to be combined with the therapeutic GOI; this can be achieved primarily by one of the following three ways:

1. Transiently by multiple plasmid transfection of the transfer vector containing the gene of interest along with the packaging and envelope plasmids simultaneously into an appropriate cell line such as 293T cells;
2. Transiently by single plasmid transfection of a transfer vector containing the GOI into an appropriate packaging cell line expressing the packaging and envelope genes;
3. Stably from a vector producer cell line. Scale, yield, purity, and quality are critical considerations in viral vector manufacture and upstream and downstream process optimization is essential for scalable manufacture of quality viral vector for clinical applications.

Both gammaretroviral vectors (GRVV) and lentiviral vectors (LVV) originate from retroviruses and are widely used for genetic modification of CAR-T cells. This article gives a broad overview of GRVV and LVV manufacture.

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VIRAL VECTOR SYSTEMS

Virus-derived vector systems (viral vectors) have been used successfully for decades in gene therapy and cell therapy as a gene delivery vehicle [1]. There are several different types of viral vectors to choose from, of which the main players in the gene and cell therapy field are lentivirus (LV), gammaretrovirus (GRV), adenovirus (AV), and adeno-associated virus (AAV). Gammaretroviral vectors (GRVV) and lentiviral vectors (LVV) derived from Murine Leukemia virus (MLV) and HIV respectively are two of the most common retroviral vectors. Important factors to consider during selection of a viral vector system for a particular application are payload capacity for the therapeutic GOI (insert size), immunogenicity, cellular tropism and efficiency of uptake by the target cells, duration of gene expression, and ease and scale of manufacturing. Thomas *et al.* have reviewed the different viral vector systems, integrating vs non-integrating, enveloped vs non-enveloped, viral DNA genome vs viral RNA [2]. Likewise, Patel *et al.* highlight advantages and disadvantages in each system [3]. Each of the vector systems is unique with its own characteristics making it suitable for some applications and unsuitable for others [4].

The persistence of therapeutic gene expression is a key consideration in choosing a viral vector for an application. Viral vectors can be broadly classified into two categories: Integrating and non-integrating. Gammaretroviral and lentiviral vectors, both of which are retroviruses with an RNA genome, are integrating and generally result in persistent expression of GOI in dividing cells while AAV and Adeno virus are non-integrating DNA virus which result in transient expression in dividing cells. Another advantage of gammaretroviral vector and lentiviral vector is their gene insert size capacity, which is about 9kb. Non-Integrating viral vectors will not be discussed in this article. The main focus of this article is integrating retroviral vectors GRVV and LVV.

INTEGRATING VIRAL VECTOR: RETROVIRUS

Retroviruses are enveloped RNA viruses with two copies of a single stranded RNA genome. The envelope plays a very important role in determining host cell (hc) specificity. The envelope proteins help in entry of cells either through direct membrane fusion or receptor mediated endocytosis facilitated by the envelope glycoproteins binding to their cognate receptors on the host target cells [5].

Retroviral vectors are popular choices for gene therapy and cell therapy because it allows long term stable gene expression by integrating into the host cell genome. However, there is a risk too, integration might cause insertional mutagenesis, leading to upregulation of proto-oncogenes and malignant transformation of host cells [6]. GRVV tend to integrate near gene regulatory regions like transcriptional start sites posing a higher genotoxic risk than lentiviral vectors which tend to integrate into the body of the gene. Another major difference between gammaretroviral vector and lentiviral vector is, gammaretroviral vector preferentially transduces dividing cells, they cannot transduce non-dividing cells, while lentiviral vectors can transduce both dividing and non-dividing cells. Gammaretroviral vectors have a simple genome structure which consists of the following protein coding genes *gag*, *pol* and *env*. *Gag* encodes for the capsid proteins, *Pol* encodes for viral enzymes (reverse transcriptase, integrase and protease) and *Env* codes for the envelope proteins [7–9]. Lentiviral vectors have a more complex genome. In addition to gag, pol and envelope, the HIV genome encodes for six additional proteins: two regulatory proteins *Rev* and *Tat* and four accessory proteins *Vpr*, *Vpu*, *Vif*, and *Nef* [5].

To mitigate the adverse effects associated with pathogenicity of wild-type retroviruses and other potentially harmful effects, retroviral vectors have been made safe by paring down the viral genome to only the essential genetic elements that are required for efficient packaging into viral particles. In addition to removing

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the non-essential genetic elements to make the viral vector safe, the viral genome is split and the packaging genes are provided in trans either through a packaging cell line for generation of a stable vector producer cell line or into different plasmids for transient production of viral vector. This reduces chances of recombination and makes the viral vector replication incompetent, thus increasing safety.

The native envelope of the virus is also replaced with envelopes from other viruses like the glycoprotein of vesicular stomatitis virus (VSV-G) envelope, a pantropic envelope most commonly used in lentivirus, GALV (Gibbon ape leukemia virus), RD114 (feline endogenous retrovirus), ecotropic (murine leukemia virus), amphotropic (murine leukemia virus) and xenotropic (murine leukemia virus) [7]. When viral vectors were being developed as gene delivery vehicle, with each modification a new generation was created where the viral genome was split increasingly and separated to increase the number of viral genes provided in trans. This split packaging gene design minimized probability of recombination to form replication competent viral particles, thus making them safer for clinical use. The commonly used method for transient production of gammaretroviral vectors consists of a 3 plasmid system, where the viral genome is split 3 ways. The transfer vector contains the long terminal repeats (LTRs), the packaging signal Ψ and the polypurine tract (PPT) of the retroviral genome along with the GOI. The packaging gag/pol and envelope genes are provided in trans by two other plasmids. The envelope determines the pseudotype of the viral vector which is chosen based on the target cell and is derived from a different virus.

The third generation of the more complex lentiviral vector consists of a 4 plasmid system where the four accessory proteins *Vpr*, *Vpu*, *Vif* and *Nef* are removed and the vector genome is whittled down to encode only 3 of the 9 HIV proteins making them safe as gene delivery vehicles. Further, the 3 HIV genes and the envelope (from a different species) are split into the:

i. Transfer vector containing the LTRs, packaging signal Ψ, Rev response element (RRE) and PPT from the viral genome along with the GOI
ii. Rev regulatory element
iii. Gag/Pol packaging plasmid
iv. Envelope plasmid.

Fourth generation lentivector systems splitting the viral genome further into a five plasmid system have also been developed and are available from companies such as Takara Bio and Dharmacon [10]. However, this approach requires more plasmids, which may reduce the transfection efficiency. In addition, codon optimization to further reduce homology between the transfer plasmid and packaging plasmids was also developed, but this resulted in reduced viral titers. Berkhout *et al.* redesigned the transfer plasmid by placing the viral cis acting elements downstream of the 3'LTR so that these elements would not be incorporated into the host cell genome, thus making the vector system safer. However, the fourth generation of lentiviral vector system needs more development to make it as efficient a gene delivery tool as the third generation system [11,12].

The first approved gene therapy vector was a Moloney MLV gammaretroviral vector for SCID-X1 (human severe combined immunodeficiency X-linked) patients [13]. Of the five CAR-T products currently approved by the FDA (Yescarta®, Kymriah®, Tecartus™, Breyanzi® and Abecma™), two were engineered using gammaretroviral vector and three were engineered using lentiviral vector In addition to long-term stable expression, lentiviral and gammaretroviral vectors are attractive as gene delivery systems because of minimal immunogenicity and high transduction efficiency.

MANUFACTURING PROCESS FOR RETROVIRAL VECTORS

There are two main manufacturing platforms for gammaretroviral and lentiviral vectors:

Transient process, which involves plasmid transfection (multiple or single) into an appropriate cell line, and stable process, where a stable cell line producing viral vector is generated from a packaging cell line expressing the packaging and envelope genes.

The main point to consider during choice of manufacturing platform is the stage of the project. At the early stages, during research and development phase, the transient process is preferred because it is faster and easier to implement. Using this transient platform, multiple viral constructs can be tested simultaneously for functionality before selection of the final construct. Once the vector construct is finalized for a project, generation of a stable viral vector producer cell line with the vector construct of choice can be initiated. It takes about a year to generate a cGMP compliant master cell bank (MCB), but a stable vector producer cell line would make vector manufacturing for clinical and commercial purposes easier.

Typically, early on during research and development, small-scale productions are done and during this phase, process optimization studies are conducted to improve yield and quality of vector. At this stage, research grade materials and reagents can be used that do not have very stringent quality requirements. As the project transitions from R&D to pre-clinical, clinical, and finally, commercial, not only does the manufacturing scale increase (which might require additional process optimization), but GMP grade reagents, cell lines, and plasmids are required to meet strict quality and regulatory requirements recommended by the FDA [14]. As a result, costs increase, which has to be factored in to determine the optimal production technology for cost-effective viral vector manufacturing.

The manufacturing process for both transient and stable platforms can be divided into an upstream part and a downstream part. Upstream Process is the production of the viral vector from an appropriate cell line and harvest of the bulk viral supernatant. Downstream process involves purification to eliminate contaminants from process- and product-related impurities, and concentration to produce a pure, potent viral vector that is efficient in gene transfer.

Upstream process

Upstream Process starts with the appropriate producer cells being grown and expanded in culture systems. The cells can be grown in suspension systems (e.g., shakeflasks, bioreactors) or adherent systems (e.g., tissue culture flasks, cell factories, roller bottles, and fixed bed bioreactors like the CellCube from Corning, or the iCELLIS™ from Pall) [15]. Adherent cells are grown in serum-containing medium, while suspension cells are adapted to grow in serum-free medium [16].

Gammaretroviral and lentiviral vectors are manufactured predominantly using HEK293 cells and 293-derived cells like 293T cells. Gammaretroviral vectors can also be derived from NIH-3T3-derived PG13 cells. Yescarta®, one of the first FDA approved CAR-T cell therapy products for treating B-cell acute lymphoblastic leukemia, was manufactured with gammaretroviral vector produced from a stable gammaretroviral producer NIH-3T3 cell line.

Gammaretroviral vector (MLV) can be manufactured transiently by triple plasmid transfection with the transfer plasmid carrying the GOI, packaging plasmid carrying Gag/Pol packaging genes, and an envelope plasmid into a conducive cell line as mentioned above, or by a single plasmid (transfer plasmid) transfection into a packaging cell line such as the Amphotropic and Ecotropic cell lines (Cell Biolabs), 293Vec cell lines (BioVec Pharma), or PG13 cell line (ATCC) that stably expresses gag-pol and env, eliminating the need to deliver the packaging genes in trans by specific plasmids.

Use of single plasmid transfections simplifies the upstream process, in addition to reducing the cost of goods (COGs), mainly in the form of plasmid costs, thus de-risking the supply chain.

For lentiviral vectors, usually third generation lentivectors are used for clinical

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applications, which involves 4-plasmid transfection with the transfer vector carrying the GOI, Gag/Pol packaging plasmid, Rev helper plasmid, and the envelope plasmid into an appropriate cell line (eg. 293T cells).

For transient systems, plasmids are transfected using transfection reagents such as lipofectamine, calcium phosphate, or polyethylenimine (PEI) [15]. For transient systems where cells are grown in adherent systems such as cell factories or flasks, medium is usually changed 24 hours post-transfection to remove toxic transfection reagent. A nuclease such as Benzonase™ or Denarase® can also be added to the viral harvest media during medium change, or it can be added during downstream processing to remove residual plasmids and any host cell DNA which might be released by lysed cells. Sodium butyrate [17] can also be added to increase viral titer. Bulk viral harvests are collected at various time points starting from 48 hours post-transfection, and can go up to 96 hours based on whether the cells are adherent or growing in perfusion-based bioreactors.

Viral vectors can also be manufactured from a stable vector producer cell line, which produces vector constitutively. However, generating vector producer stable cell lines is time consuming and can be challenging. The advantage of making a stable producer cell line is that, once made, it is easier to scale for large-scale manufacture. It is also more cost-effective, with increased reproducibility and safety.

It is easier to make a stable gammaretroviral producer cell line because it has a simpler genome. Only three protein coding genes are needed: *Gag/Pol*, *Env*, and the transgene of interest. Additionally, there is no toxicity associated with the envelope protein. In contrast, lentiviral vectors are harder to generate because of the larger, more complicated genome and toxic effects of VSV-G, which is the most popular envelope used for lentiviral vector.

To make a vector producer cell line, a vector packaging cell line expressing the helper genes gag/pol and env is transfected with the transfer vector. In addition to the LTRs, Ψ packaging signal, PPT, and GOI, the transfer vector also contains an antibiotic resistance marker which enables selection of a stable vector producer cell line expressing the GOI along with the antibiotic resistance gene [18,19]. The availability of different vector

TABLE 1

Advantages and disadvantages of transient vs stable vector production systems.

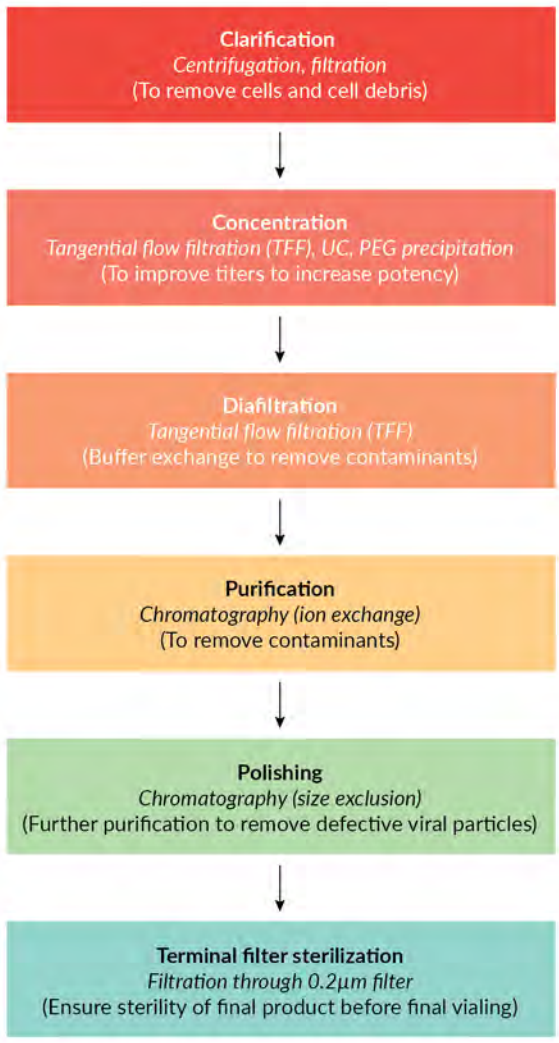
	Advantages	Disadvantages
Transient system	1. Flexibility – multiple genes can be tested simultaneously in small scale during R&D 2. No lead time for manufacture of cell line	1. Scalability 2. Lot-to-lot variability 3. Impurities: plasmids and transfection reagent 4. Cost of plasmids and transfection reagent
Stable system	1. Scalability: easier to scale up 2. Higher yield from a single manufacturing run: increased number of bulk viral harvests 3. Higher lot-to-lot consistency 4. Cleaner viral harvest because of absence of plasmids and transfection reagent	1. Long lead time needed to make a stable producer cell line 2. Cost and lead time for making a cGMP compliant MCB

packaging cell lines with different envelopes for the gammaretroviral system makes it easier to make stable gammaretroviral vector producer cell lines, thus making it advantageous for large-scale manufacture of a variety of pseudotyped viral vectors to meet clinical and commercial needs [18,20].

In the case of lentiviral vectors, constitutive lentiviral vector producer cell lines are more difficult to generate because of VSV-G and Rev, which are toxic to the cells [21]. Inducible stable lentiviral vector packaging cell lines like 293SF PacLV have been generated where expression of VSV-G and Rev are controlled by inducible promoter systems [22,23].

FIGURE 1

Manufacturing process flow overview for gammaretroviral and lentiviral vector.



For viral vector production from a stable vector producer cell line, the cells are thawed and expanded. The cells are then seeded at an optimal density such that the cells are in the exponential growth phase during viral harvest to be able to produce high levels of viral vector.

Table 1 summarizes pros and cons of the two different manufacturing platforms: stable vs transient.

Downstream process

The bulk viral harvest supernatant produced in the upstream process has to go through downstream processing to improve potency and quality for therapeutic use. Gammaretroviral and lentiviral vectors are fragile and are prone to inactivation under stress. Hence, number and design of unit operations in downstream processing has to be carefully planned to maximize recovery and yield.

The main stages in downstream processing includes clarification, concentration, purification, and formulation, as shown in the flow diagram. Depending on the application and type of viral vector, downstream processing includes all or a combination of the steps in Figure 1.

Clarification step removes cells and cell debris. Concentration and diafiltration by tangential flow filtration (TFF) removes salts, serum, and low molecular weight contaminants. Diafiltration also allows viral vector to be formulated in final buffer. Purification by chromatography removes impurities.

For viral vectors produced at small-scale, clarification step can be done by centrifugation or filtration, and concentration can be done by Ultracentrifugation (UC) or Polyethylene glycol (PEG) precipitation. The % recovery in terms of infectious viral units is high from UC and PEG precipitation steps. However, centrifugation or UC is not scalable. In addition, UC and PEG precipitation brings down impurities to a level that would not meet regulatory standards for clinical use.

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For larger scale viral vector production used for clinical application, downstream processing varies based on the type of viral vector, the envelope of the viral vector, and the application. Clarification is done by filtration though an appropriately sized filter. Usually, a filter train with decreasing pore sizes is used to prevent filter clogging and maximize recovery. Concentration is usually done by TFF using hollow fiber modules or cassettes with a 500 kD or 750 kD MW cutoff for GRVV and LLV, as a result of which, impurities smaller than the MW cutoff can diffuse out into the permeate through the pores. Viral vector can be further purified by chromatography. In many cases, particularly for viral vectors used for *in vivo* application, purification by chromatography (ion exchange, size exclusions or affinity) is an added step during downstream processing. If required, a terminal small pore sized filtration step can also be added to ensure microbiological sterility and further removal of any particulate matter [23,24] Formulation is the final step in the downstream process, which is an important factor for vector stability [25].

At each step during downstream processing, attention should be paid to yield and quality of the vector by measuring viral titer and impurities including but not limited to: hcDNA, hcProtein, BSA, endotoxin, and mycoplasma. In addition to titer and impurities, final vector products are also assessed for safety by testing for sterility, adventitious agents and replication-competent retrovirus or lentivirus (RCR/RCL). Quality of the final vector product is determined by the analytical tests designed to assess safety, purity, and potency.

Downstream optimization requires careful evaluation at each step to minimize loss and improve quality, purity, and potency of the vector.

PROCESS OPTIMIZATION CONSIDERATIONS

To ensure optimal yield of viral vector, the following should be evaluated during process development studies.

Transient system needs more upstream optimization than vector production from a stable producer cell line because of the additional transfection step. For clinical applications, moving away from FBS is an important process optimization consideration because of the risk of pathogen contamination and FBS lot-to-lot variability, which could have an impact on the reproducibility of a quality final product [26].

Upstream optimization steps to evaluate:

1. Critical starting materials – cell bank, FBS, plasmids (for transient system)
2. Chemically defined media to move away from FBS
3. Plasmid ratio (for transient system)
4. Transfection reagent (for transient system)
5. Plasmid vs transfection reagent ratio (for transient system)
6. Cell seed train for expansion of cells
7. Cell seeding density
8. Characterization of cell growth in different media formulations
9. DNA to cell ratio (for transient system)
10. Number and time of bulk viral harvests (for stable system)

Downstream optimization steps to evaluate for maximal recovery:

11. Appropriate filters
    a. Filter trains
    b. Material of filter membrane
    c. Surface area of filter (optimal volume of viral supernatant to filter surface area to minimize loss)
    d. Pore size of filter membrane

e. Flow rate as measured by LMH (Liters/m^2/h)

12. Optimal TFF conditions

a. Hollow fiber module or cassette

b. Molecular weight cutoff

c. Buffer and pH of buffer

d. TFF parameters (flow rate, shear, and transmembrane pressure [TMP])

13. Optimal chromatography conditions, including but not limited to

a. Resins

b. Buffers, pH

c. Flow rate

d. Elution conditions

14. Post-concentration filter

a. Material of filter membrane

b. Size and pore size of the filter

c. Flow rate as measured by LMH (Liters/m^2/h)

15. Final formulation

TRANSLATION INSIGHT

- Viral vector manufactured from a vector producer cell line facilitates consistency, ease of manufacture, scalability, and purity. There are many vector packaging cell lines expressing gag/pol and env proteins available for gammaretroviral vector. Historically, production of lentiviral vector producer cell line has been more of a challenge because of the more complex genome and VSVG envelope (rhabdovirus vesicular stomatitis virus) used to pseudotype most lentiviral vectors. Rev and VSVG are known to be toxic to cells and so are difficult to integrate into a cell line to make a constitutive packaging cell line. Lentivector packaging cell lines can be made by modifying other, less toxic envelopes, which are commonly used in gammaretrovirus. For example, modified 4070A (amphotropic murine leukemia virus), GALV (gibbon ape leukemia virus), and RD114 (feline endogenous retro virus) have been used in transient production of lentiviral vector with positive results [27,28]. Broussau *et al.* generated an inducible lentiviral packaging cell line, 293SF-PacLV, where expression of Rev and VSV-G are tightly regulated by Tet and Cumate double switch system [22]. This cell line also grows in suspension in serum-free medium, making it ideal for scale-up and large-scale production for clinical and commercial application. Recently, Manceur *et al.* used this 293SF-PacLV packaging cell line to generate an inducible GFP-expressing lentiviral stable producer cell line by transfecting the cell line with a GFP plasmid [16]. This stable GFP lentiviral producer cell line, called clone 92, produced high-titer virus. By optimizing upstream process steps like cell density and media replacement, the yield of GFP viral vector was additionally increased.

- Most of the lentiviral vector production to date is transient. For clinical applications, there are several disadvantages to using a transient platform, primarily batch-to-batch variability, COGs, and potential for contamination due to use of plasmids and FBS. In addition, the window for collection of viral vector in transient transfection is smaller. Transitioning from a transient to a stable lentivector producer cell line would be a step forward because it would increase the reproducibility of the upstream process, in addition to reducing the cost of goods (mainly plasmid and FBS costs), thus de-risking the supply chain. In addition, lentiviral vector would have a broader application in terms of choice of target

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cells for clinical applications, because of its ability to transduce non-dividing cells and it being less genotoxic than a gammaretroviral vector. Using the inducible double switch system described by Broussau *et al.* [22] and Manceur *et al.* [16], or a similar approach, manufacture of a stable lentiviral producer cell line would be more viable and could be used for making viral vector for pre-clinical and clinical applications. The additional advantage of being able to grow and scale-up the manufacture of stable cell lines in suspension cell cultures in serum-free medium makes it attractive, and is probably going to be the next trend for improved viral vector manufacturing [29]. There are a number of companies including Patheon, Oxgene, and CEVEC, that offer custom services for generating lentiviral vector producer stable cell lines. Oxford Biomedica offers high-yielding lentiviral producer cell lines. The next step forward for use of retroviral vectors as gene delivery vehicles in gene and cell therapy would be an increased adaptation of the stable viral vector manufacturing platform to generate stable lentiviral vector producer suspension cell lines grown in serum-free medium.

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CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING

SPOTLIGHT

INNOVATOR INSIGHT

Facilitating gene therapy development with solutions to four capsid analytical challenges

Susan Darling

Access to robust analytical processes for viral vectors that support both the development, and the production phase is a challenge today, given the short turnaround time for obtaining analytical results. The functional cell-based assays and infectivity studies that have been used in the first years of gene therapy development can take days or weeks to generate results. However, in the downstream process, the decision to progress a batch must often be made within 24 hours or less. Key decisions about the affinity capture and anion exchange steps require analytics to answer the question about whether to progress a batch and how so. Today, manufacturers must use limited information for the decision to move ahead with a process and thus risk losing time and costly product.

With several gene therapies already on the market and hundreds more advancing rapidly through the clinic, next generation methods are essential to ensure successful commercialization. Access to simplified and rapid biophysical assays would provide more comprehensive information in a quicker manner to facilitate decision making on the right time scale.

New robust assays based on mass spectrometry (MS) and capillary electrophoresis combined with laser-induced fluorescence (CE-LIF) detection and other detection methods, such as UV, can rapidly provide accurate and reproducible results for both the protein and genetic components of viral vectors. Below are discussed techniques for capsid protein analysis and purity determination, viral vector genome integrity analysis, and the determination of empty and partial versus full capsids.

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NEED FOR ROBUST ANALYTICAL SOLUTIONS

Most gene therapies use viral vectors such as adeno-associated virus (AAV), lentivirus and adenovirus to deliver genetic material into cells. Whether delivered to the cells *ex vivo* or *in vivo*, the new genetic material replaces or restores the normal phenotype of missing, non-functional or incorrectly functioning genes to treat a range of diseases.

AAV is widely used as a gene delivery vehicle because it is non-pathogenic, exhibits low immunogenicity, and can readily enter a variety of cell types. This small, icosahedral virus (~20–25 nm, ~5.9 megadaltons) comprises a protein shell (i.e., capsid) encompassing a single-stranded DNA that is approximately 4.8 kilobases in size. The viral capsid is a 60-mer typically made up of three viral protein monomers (VP1, VP2, VP3) with respective molecular weights of 87, 73, and 61 kilodaltons assembled in a ratio of approximately 1:1:10.

A common process for making therapeutic recombinant AAVs (rAAVs) involves transfecting host cells with three plasmids, one of which contains the entire rAAV genome and two helper plasmids that contain *Rep* and *Cap* genes that enable the host cells to make virions. After manufacture, the rAAVs are purified by immune purification or ion exchange chromatographies and lysis of the host cells, followed by dialysis and buffer exchange, then aseptic filling.

During AAV production, the capsid viral proteins participate in the packaging of both the capsid and genome. They also determine the efficacy of a gene therapy, playing roles in receptor binding during cell entry, intracellular trafficking and genome release.

Correct expression of the viral vector capsid with the right size, peptide sequence and post-translational modifications (PTMs) is essential. The purity of the capsids with respect to host-cell protein and other genetic contaminants is critical to avoid the potential for immunogenicity and off-target effects. It is also important to minimize the number of empty and partial capsids, which can lower infectivity and thus lead to low protein production.

Many experimental conditions can influence the overall outcome of the production process. Rapid and robust analytical methods are therefore needed for effective in-process monitoring and final product release to ultimately produce a homogenous product that meets safety, strength, identity, and purity requirements.

CAPSID PROTEIN ANALYSIS

The AAV capsid is the primary interface between host and virus. Since post-translational modifications have the potential to impact the binding and subsequent infectivity of capsid proteins to a host cell, any imperfection affects the performance of the viral vectors. The three viral proteins produced in the viral vector manufacturing process differ only slightly in length and the N-terminus. They can also be generated in multiple variants due to different PTMs, which can impact efficacy. A rapid, robust method is therefore needed to fully characterize the capsid proteins, including their ratios and the presence of desirable and undesirable PTMs, regardless of concentration and often using small sample quantities.

Liquid chromatography (LC) combined with mass spectrometry (MS) can be used to characterize capsid viral proteins. Specifically, quadrupole time-of-flight MS (Q-TOF MS) enables rapid characterization of AAV capsid proteins. SCIEX has developed a simple digestion strategy that eliminates the need for dialysis or spin filters for sample preparation.

Digested samples analyzed using a SCIEX X500B QTOF System coupled to an ExionLC™System provided MS and MS/MS data for low-abundance peptides and PTMs (glycopeptides, deamidation sites, disulfide bonds, etc.) at the required sensitivity to achieve nearly complete sequence coverage, thus allowing confirmation of both C and N-termini and identification of modifications, along

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with their localization and relative quantitation [1,2]. Such robust analytics deliver rapid, accurate results to strengthen gene therapy development and commercialization [3].

Proprietary SCIEX SWATH®-based LC-MS/MS can also identify and quantify thousands of host-cell proteins and other contaminants in a single run.

CAPSID PURITY DETERMINATION

A rapid, robust, reproducible, and highly sensitive biophysical method is required for in-process evaluation of capsid protein purity at the low AAV concentrations found in most gene therapies (~50 ng/mL). The traditional method for determining AAV capsid viral protein purity involves SDS-polyacrylamide gel electrophoresis (SDS-PAGE) technology. There are severe shortcomings to this method, including a limited quantitation capability due to inherent sample preparation artifacts, a slow migration time and staining variability. Migration times for reversed-phase high-performance liquid chromatography (HPLC), meanwhile, vary significantly with serotype.

CE-SDS using a capillary gel electrophoresis mode, which has been used extensively for the purity analysis and quantitation of therapeutic proteins, offers advantages over conventional slab gel technology including high resolving power, better quantitation, excellent reproducibility, and automated operation, even for the lower concentrations of viral proteins found in AAV samples. It can also provide higher resolution than HPLC for protein separation.

For purity of AAV products with titers greater than 1 x 10^{13} genome copies per mL (GC/mL) or lower titer but sufficient sample volume, a PDA or UV detector can be used. Ultra-high sensitivity can be achieved using a fluorescent dye for sample labeling and laser-induced fluorescence (LIF) detection, enabling rapid analysis (~15 minutes) of in-process samples with AAV titers as low as 1 x 10^{10} GC/mL and limited sample amounts. In both cases, the sample prep is straightforward, and the method offers excellent resolving power, good repeatability, and high linearity of absorbance response to sample concentration.

GENOME INTEGRITY ANALYSIS

The ability to determine the integrity of the genomes used in viral vectors for gene therapies is crucial, as their efficacy and safety depend on the presence of the intact genome in the carrier capsid. For AAVs, the transgene in the AAV genome cassette could be missing (empty or partial capsid) or truncated, or the capsid could contain contaminant products instead of the transgene.

There are several technologies currently in use for this determination, such as denaturing agarose gel electrophoresis, Southern blot, quantitative polymerase chain reaction (qPCR), HPLC, and Next Generation Sequencing. While these techniques all have specific strengths and some are low cost, they are time-consuming, have low precision, and all of them generate large amounts of toxic waste. Some cannot detect fragments that do not contain the target sequence, do not provide size determination, or are very expensive to implement.

Here again, CE in the capillary gel electrophoresis mode with LIF detection is a rapid, automated biophysical method for genome size analysis of double-stranded DNA (dsDNA), including restriction fragment analysis of its vectors, as well as single-stranded DNA (ssDNA) and RNA, and offers higher resolution than HPLC. Fragments differing by as few as 10 base pairs can be separated and detected using UV or LIF identification. Reconstitution of the gel to a larger volume allows for determination of plasmid stability via plasmid isoform analysis (relative abundance of supercoiled and open circular isoforms over time).

SCIEX PA 800 Plus allows for a simple sample preparation method, CE-LIF, designed to digest contaminant fragments outside of the AAV capsid without degrading the

viral proteins or causing interference. This is an ideal rapid biophysical analytical method for AAV genome integrity and purity analysis that can be done in four steps.

As part of its CE portfolio, SCIEX offers robust and accurate tools for rapid genomic analyses using the GenomeLab GeXP™ system, which is capable of Sanger DNA sequencing and quantitative polymerase chain reaction in one system. It can do genotyping and single nucleotide polymorphism analysis, as well as short tandem repeat analysis and DNA profiling. The ability to conduct these analyses in-house rather than sending them out to a lab provides better data control and affords more rapid decision-making.

EMPTY VS FULL CAPSID DETERMINATION

In addition to the AAV full capsid containing the transgene, product-related capsid impurities can include an empty capsid, or virus protein shells without the vector genome, a partial capsid containing transgene fragments, and an 'other' capsid, which contains any sort of nontarget, extraneous host-cell nucleic acid. The contamination of packaged genome-related impurities affects the efficacy and the safety of the vector product, increasing the potential immunogenicity and can inhibit the transduction of the full capsid by competing for vector binding sites on the cells.

The analysis of empty and partial versus full capsids is thus one of the critical quality attributes for AAV products. There are multiple technologies used for determining the ratio of AAV full and empty viral capsids. The quick and easy methods (qPCR/ELISA and spectrophotometry) suffer from poor accuracy, while electron microscopy is too time-consuming, ion exchange chromatography (IEX) does not provide good resolution and charge-detection, and MS is not yet commercially available. Analytical ultracentrifugation (AUC) is the gold standard, but requires large sample sizes, is high cost and requires highly trained operators.

Capillary isoelectric focusing (cIEF), on the other hand, is a fast, easy-to-perform and robust biophysical method that is effective for the reliable separation and quantitation of full, partial and empty AAV capsids. Separation is achieved based on the charge variance of the isolectric points, with full capsids having lower pl values than empties. SCIEX has developed a robust cIEF-based method for AAV full and empty capsids analysis that can be completed in less than an hour. This method shows excellent resolution between full, empty, and partial capsid peaks and is also capable of analyzing different AAV serotypes [4].

CE-LIF can also be used for full/empty capsid analysis in combination with genome integrity analysis. SCIEX has developed a fast, size-based screening workflow for AAV that involves purification of the AAV sample with the QIAquick PCR kit straight to nucleic acid, followed by separation and analysis. This method provides very good separation of intact and partial genome peaks and small size impurities in just 30 minutes (10 minutes for prep, 15 minutes for separation), allowing rapid analysis of in-process samples.

CONCLUSION

Economical, rapid and robust biophysical methods for the analysis of viral vector capsids – both in-process samples and final products – is essential to ensuring safe and effective gene therapies. SCIEX has developed MS and CE-LIF solutions that provide the critical information required for characterizing AAV viral vector proteins, determining AAV capsid purity and genome integrity and separation and detection of full, partial and empty AAV capsids.

These methods offer excellent resolution and sensitivity with minimal preparation and can be automated for rapid analyses. While they have been developed specifically for AAV samples, including different serotypes, these methods could be modified to work with other viruses.

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FASTFACTS

The Corning® Ascent™ Fixed Bed Reactor System: designed for high yield, scalable viral vector production and other applications for cell and gene therapies

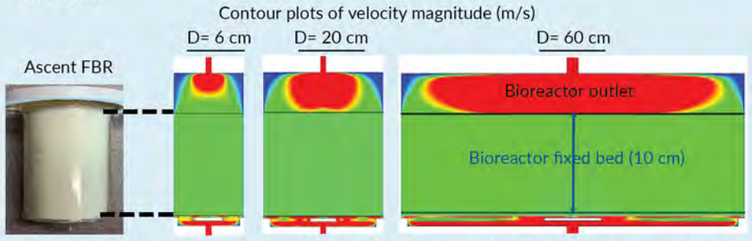
The cell and gene therapy sector is growing rapidly, with more than 1,000 ongoing clinical trials. Viral vectors have emerged as leading gene delivery tools – but the demand for these vectors will soon exceed the capacity of CDMOs to produce them. Many manufacturers continue to utilize adherent cells, and there is a high demand for scalable, high-density adherent technologies designed to meet the requirements of cell and gene therapy manufacturing processes. To address this demand, the Corning Ascent Fixed Bed Reactor (FBR) System was designed to intensify adherent cell-based bioproduction, while increasing yield and reducing cost.

ACHIEVING UNIFORMITY AND HIGH YIELD

A uniform laminar fluid flow through a bioreactor fixed bed is critical for a homogenous environment. To assess fluid flow, computational fluid dynamics modeling across three different reactor sizes and dye residence time distribution (RTD) analysis were performed **(Figure 1).**

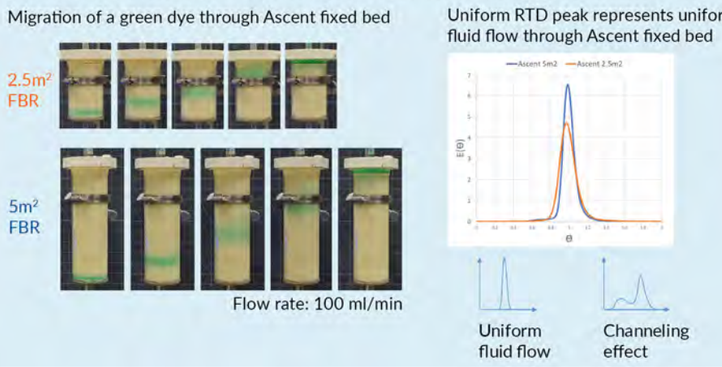
Figure 1. Uniform fluid flow in the Ascent FBR as demonstrated by computational fluid dynamics modeling and dye residence time (RTD) distribution analysis.

Computational fluid dynamics modeing demonstrating uniform laminal fluid flow across fixed bed sizes



Uniform fluid flow is enabled by special design of distributor plate at the base of the vessel and mesh structure and orientation

Uniform fluid flow in Ascent™ FBR is confirmed by dye resistence time distribution analysis



The impact of uniform flow on HEK293 cell seeding and growth was then evaluated. Using crystal violet staining, cells were observed to be growing in uniform monolayers distributed from the top to the bottom of the reactor. This homogenous cell distribution is critical for optimal and consistent viral vector yield, and as seen in the table below, results in consistent rAAV2 yield.

Consistent rAAV2 vector yield across multiple runs and bioreactor sizes.

Day 0: seed HEK293T cells at 22,000/cm²		Day 3: triple transfect cells with PEI	Day 6: harvest and lyse cells/ collect medium		
Runs	**Ascent FBR surface area**	**Ascent FBR diameter**	**Cells/cm² at harvest**	**% GFP+ cells**	**Bulk AAV2 VG/cm²**
Run 1	0.2 m2	29 mm	407,500	89.9	1.74×10^{10}
Run 2	0.2 m2	29 mm	373,125	93.4	3.00×10^{10}
Run 3	0.2 m2	29 mm	376,250	89.3	2.16×10^{10}
		Average	**385,625**	**90.9**	**2.3×10^{10}**
Run 4	0.7 m2	60 mm	357,832	92.3	N/A
Run 5	0.7 m2	60 mm	432,153	94	3.19×10^{10}
Run 6	0.7 m2	60 mm	479,351	87	2.98×10^{10}
Run 7	0.7 m2	60 mm	395,062	88.5	1.86×10^{10}
		Average	**416,100**	**90.5**	**2.68×10^{10}**

Assuming 500m²SA for production-scale Ascent vessel and linear scalability, we would expect 1.17×10^{17} bulk VG/vessel.

EFFICIENT CELL HARVEST

Another key benefit of the Ascent bioreactor compared to other FBR technologies is the ability to harvest viable cells from the bioreactor. HEK293 cells grown in the Ascent FBR were successfully harvested, as demonstrated by crystal violet staining before and after cell harvest. More than 90% of cells were harvested, with a viability of above 90%. In addition to increasing yield in viral vector production, this feature of the system enables its use in other applications, including the production of stem cell therapies.

HIGHER YIELD AT LOWER COST

Side by side comparison of the Ascent system to other FBRs demonstrated 3-fold higher rAAV vector genome yield per cm², and a 30% reduction in culture medium use (see table below). Collectively, these benefits result in a significant reduction in manufacturing cost per dose.

Ascent™ FBR demonstrated 3-fold higher rAAV2-GFP vector yield/cm² compared to other FBR in side-by-side evaluation

Bioreactor surface area (cm²)	6,700	5,300	
Total cell number/vessel	3.47×10^9	3.73×10^9	Can harvest cells
Transfection efficiency (%)	90	NA	Can assess transfection efficiency
Total bulk AAV GC/vessel	12.6×10^{13}	3.24×10^{13}	~4x higher total VG yield/ vessel
Bulk AAV GC/cm²	18.6×10^9	6.12×10^9	~3x higher bulk VG/cm²
Bulk AAV GC/cell	36,300	8,700	~4x higher specific cell productivity
Total media used (mL)	2,000	3,000	~30% less media consumption

Based on our cost model, these performance benefits led to up to 65% reduction in VG cost/GT dose for Ascent FBR at 1x1018 annual VG scale.

MEETING INDUSTRY NEEDS

The Corning Ascent FBR system provides a highly scalable platform for high-yield adherent cell culture. The system maximizes cell density while maintaining homogenous cell distribution and fluid flow, which in turn maximizes cell health and vector yield. As the Ascent FBR enables viable cell harvest, the system can also be used for a range of applications beyond viral vector production.

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CELL & GENE THERAPY INSIGHTS

FASTFACTS

The EuLV system: lentiviral vector production based on stable producer cell lines

In recent years, the manufacture of viral vectors has emerged as a bottleneck for the burgeoning cell and gene therapy industry. Consistent, large-scale vector production remains a challenge, and solutions are required to meet both upstream and downstream manufacturing needs. The EuLV system is Eureka Biotechnology's answer to the challenges currently facing lentiviral (LV) vector production.

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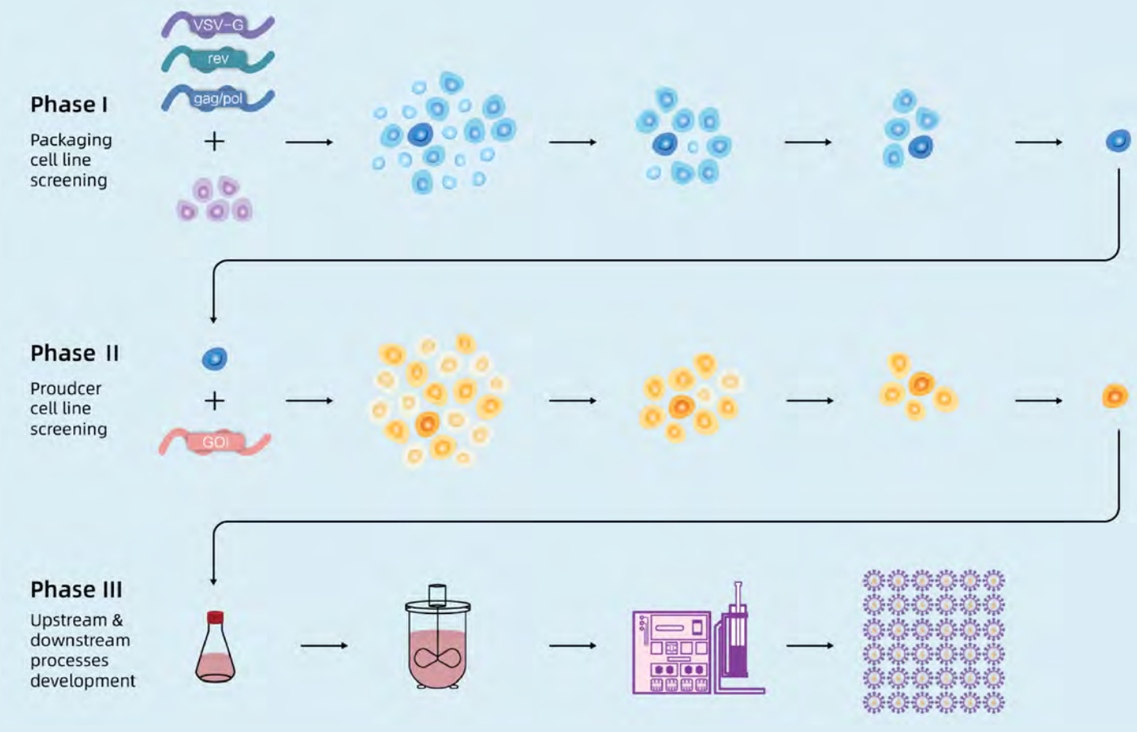
THE EULV SYSTEM

The EuLV system is an inducible stable producer cell line with scalable process technologies, which is adapted to high cell-density suspension culture in a chemically defined medium. The cell line does not require plasmid transfection – all the required packaging genes, as well as the gene of interest (GOI), are stably inserted into the genome of the producer cell lines, and the production of LV is achieved by chemical induction. Using stable cell lines to produce LV is an attractive option for manufacturers, as it increases the overall quality of the viral vector product by increasing safety and reproducibility, while also reducing production costs.

Figure 1. EuLV Technology Roadmap.



EULV DEVELOPMENT

The EuLV system involves three main phases **(Figure 1)**. In phase I, all LV packaging genes (e.g., env, gag/pol and rev), and components of molecular switches, are stably inserted in the genome of 293T cells to construct the EuLV packaging cells. In phase II, the customized GOI is stably inserted into the genome of the best clone of the EuLV packaging cells to construct the producer cells, and monoclonal cell lines are screened and tested. In phase III, EuLV producer cells are cultured at large scale in a fed-batch manner, and LV vectors are produced by the addition of an inducer.

EULV SYSTEM ADVANTAGES

Compared with a conventional plasmid transfection method, LV production using the EuLV system is simple, with much less batch-to-batch variance **(Table 1)**. After a systematic and comprehensive evaluation during producer cell line screening, the LV produced in EuLV cells was demonstrated to be more homogenous, with a significantly lower empty/full capsid ratio. The titer in culture medium can reach up to $8x10^8$ TU/mL, which is at least ten-times greater than when using a traditional four plasmid transient transfection method. Notably, the system significantly reduced production costs, which is a crucial consideration for gene therapy products being developed towards commercialization.

Table 1. Advantages of the EuLV system.

	Plasmid transfection	EuLV system
Production method	Plasmid + transfection reagent	Stable cell line + inducer
Culture method	Adherent or suspension culture	Suspension culture
CDM medium	No, may require serum	YES
Process stability	Variable	Stable
Virus homogeneity	Low	High
Virus specific activity	Around $1x10^5$ TU/ng p24 (ELISA)	$2x10^6$ TU/ng p24 (ELISA)
Titer in culture medium	Low ($1-5x10^7$ TU/mL)	High (up to $8x10^8$ TU/mL, GOI dependent)
Yield after purification	Variable	$1x10^{11}$ TU per liter of culture
Cost of production	High	Signification reduced

FROM GOI TO PRODUCER CELLS IN 4 MONTHS

Eureka Biotechnology has now begun to provide a CRO service for the EuLV system. Clients can simply provide a GOI, and monoclonal cells can be delivered in just 4 months. Optional services include GOI optimization, small-scale production, process development, and clone characterization with a 3-month stability study. By removing the need for plasmid transfection, the EuLV approach can eliminate several challenges facing LV vector production, improve scalability, and greatly simplify the manufacturing process while also reducing cost.

CELL & GENE THERAPY INSIGHTS

For all queries regarding the EuLV system, please contact enquiry@eurekabio.com

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CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING

SPOTLIGHT

INTERVIEW

Current and future perspectives on lentiviral vector bioprocess and bioanalytical tools

PAUL CARTER is the Head of Vector Processing at Quell Therapeutics Limited and has over 30 years' experience in the pharmaceutical industry, working across cell and gene therapy, biopharmaceutical and small molecule R&D. Prior to joining Quell, he was leading GSK's Downstream Process Development Group in Cell and Gene Therapy.

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Q How has the pandemic impacted vector bioprocessing in practice, and what associated challenges do you expect the industry to face on an ongoing basis?

PC: The COVID pandemic has really pushed the biomanufacturing industry, requiring highly accelerated activities for the development and manufacture of vaccines for COVID-19. These use the same types of technology and manufacturing plants that can be used for viral vector manufacture. So, if there was a vector manufacturing capacity challenge already, it has just been exacerbated by the need to rapidly produce COVID-19 vaccines.

I think the COVID pandemic has raised the profile of the bioprocessing industry, due to its ability to deliver much needed vaccines, so there has also been an upside. However, in general, it has highlighted the capacity crunch in biomanufacturing with specific aspects, such as filling, making the news due to vial supply shortages.

One aspect which is maybe less obvious is the pressure on testing capacity. The laboratories required for release testing of COVID-19 vaccines are the same ones being used for viral vector batch release assays. This increases the potential for delays, as the respective CROs are being pushed very hard. Some of them, for example, have been addressing this challenge by rapidly putting new facilities in place. Throughout this, I am sure, COVID-19 vaccine release assays are being prioritized, which is entirely appropriate.

There are multiple points within the biomanufacturing industry that are being stretched, due to the technology requirements for COVID vaccine and viral vectors manufacturing being the same. However, I do believe that the industry is responding extremely well, with more and more manufacturing capacity being brought online within a short space of time.

Q Indeed, meeting the capacity shortfall has been a constant theme for viral vector-driven gene therapy over recent years – can you comment further on how the sector is doing in this regard, for you, and what related trends/developments you expect to see moving forward?

PC: Suppliers have realized there is a need for viral vector manufacturing capacity at larger scale and are making the transition from static 2D bioprocesses and scaling up to adherent bioreactors such as the iCELLis®. There are some nice examples where companies have taken their process, scaled it up from cell stacks to an adherent bioreactor and successfully increased their volumetric productivity.

There is also an increasing focus on suspension cultures, which can substantially simplify the process scale-up, providing the option to scale-up to hundreds and potentially thousands of liters. It does come with its own set of issues, though. For example, how easy is it to do transfections at very large-scale? Even with some of the new polyethyenimine (PEI) transfection protocols and reagents it is difficult when reaching many hundreds of liters, simply due to the dynamics of mixing at that scale.

"There is ... an increasing focus on suspension cultures, which can substantially simplify the process scale-up."

There is a lot of work going on to develop both producer and stable cell lines, which will reduce or eliminate the need for plasmid DNA. Reducing the need to make large amounts of plasmid DNA shortens the supply chain and removes a lot of complexity and cost from it. Stable cell line processes, which use small molecule inducers such as doxycycline or cumate to initiate vector expression, are much easier to work with than

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> "...the COVID pandemic has raised the profile of the bioprocessing industry, due to its ability to deliver much needed vaccines, so there has also been an upside. However, in general, it has highlighted the capacity crunch in biomanufacturing with specific aspects, such as filling, making the news due to vial supply shortages."

transient transfection processes, as the need to make large transfection mixtures, which can be extremely challenging and a source of process variability, is avoided.

There is also work underway to improve culture media and feeds, as well as implementing perfusion systems. These are both ways to get to higher cell densities and potentially increase cell culture productivity. In summary, there are multiple approaches being investigated to try to address the capacity shortfall.

Q Can you go deeper on progress in addressing the traditionally high cost of goods for LV vectors, and how further improvements in this regard might be achieved?

PC: It is mainly about increasing the batch size, as we have discussed, with the aim to reduce the cost per unit of vector. As the field is looking to scale up from tens to hundreds of liters, there are significant cost savings to be made. In addition, moving from static 2D to 3D cultures in bioreactors enables a lot more control over the upstream process with potential for increased reproducibility.

These process controls can enable higher cell densities and, hence, vector productivity. Better control of the production system may also lead to an increase in infectivity, positively impacting the quality of the vector being produced.

Moving to suspension culture, or even perfusion culture, can lead to significantly higher cell densities and higher productivity, especially if the period during which the cells are producing vector can be extended.

Improving upstream productivity by making more vector per unit volume is one way to decrease vector cost of goods. Reducing losses during downstream processing is another option. In this context, building a better understanding of the individual downstream processing steps and their impact on vector quality and yield is important. It provides an insight into the unit operations which have the greatest impact on cost of goods, thereby enabling targeted process development activities to reduce the latter.

In summary, multiple things are being done to address the high cost of goods for vector and significant progress is being made here.

Q Where have you seen positive advances in terms of enabling technology innovation/evolution in downstream vector bioprocessing over recent times?

PC: In vector downstream processing, people are starting to look at chromatography media in more detail, especially as there are no new vector specific media being released, there is a drive to better utilize the various ion exchange media already on the market. We are gaining a greater understanding of how these interact with viral vectors, which is helping in the drive to increase process efficiencies.

Then there are some media specifically designed to provide a very gentle way of processing viral vectors. Especially if you are looking to apply a second chromatography step as an intermediate purification to reduce host cell protein and DNA contaminants. These enable good removal of the key contaminants without impacting vector infectivity.

Additionally, the manufacturers of ultrafiltration (UF) devices used for vector processing are beginning to understand that the vector processing industry has specific needs. Consequently, they are looking to increase their portfolio of products with larger molecular weight cut-offs and changing some of their screens to options more appropriate for viral vector bioprocessing. Some of the filter manufacturers, for example, have realized that it is possible to sterile filter viral vectors, and are now looking into optimizing some of their membrane technologies. The same is also happening with the UF devices: making units that allow easy scale-up and scale-down makes process development a whole lot easier.

Q And how about on the bioanalytical side? Any recent improvements that have made a difference to you?

PC: The automation of capsid protein measurements using microfluidics is starting to be used more widely – it is significantly quicker and much less labor intensive than ELISA assays. It also results in greatly reduced variability, which is key when working with viral vectors.

The transition to digital droplet (dd)PCR or digital (d)PCR for titer and vector copy number determination is making those experiments easier and more straightforward to automate, so workflows with much higher throughput than fluorescence-activated cell sorting (FACS) titration methods can be put in place.

We are also looking at viral vectors in more detail, specifically their aggregation status. As I mentioned earlier, there have been issues with the sterile filtration of viral vectors. However, if the aggregation state of the viral vector is well understood, e.g. through the use of technologies such as DLS (dynamic light scattering), a good understanding of the impact of the various processing steps on the vector can be gained.

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Finally, having rapid analytics, rather than waiting for results, makes a huge difference and is helping to reduce process development cycle times.

Q What would be on your wish list in terms of future bioprocessing and bioanalytics innovation?

PC: Having a Protein A type of reagent for VSV-G pseudotype viral vectors would be a real industry game-changer. Some kind of affinity ligand to allow rapid capture and release, enabling multi-cycle chromatography and allowing us to purify our lentiviral vectors using more gentle conditions. Developing a VSV-G affinity ligand certainly presents some challenges. However, I do know there are companies that are interested in looking into how this could be achieved.

Further, having a better way of measuring lentiviral vector infectivity would be helpful. Currently, we derive infectivity from infectious titer and the concentration of p24. Having quicker and simpler assays would make a difference.

Finally, it would be great if the lentiviral vector field had something like the rapid chromatography empty/full assays you see for AAV. I think lentivirus biology would make this very challenging, but I would like to see people working on it.

Q What would an optimal separation and purification system for lentiviral vector look like?

PC: It's really about going back to first principles and asking, "what do we want to do at the different steps in the process?" If we assume that we have cells or cell debris to remove, then the first step to consider would be some kind of harvest filtration that will clarify the material, allowing us to run a chromatography step early in our process.

Secondly, what is the best way to remove the water when we are working at volumes of hundreds of liters? The main aim here is to decrease the volume, so a bind/elute chromatography step would be ideal for that. I have already mentioned the potential positive impact of an affinity step, if realized, as it would enable rapid material concentration whilst still maintaining infectivity.

The next step in the process would be a gentle, intermediate purification to deal with host cell protein and DNA contaminants. Once most of the purification has been done, we need to assess whether to concentrate further and how to formulate the vector product. I would choose an ultrafiltration (UF) step, as these can be set up to concentrate the material, followed by diafiltration (DF) to formulate the lentiviral vector.

"...it would be great if the lentivral vector field had something like the rapid chromatography empty/full assays you see for AAV."

Further, I am in favor of not trying to run a fully closed process to assure sterility, but prefer a defined sterile filtration step at the end of the process. It makes life a lot easier as it provides certainty that the product is sterile, because it has been passed through sterilizing grade filter(s). It is an incredibly important step, especially as the process scale increases.

Following that, container selection, whether to use bags or vials (according to how the vector addition is integrated into the cell process) needs to be considered.

And finally, the cryopreservation protocol, taking into account the amount of vector and the required freezing rate to maintain infectivity, needs to be optimized.

Of course there are other aspects such as temperature control during processing that are very important. However, the ones mentioned and how they could be combined for optimal performance are key in relation to controlling vector infectivity throughout the process.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING

SPOTLIGHT

INTERVIEW

Keys to success for in-house lentiviral vector tech transfer and manufacture

NICOLE NUÑEZ, PhD, joined Eureka Therapeutics in 2018 as a Process Development Scientist, and co-led the process development, manufacture, tech transfer, vendor management and regulatory compliance of lentiviral vectors to be used in the production of T-cell based therapies. Nicole currently serves Eureka as a Regulatory Scientist focusing on the development and implementation of regulatory strategy for the company's T-cell therapy and vector products.

LIAM POWERS joined Eureka Therapeutics as an intern in 2016 and is currently a Process Development Research Associate. His main focus has been the optimization and development of lentiviral vector production and purification systems. He led the engineering of Eureka's GMP vector process and provided oversight in tech transfer and manufacturing operations.

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Q What are you working on right now?

NN: Eureka is currently working on the build-out of our new GMP facility that is going to be located in the Greater Bay area. This is going to accommodate manufacturing of both our lentiviral vectors and our T-cell therapy products.

In addition, we are engaged in setting up our regulatory strategy for the lentiviral vector. We recently filed a master file with FDA for our lentivirus manufacturing process. It has been accepted by the Agency and can potentially support all of our future T-cell programs.

LP: My work is mainly focused on process development for lentivirus and T-cell therapy processes. Some of my experiments look at T-cell transduction, but equally, a lot of my work involves how to develop a precise, accurate and robust biological assay to determine lentiviral vector potency.

Q Can you tell us more about Eureka's approach to lentiviral vector manufacturing? What differentiates it from other current efforts in the cellular immunotherapy space?

NN: We have spent considerable time and attention on ensuring the design of our plasmids and manufacturing process result in high titers and maximize yield, which in turn means we can limit the scale of production required.

It is often the case for biotech companies relying on viral vectors, especially as a reagent in cell therapy manufacture, that the vector is seen as a bottleneck in the process. We therefore took it upon ourselves to develop our own proprietary process, eliminating the need for external vector CMO support. This has allowed Eureka to have greater control over the process, faster turnaround time, the ability to accommodate new programs that require vector, and best of all, we can do all of these things in accordance with our own timeline.

LP: Our overall approach is to control the steps of the process for ourselves, instead of relying on many different partners to whom we would be beholden in the future. That involves us investing a lot of energy upfront and to really take the time to optimize our own process.

Specific areas of focus in this regard include plasmid optimization – making sure they can accommodate the transgenes and finding different combinations that work better – and optimization of the transfection and harvest steps. Additionally, we have prioritized the ability to make our own cell line. And lastly, as Nicole mentioned, we have worked to build the required knowledge on the regulatory side to allow us to operate independently in that regard.

Q What are the key challenges or bottlenecks that you have faced in lentiviral vector bioprocess development to date, and how have you addressed them?

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> "When you are doing tech transfer, a lot of the work is around identifying gaps and doing risk analysis... one of the biggest takeaways or pieces of advice I could offer others ... is to document every single conversation that you have regarding your manufacture, your QC strategy, the tech transfer, the process development, etc."
>
> Nicole Nuñez

NN: One issue or bottleneck we have found, which again primarily relates to working with CMOs, is that the yield is not reliable. You could work with two different CMOs and get totally different yields, depending on their process. When I'm talking about yield, this really comes down to the functional titer because obviously, there are for the most part, comparable ways to measure and quantitate physical titer – such as by performing p24 ELISA, for example. However, this doesn't give you an idea of how many live, active lentivirus particles are present.

I think this has been a little bit of a pain point. It's sometimes really hard to evaluate a CMO and how their viral vector is going to handle your transgene. How is it going to produce, and are you going to end up with enough product to use in your clinical study, for example?

LP: In terms of alleviating bottlenecks, I think switching from an adherent cell line to a suspension cell line was the main thing that really allowed us to scale-up for the first time.

Q What are the keys for you in ensuring tech transfer success, especially in the currently challenging environment given the pandemic?

NN: Liam and I both led the tech transfer of our lentiviral vector programs into the GMP sphere. What was key was the interplay of multiple departments – working closely with our process development teams, colleagues from manufacture, quality control, quality assurance, regulatory, contracts, finance, and our leadership teams. All contributed to the planning and execution that allowed us to have a successful launch of our GMP lentivirus manufacturing programs.

> "One thing that I think really helped our success was we had a small and specialized team focused highly on the tech transfer. And the main attributes of that were being flexible and agile..."
>
> - Liam Powers

When you are doing tech transfer, a lot of the work is around identifying gaps and doing risk analysis – preferably before the gaps become an issue, of course – and trying to identify what issues we need to tackle now, and what we need to tackle next.

I think one of the biggest takeaways or pieces of advice I could offer others involved in this type of tech transfer process is to document every single conversation that you have regarding your manufacture, your QC strategy, the tech transfer, the process development, etc. You never know when even the smallest discussions might come up again down the road, and if and when they do, you are really going to be thankful that you took diligent notes on why you made the decisions that you did.

The good thing with GMP is that you are always documenting everything, obviously. But when you are upstream of GMP – during tech transfer, process development, or even in research – when you're having those early conversations, you should also always document them. It is not as though documentation sets a precedent at that early stage. And it's important to make a note of why you decided to make this filter change, why you decided to do this QC assay over that one, etc. Later on, when you go to a conference, or a regulatory agency approaches you with these questions, you are going to have the answers already fleshed out because you have thought about them already, even if it was several years ago.

LP: I think Nicole is absolutely correct. There are so many different factors we are all trying to bring together for a successful tech transfer.

One thing that I think really helped our success was we had a small and specialized team focused highly on the tech transfer. And the main attributes of that were being flexible and agile, because different issues arise and sometimes you have to respond to them quickly.

At the same time, this focused team allowed us to keep a really close working relationship with the staff at our GMP facility. I really feel like the relationship we fostered and the close communication between our side and their side really made the whole thing go smoothly. I actually think that relationship might be one of the most important things in tech transfer – in addition to all the process aspects, of course.

Q What recent developments in terms of bioprocess and analytical tools have had the greatest impact on your work of late – and where would you like to see further innovation in this regard?

LP: We haven't placed too much focus on incorporating recently developed analytical techniques or technology in our process development. That is because for

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measuring and establishing what a good process is, we rely mainly on our functional titer assay, and I don't think there is any proxy technique that can really compare.

But in terms of future innovation that I would like to see in the lentiviral vector field, it would be that the different major players could come together and make an established way of interpreting what a transducing unit is. There is so much confusion among different labs currently, in terms of both how they each measure it and how the results translate from one lab to another. I think if everyone could agree on one standard way that would help us all to innovate in our own particular areas in the future.

NN: I would echo Liam's comment on the need to standardize in this field, which brings me back to my earlier point about the challenges in evaluating CMOs to do your vector production – there isn't a guideline out there or a universal method. So I think a lot of effort needs to be spent both on developing your process and on evaluating your process at the end because ultimately, those will be the assays you are going to be using for QC release of your viral vector.

LP: I also think the required production scale requirements of these gene therapy vectors don't match up particularly well with what the tool vendors are currently supplying. They are mainly geared towards antibodies or other biologics, which are produced at larger scales. If vendors could make their products at a more moderate scale – between 2 and 20-liter batches, for instance – that would probably help other small companies like Eureka to get started.

Q What would an optimal separation/purification system for LVV look like?

LP: In terms of the whole process, I'll just comment that the processing time is key – you want to keep it as short as possible because the virus is degrading throughout your process. Ideally, one day from culture harvesting to vialing would be best. I think fully automated systems would help create that scenario.

Regarding purification specifically, I think ultrafiltration is the most essential step. That can do the bulk of the required separation, although for certain applications where a high purity grade is required –injectables, for example – you would need to add a chromatography step as well.

We also think that you need to have a sterile filtration step at the end because it reduces risk and will improve your lot-to-lot consistency.

"Ideally, one day from culture harvesting to vialing would be best. I think fully automated systems would help create that scenario."

- Liam Powers

Q Finally, can you summarize the chief goals and priorities, both for yourselves in your own work

and for Eureka Therapeutics as a whole, over the coming 12–24 months?

NN: For Eureka Therapeutics as a whole, our main goal is to get proof-of-concept clinical data from clinical studies using our lead T-cell therapy products in liver cancer indications, which of course in part, relies on the successful manufacture of our lentiviral vectors.

For me personally, I recently transitioned from being a process development scientist to a regulatory scientist. I will be focusing on the intersection of manufacturing and QC work with the regulatory sphere, regulatory writing, submissions, communication with FDA, the management of our viral vector master file, and so on.

This is still a new field, relatively speaking, and it is constantly evolving. When it comes to the regulatory agencies it's really hard to predict what they are going to want and when they are going to want it. Every time a press release or news story comes out about a safety issue with someone's viral vector product, I believe you should always stop, pause, and really look at your own viral vector program. Ask yourself, does that situation apply to us and if so, do we have any risk mitigation in place? I think you always need to be thinking forward on the regulatory front.

The regulatory environment will continue to develop, and I think it's important that companies spend a considerable amount of time thinking about their strategy, with the realization that it might not fit every single program the company has for viral vector development moving forward.

LP: For me, it's continuing to support and develop our capabilities in our clinical trials and also our GMP manufacturing.

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CELL & GENE THERAPY INSIGHTS

VIRAL VECTOR BIOPROCESSING

SPOTLIGHT

INNOVATOR INSIGHT

Improving upon legacy vector and plasmid bioprocess technology for tomorrow's advanced therapies

Henrik Ihre, Peter Guterstam & Mats Lundgren

It is undeniable that plasmids are of great importance to the biotechnology industry. Plasmid technology not only supports existing modalities but is key for the development of next-generation cell and gene therapies, along with the emerging field of mRNA therapies. Modern purification technologies for plasmid DNA and viral vectors are poised to improve both productivity and speed compared to legacy methods. This article will focus on the importance of plasmids, along with modern processing methods for plasmid DNA and vectors, including solutions from Cytiva that can offer flexible, robust results with significantly improved productivity as compared to conventional methods.

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PLASMIDS: THE BASICS

Plasmids are a natural part of many cells, and are comprised of small extrachromosomal plasmid DNA (pDNA) molecules. They are carriers of genetic information, enable functions including communication between cells, and provide properties such as resistance to antibiotics. Within the field of biotechnology, plasmids are predominantly of interest as

vectors that can be used as tools to clone, amplify, and express genes. GMP-grade plasmids can also be used for *in vivo* applications such as DNA vaccines and gene therapy.

Plasmids come in many different shapes, forms, and sizes. Supercoiled plasmids in the size range of 5–20 kbp are of key interest to the biotechnology industry, and are most commonly used in bioprocess applications. Driven by new modalities, growth and market interest in plasmids has increased significantly in the last few years. As of 2020, the global plasmid market represents more than $80 million per year, and is growing at a rate of over 15% per year.

Plasmids are of key importance to the cell and gene therapy industry, and represent the starting point for many different modalities. They are used as a platform for many approaches found in laboratories around the globe. The entire biopharmaceutical industry has been built on the invention of recombinant DNA technology, which requires plasmids for the development of new expression systems. In both cell and gene therapy, plasmids are used to transfect cell lines to produce different viral vectors of interest. In order to develop mRNA-based therapies and vaccines, plasmids are used as templates for the enzymatic production of mRNA. For so-called DNA vaccines, GMP-grade plasmids is the active pharmaceutical ingredient (API).

Although plasmids are a common denominator within the cell and gene therapy industry, plasmids themselves are not one-size-fits-all, and one plasmid process cannot fit all needs within the industry. Requires volumes and quality of plasmids vary greatly depending on application area (Figure 1).

It is important to note that DNA vaccines are still in the early stages of development. If they achieve high success on the market, this may potentially drive the need for significant volumes of plasmids, as for this type of application the plasmid is the API, and purity requirements are therefore extremely high.

Quantity is not the only consideration – today, the field of gene and cell therapy requires not only large quantities of plasmids but also several different plasmids for each

FIGURE 1

Scale and amount of plasmid needed by application area, arranged from largest to smallest required volumes (L-R).

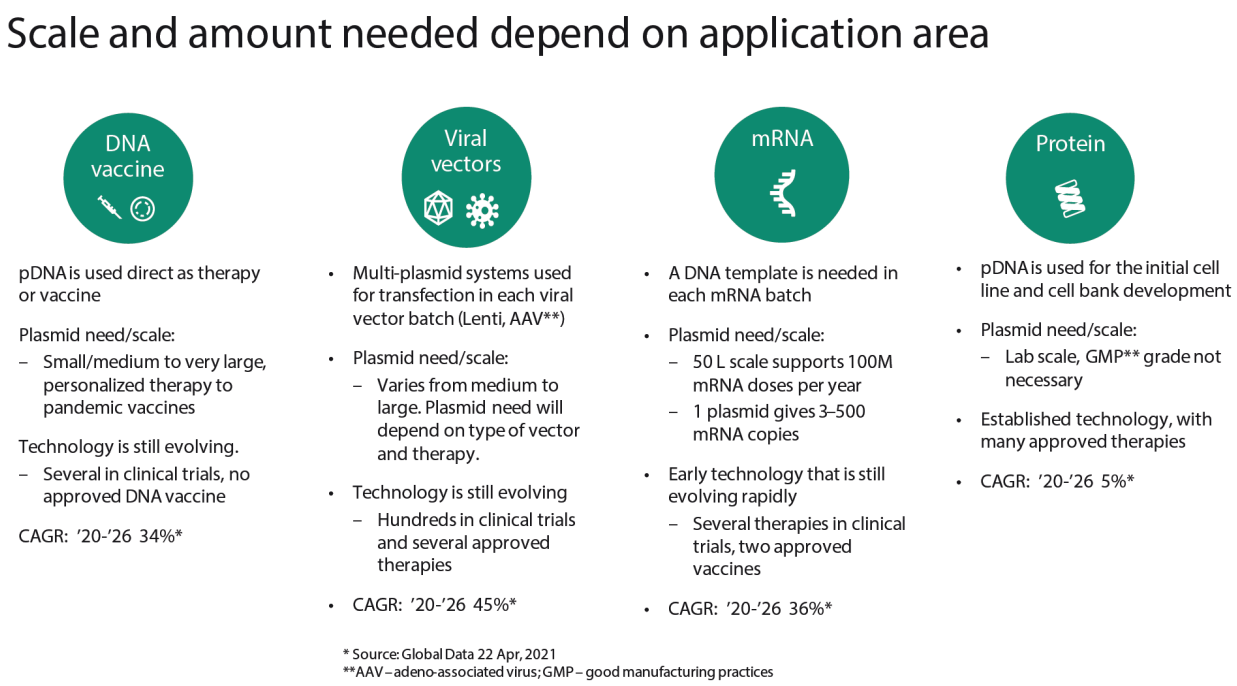

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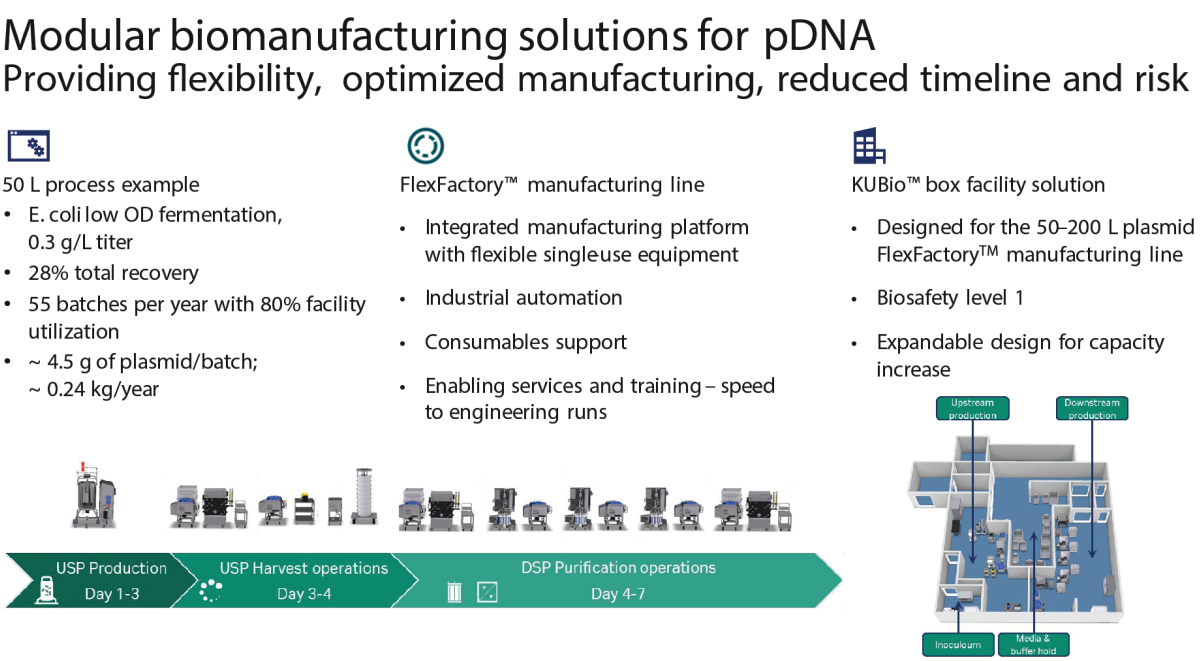
FIGURE 2

Modular biomanufacturing process for pDNA.



cell transfection. It is not uncommon for each viral vector to require up to three or four different plasmids – not including the plasmid containing the gene of interest – to develop a functional viral vector.

The emerging fields of mRNA-based vaccines and therapies require a plasmid as a template for the enzymatic *in vitro* transcription reaction. Each plasmid template may give rise to several hundred copies of mRNA, meaning smaller volumes of plasmid could generate larger quantities of mRNA molecules. When considering the plasmids needed for the design and development of new expression systems for different recombinant proteins, only very small amounts of plasmid are required.

There are also newer technologies that may change the future plasmid landscape. The current cell lines designed for viral vectors may be modified in such a way that only the plasmid containing the gene of interest is required, which could drive down volume demands. Rolling circle amplification (RCA) is an alternative process to the more traditional *E. coli*-based expression systems, which would allow for truly cell-free expression, and this option may be more suitable for small-scale GMP and personalized applications, while also potentially allowing for shorter timelines. Other options could include synthetic DNA templates or re-use of templates. Self-amplifying mRNA techniques are also reasonably advanced, and could, if they are shown to be successful in clinical trials, mean less need for plasmid as template for production of mRNA.

LARGE-SCALE PLASMID BIOMANUFACTURING OPTIONS

A biomanufacturing enterprise includes the process, facility, resources available, and infrastructure. These elements are integrated, and influence each other in significant ways. When designing a plasmid process, the primary objective is typically to manufacture a certain mass of supercoiled plasmid to the right specifications for the intended application. Challenges include meeting the different requirements needed for different plasmids and

BOX 1

Fibro chromatography in downstream processing of AAV.

Due to the importance of AAV to the field of gene therapy, Cytiva has prioritized the development of a new AAV capture product. Higher capacity, process intensification, and improved productivity in AAV manufacture are all prerequisites to allow for the treatment of large patient populations, and make promising and curative therapies more accessible in a sustainable and effective manner.

Low titers are a key challenge in downstream processing of AAV, and may result in the need to load very large sample volumes onto a capture column. As a result, it may be necessary to oversize the affinity resin column to allow processing in a reasonable timeframe. An alternative is to concentrate the feed with a tangential flow filtration (TFF) step, but this takes time and will impact overall recovery.

Resins have a great capacity for proteins and other smaller compounds that can access all of the available ligands within the pores of a resin chromatography bead. When purifying larger entities, only a limited percentage of the target entities can access the ligands that are on the interior of the chromatography bead, and therefore capacity drops with entity size. Some biological entities such as lentiviruses, AAV, exosomes, plasmids, mRNA, and liquid nanoparticles (LNPs) are much larger and can only bind on the outer surface of the bead, resulting in relatively low resin capacity.

In contrast, Fibro chromatography uses electrospun cellulose fibers, which generate a structure that is more porous than a chromatography resin, allowing AAVs and other large entities to bind throughout the material. As seen in Figure 11, this provides relatively stable capacity regardless of target entity size.

applications, meeting yield and purity goals, and ensuring access to manufacturing capacity. Designing a process to meet scale and purity goals, leveraging modular and single-use strategies to provide flexibility, and using integration solutions to ensure compliance and efficiency, are all strategies that can be employed in plasmid GMP manufacturing to ensure success. Cytiva's FlexFactory™ platforms and KUBio™ facilities are offerings that can be built around a process and its specific mass balance, and process design services are available to better support process understanding.

From a downstream process perspective, there is typically a complex and challenging feed from the *E. coli* reactor, that may contain up to 3% of the desired plasmid along with impurities that need to be removed. Cytiva has a downstream process in place which is based on three key chromatography steps: achieving RNA reduction by size-exclusion chromatography, a thiophilic step to separate the active supercoiled form from the non-active open circular form of the plasmid, and finally, an ion exchange step to remove endotoxins.

Figure 2 details a modular biomanufacturing process for pDNA based on the FlexFactory solution from Cytiva. If a facility is in place, the FlexFactory solution can be fitted and designed into that existing facility. If a facility is not available a KUBio box facility solution, comprising of a specifically designed FlexFactory solution within a KUBio box, can be used.

PROCESS INTENSIFICATION WITH FIBER-BASED PLASMID PURIFICATION

Looking to the future of plasmid production, there are incoming technologies and formats which may support higher productivity processing of both plasmids and other large target molecules.

One of the key challenges with purification of large target molecules is that they may not be able to enter the pore structure of a porous bead, resulting in low overall capacities and productivities. The new Fibro technology recently launched by Cytiva may offer a solution for such large target molecules and processes (see Box 1).

Case study: conventional bead format versus novel Fibro format

Significant benefits can be achieved by coupling the same ligand to a different porous structure, allowing for fuller access to the

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surface area. As shown in Figure 3, a plasmid process based on the conventional bead format and the novel Fibro format were compared.

A 2.5-liter ready-to-process column packed with Capto™ PlasmidSelect and a 600 ml Fibro cassette modified with the same Capto PlasmidSelect ligand were compared, using a feed of 50 liters of 6 kbp plasmid, at 0.1 g/L. Fibro plasmid purification offered up to 40 times higher productivities due to the rapid cycling combined with the high accessibility of the surface structure. This novel format could be suitable not only for plasmids, but for large target molecules in general, and may offer significant improvements in both productivity and process economy.

BIOPROCESSING OF ADENO-ASSOCIATED VIRUS (AAV) VECTORS

Viral vectors can be used for a variety of purposes, including vaccines, oncolytic therapies, or gene therapies. Viral vectors can also be used in cell therapies as reagents and, regardless of the final application, there are some common themes in the production process.

Everything starts with expansion of the producer cells – in this case, HEK293 cells. Once the cells have been expanded the virus must be introduced, which can be done via the infection or transfection route. The virus then propagates in the cells, before being released and purified. Finally, there are a series of formulation, fill and finish steps. Cytiva's AAV production process is shown in Figure 4.

Briefly, a triple plasmid transfection system is used to transfect HEK293 cells with the green fluorescent protein (*GFP*) gene. A vial from the working cell bank is thawed and the cells are expanded in shake flasks to the volume required to inoculate the production bioreactor (in this case the Xcellerex™ XDR-10 single-use stir-tank bioreactor). Further downstream, scalable and robust technologies based on filtration and chromatography technologies are used for purification. A range of orthogonal analytics is used to ensure performance.

FIGURE 3

Plasmid purification using Fibro versus Capto PlasmidSelect technologies.

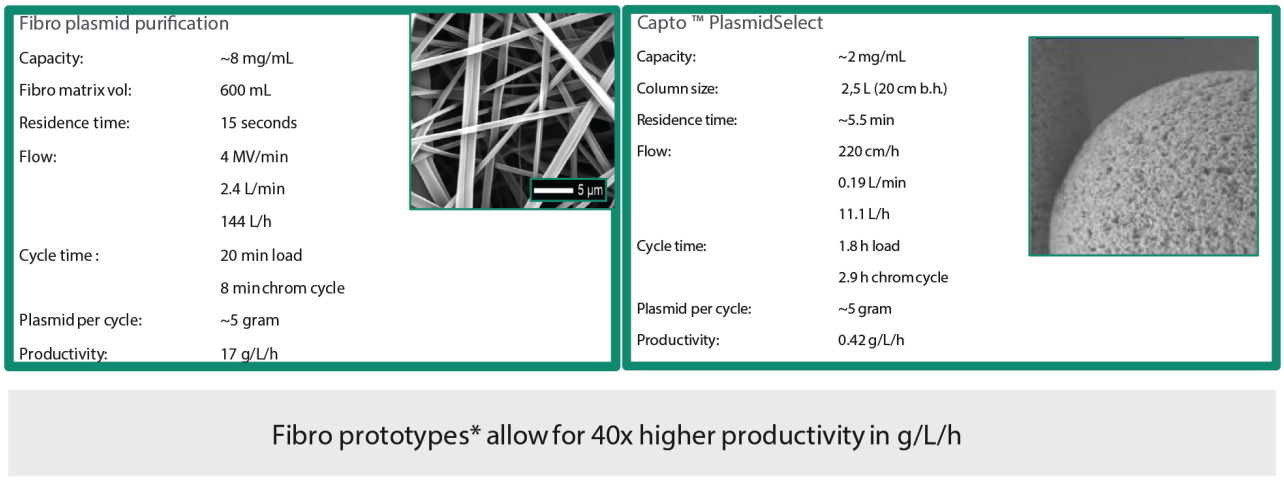
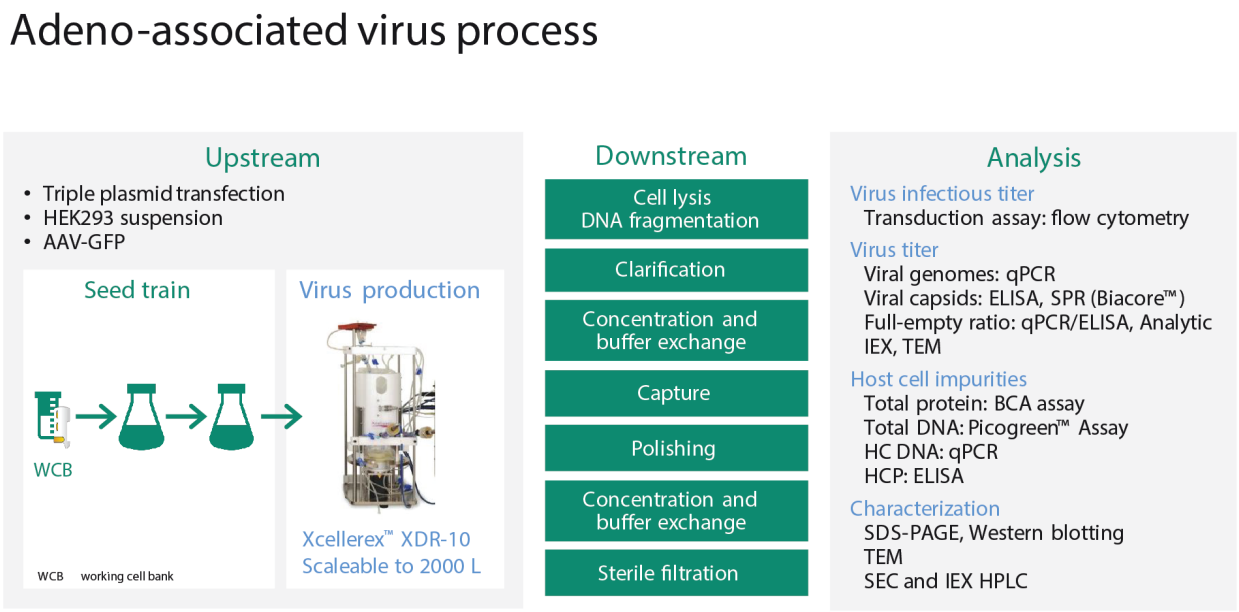


FIGURE 4

AAV production process.



UPSTREAM PROCESSING

Cytiva's upstream strategy was based on the need to adapt the host cells to serum-free suspension cell culture. A number of different animal-origin-free cell culture media were validated, since avoiding any animal-derived components offers a clear regulatory advantage.

To optimize the triple plasmid transfection procedure, design of experiments was used to assess parameters including:

- Cell density and volume at transfection
- Plasmid concentration and ratio
- Transfection reagent (PEI)–plasmid ratio
- Incubation time of mix prior to transfection
- Temperature
- Supplement
- Time to harvest post-transfection

Success criteria included transfection efficiency over 70%, and AAV titer of at least 106 TU/mL or 109 VP/mL. The resulting optimized transfection protocol can be seen in Box 2.

The optimized protocol produced around 1011 VP/mL both in the shake flasks and in WAVE™ 25 and XDR-10 bioreactors. Productivity was consistent for serotypes AAV2, AAV5, AAV8, and AAV9. The process is also effective at larger scales: Cytiva customer Homology Medicine scaled up this transfection process from 2 liters to 500 liters, with good linear scalability.

BOX 2

Optimized transfection protocol.

- VCD: 1 x 10^6/mL
- DNA (µg/mL): 0.75
- PEI/DNA ratio: 2
- Transfection volume (% of total): 5
- Incubation time: 15 minutes
- Temperature: 37°C
- DNA ratio: 1:1:2
- (Rep/cap: helper: transgene GFP)
- TOH: 72h

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DOWNSTREAM PROCESSING

A similar step-by-step optimization was followed for development of a downstream process. In particular, two chromatography steps are critical for the performance of the overall process. First, the virus was captured on a chromatography column with Capto AVB affinity resin, which binds to several different AAV serotypes, including AAV2 and AAV5. The material was loaded onto the column, then washed and eluted. Cytiva's ÄKTA pure protein purification system was used along with the Capto AVB resin packed in HiTrap™ columns. With a concentration factor of ~100-fold, recoveries of between 70% and 80% were achieved. Results achieved with this approach are shown in Figure 5, with no detectable virus in flowthrough or host cell proteins (HCP) in the eluate. Analysis of the eluate using transmission electron microscopy using the MiniTEM™ system (Vironova) was also carried out, and intact, high purity particles of the expected size (25 nm) were observed.

The Capto™ Q ImpRes, an ion exchange resin, was then used in the polishing step in order to reduce empty capsids. Polishing can prove to be a challenging step to optimize, and there are a number of critical parameters which must be evaluated and optimized. Along with ensuring the maximum amount of full capsids are present in the starting material, optimization of pH, magnesium chloride, additives, and wash gradients is required. When considering recovery of the viral genomes and percentage of full capsids, some trade-off regarding recovery may be necessary, depending on the target for enrichment of full AAV capsids.

IMPROVING ANALYTICS

Analytics are critical to process development for viral vectors. Cytiva has developed a new

FIGURE 5

Affinity chromatography with Capto ABV. Western blot overlays depicting host cell proteins (red) and viral proteins as detected with antibodies against VP1, VP2, and VP3 (green).

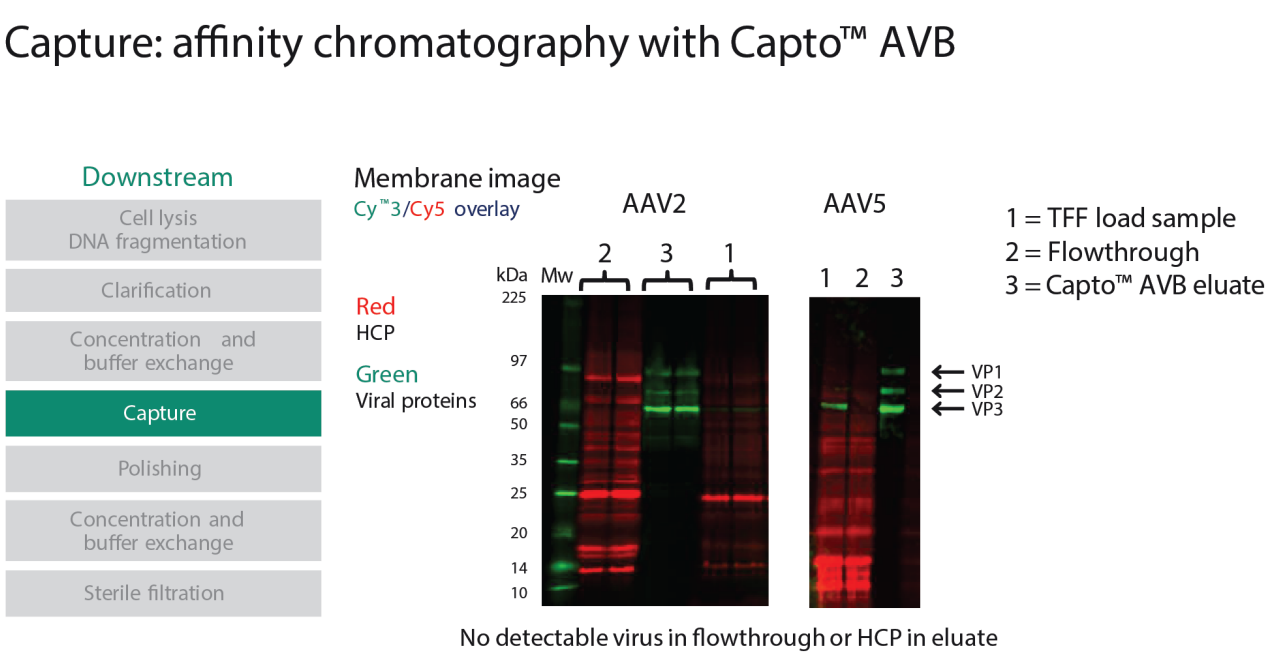
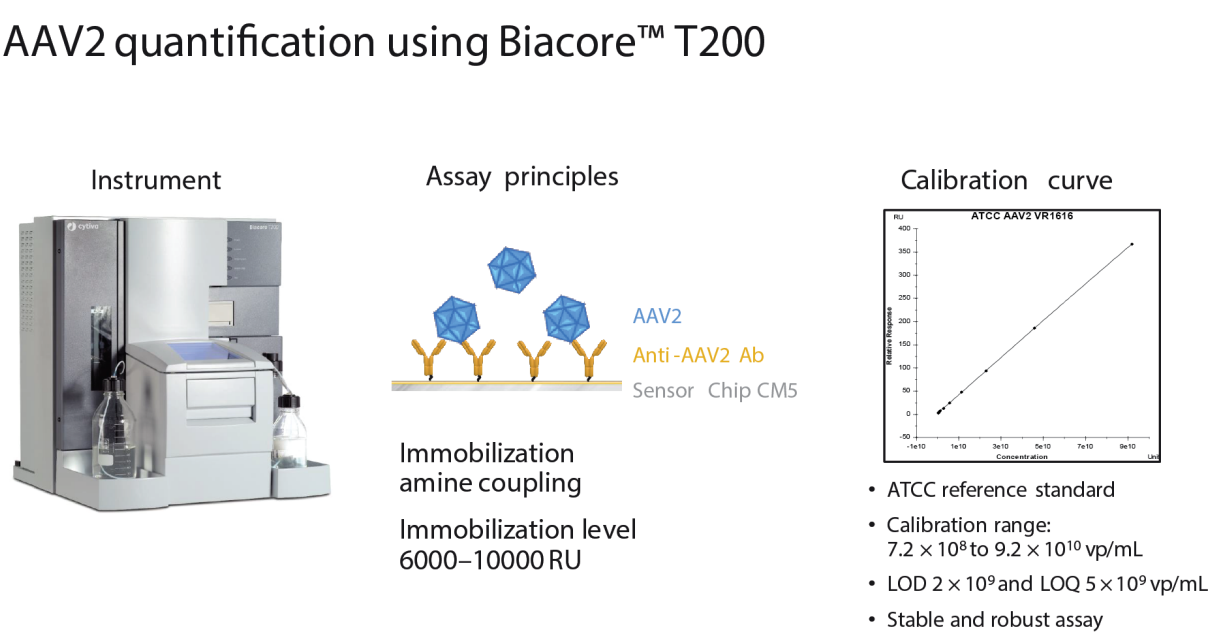


FIGURE 6

The Biacore T200 system for AAV quantification.



AAV quantification assay using the Biacore™ T200 system (Figure 6).

The assay is based on immobilizing anti-AAV antibodies to the sensor chip in the Biacore instrument via amine coupling. As the sample flows over the chip, interactions between the antibody and the virus can be detected. In the above example, anti-AAV2 antibodies were used, but other reagents can be utilized depending on the serotype being measured. To the right of Figure 6 is a calibration curve using an ATCC reference standard.

This is a stable and robust assay that has now overtaken the use of ELISA at Cytiva – Figure 7 shows results with ELISA versus Biacore using various samples. In all cases, a strong correlation between the Biacore and ELISA assay was observed.

CONCLUSION

Plasmids are the cornerstone of modern biomanufacturing. As newer modalities enter the market, the need for a variety of different plasmids, in increasing quantities, will only grow. It is key for manufacturers to understand their plasmid requirements as early on as possible, to allow these needs to inform their process design and development. Offerings from Cytiva, including the FlexFactory, KUBio box, and Fibro chromatography, offer plasmid and viral vector bioprocess solutions that can be applied at different scales, to support the continued growth and development of both the latest advanced therapies and the biotechnology industry as a whole.

Developing Fibro

A chromatography cycle using Fibro is shown in Figure 8. Recoveries and impurity removals that are comparable to available AAV capture resins were achieved with residence times of only a few seconds, and a binding capacity of over 1 x 10^{14} capsids per mL.

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Clarification is important for achieving a high flow rate, and when developing Fibro for AAV, different clarification methodologies were considered. Normal flow filtration can be suitable for AAV feeds that do not use lysed cells. If lysed cells are used, which is often the case, depth filtration followed by normal flow filtration may be needed. There are also limitations when loading very large sample volumes with this clarification method, so for challenging feeds a charged depth filter or a precipitation step can be added to the midstream methodology.

Fibro prototype for AAV5 purification: Belief BioMed collaboration

Figure 9 shows data from a collaboration between Belief Biomed and Cytiva. A clarified sample using a normal flow filter, and then an uncharged depth filter, was used. The chromatogram is similar to that shown in Figure 8, which was generated in-house. The purification performance in the eluted peak regarding removal of DNA and HCP is also similar to results seen when using capture resins.

Figure 10 visualizes the benefits of Fibro as compared to currently available AAV resins. When working with Fibro, residence times are just a few seconds long. In contrast, residence time with capture resins is often a minute or more. The porosity of the Fibro material also offers better capacity than standard chromatography resins, with capacity essentially doubled for AAV.

To summarize, the Fibro chromatography system enables significantly reduced process time for an affinity capture step. It provides an opportunity to not only speed up the loading and the capture step but also to potentially eliminate the need for TFF, which can positively impact both overall process time and yield. Fibro for AAV also provides similar recovery and purification performance as corresponding affinity resins. The Fibro system is simple to set up, and fits both laboratory equipment and GMP manufacturing instruments.

FIGURE 7

Analysis of AAV2 process samples using Biacore versus Elisa assay.

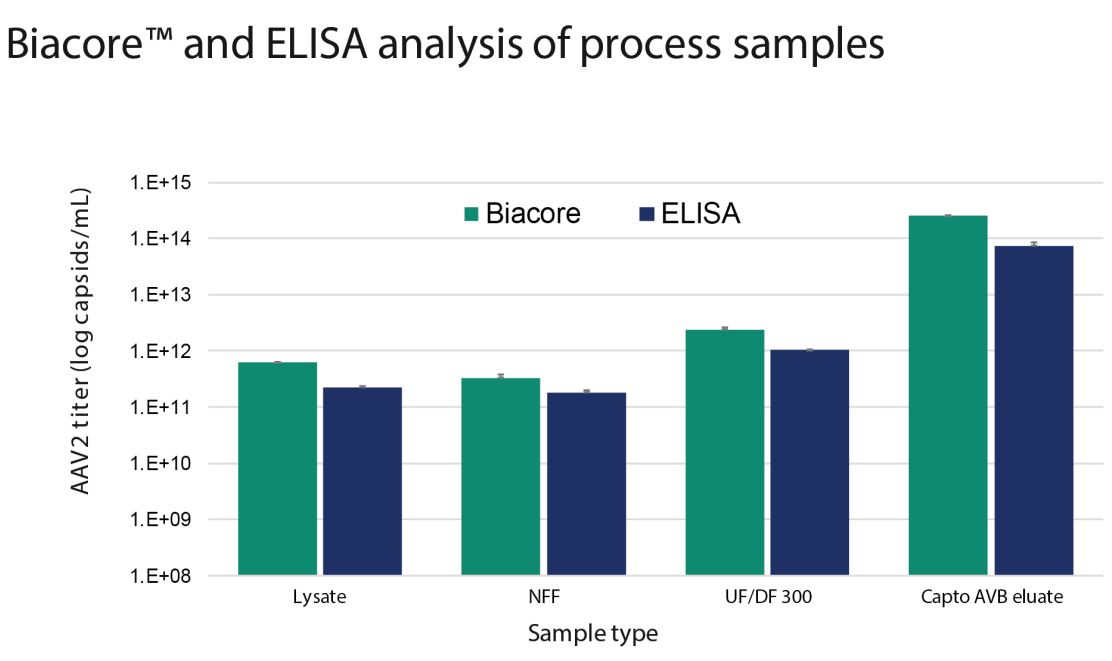


FIGURE 8

Fibro chromatogram.

High binding capacity and recovery at very low residence times

Fibro chromatography opens up new opportunities in AAV affinity capture

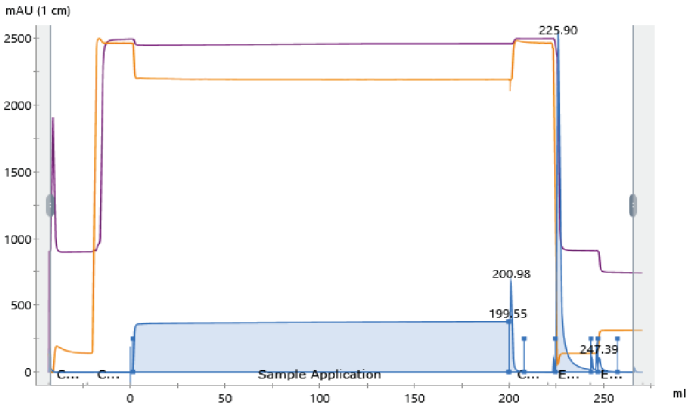
Binding capacity AAV5:
1–4 × 10^{14} capsids/mL

Residence times
1 to 5 s residence time for AAV5

Recovery:
60% to 90%

Purity:
HCP removal: > 99%
DNA removal: > 99%

HCP – host cell protein
NFF – normal flow filtration



Running conditions for AAV5:
Binding buffer : 20 mM Tris, 500 mM NaCl, 0.001% poloxamer F68, pH 8.5
Elution buffer : 100 mM glycine, 0.001% poloxamer F68, pH 3
Flow rate : 10–20 mL/min (serotype dependent)
Load: AAV5 NFF+TFF at ~1 × 10^{12} capsids/mL (~ 500 Fibro volumes)

FIGURE 9

Fibro prototype for AAV5 purification.

Fibro prototype for AAV5 purification

Biomanufacturer case study: clarified sample

- Residence time in seconds rather than minutes
- Loading time reduced from hours to minutes
- Recovery > 80% (based on vg)
- Purification performance similar to affinity resins
- Simple elution

Clarification:	NFF + uncharged DF
Load:	9 ×10^{13} vg clarified AAV5
Load volume:	500 Fibro volumes (500 × 400 µL = 200 mL)
Load rate:	25 Fibro volumes/min
Residence time:	2.4 s
Load time:	20 min
Elution:	pH 2.5

Data from collaboration with Belief BioMed

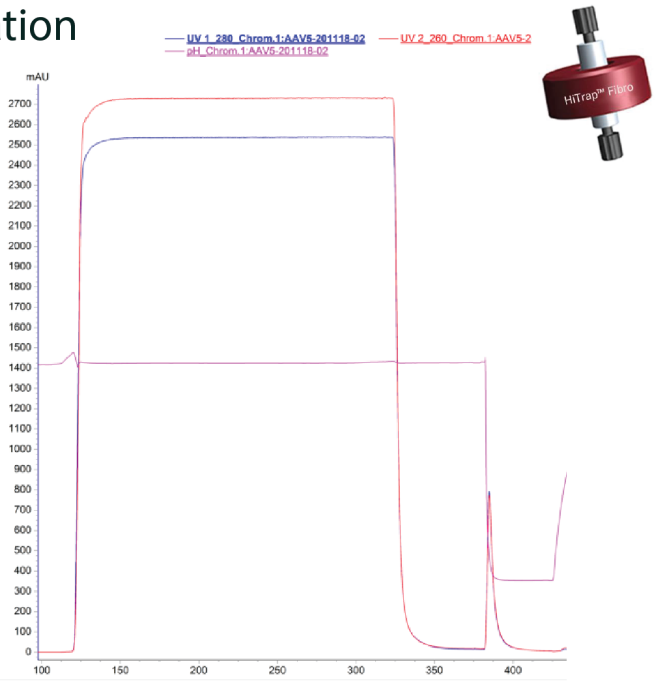


FIGURE 10

Residence times with Fibro versus currently available AAV resins.

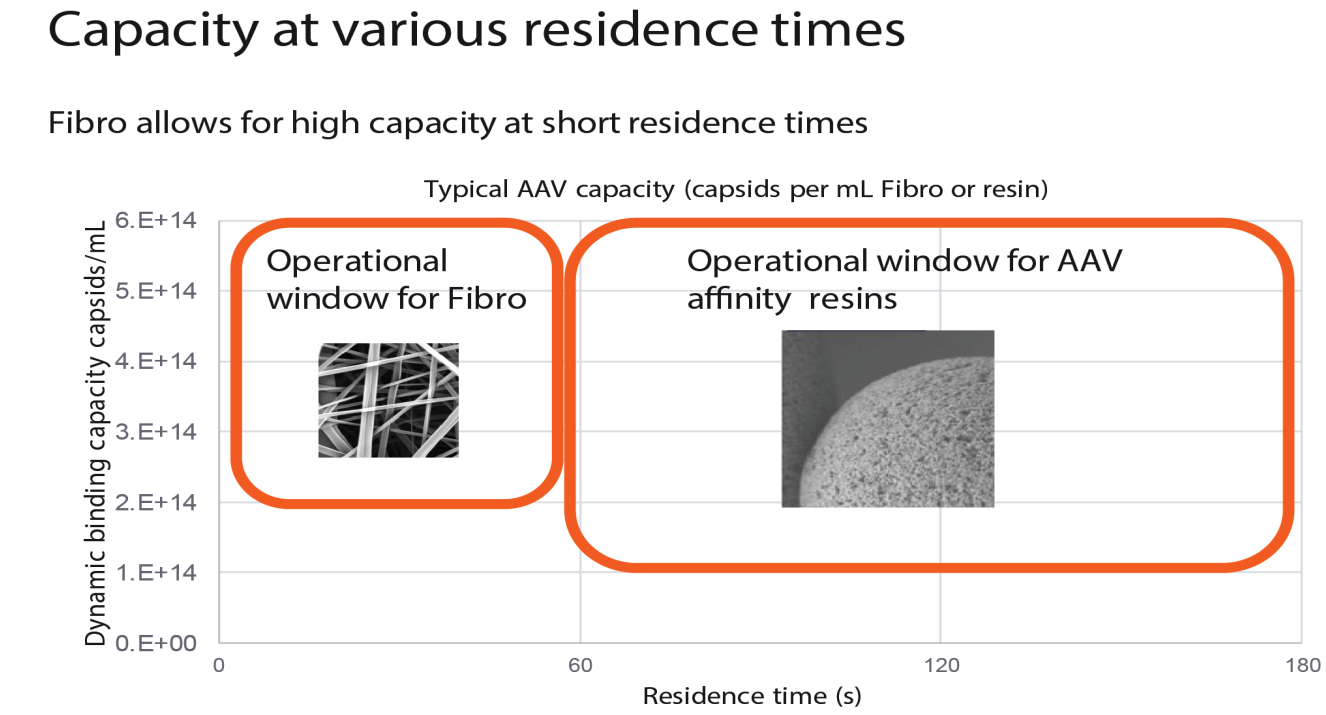


FIGURE 11

During the capture step of downstream processing, binding capacity varies with target entity size.

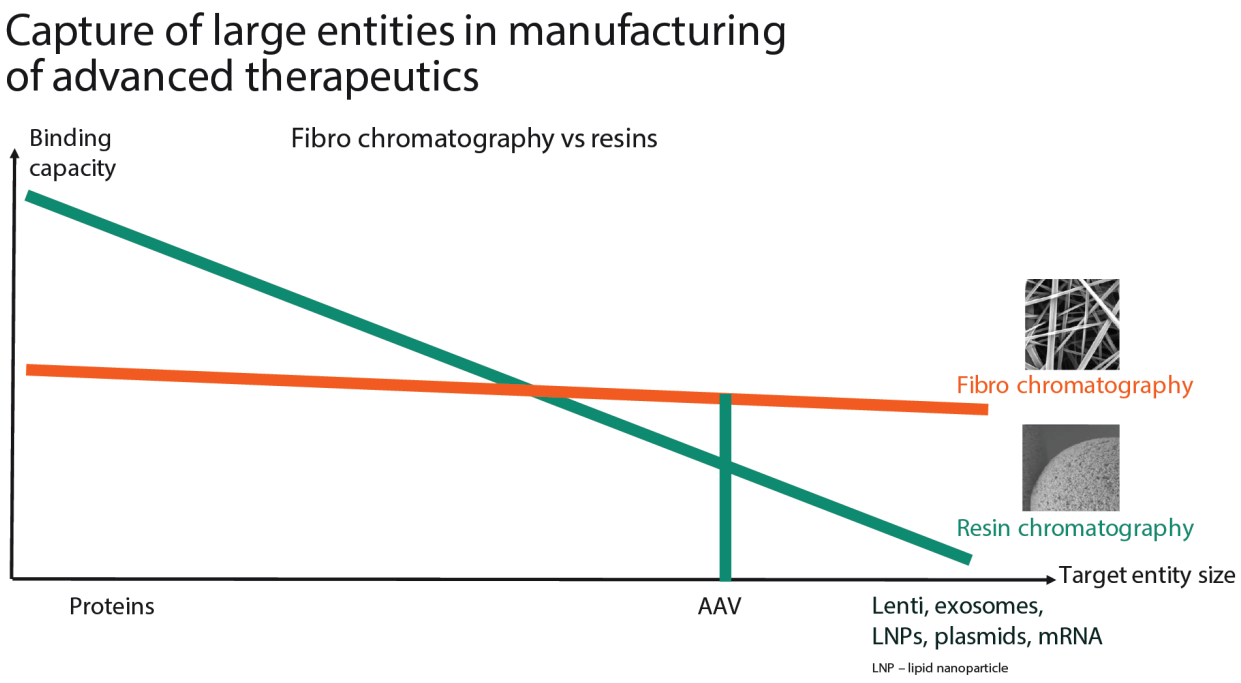


FIGURE 12

Initial screening of PlasmidSelect prototypes.

Initial Screening of Capto™ PlasmidSelect Prototypes

- 3 new resins were added to the Prototype list
- Further screening experiments performed utilising higher levels
  - ~3.8 mg pDNA mL^{-1} resin
- Whilst process yields were comparable for all prototypes greatest enrichment of SC pDNA was observed
  - No separation of OC pDNA was observed for resins G & H
  - Resin E presented SC pDNA within the wash fraction pDNA
- Following discussion with Cytiva, it was agreed to progress
  - Choice allowed for a relatively wide specification range for Ligand Density

Prototype Designation	Base Matrix	Ligand Density
Control	Sepharose ™ HP	High
A	Capto ™ ImpRes	Low
B	Capto ™ ImpRes	Medium
C	Capto ™ ImpRes	High
D	Capto ™ ImpAct	Low
E	Capto ™ ImpAct	Medium
F	Capto ™ ImpRes	V.High
G	Capto ™ ImpAct	High
H	Capto ™ ImpAct	V.High

FIGURE 13

Results of a benchmarking study showing the impact of an improved resin for large-scale pDNA production.

Impact of New Resin for Large Scale pDNA Production

Assumptions		
Criteria	**Legacy PlasmidSelect Xtra resin**	**Capto™ PlasmidSelect resin**
Quantity of Plasmid	70 g	
Load Volume	650 L	
Max Resin Capacity	2 mg mL^{-1}	3 mg mL^{-1}*
Max Bed Height	15 cm	
Load/Wash Flow Rate	100 cm h^{-1}	150 cm h^{-1}
Elution Flow Rate	50 cm h^{-1}	80 cm h^{-1}
Outputs		
Total Resin Volume	**43.6% less resin required**	
Total Buffer Volume	**49.6% lower buffer requirement**	
Total Duration	**13.7% faster processing**	

*May be increased further for some plasmids

BOX 3

Case study: Resin collaboration with Cobra Biologics

The need for a new resin in Cobra's in-house downstream platform for plasmid purification was driven by an ever-increasing demand for a variety of plasmids. This put pressure on Cobra's ability to process increasing quantities of plasmids in short timeframes. One of the key chromatography steps became the focus for improvement: the thiophilic or pseudo-affinity step, based on the PlasmidSelect Xtra Resin, which enables the separation between the open circular and supercoiled versions of plasmids.

The key parameters that were targeted were improving the flow rate, lowering the back-pressure, enabling more flexibility in column packing with taller bed heights, and improving the dynamic binding capacity, which combined would allow for improved productivity and process economy.

The legacy resin, PlasmidSelect Xtra, is designed on a base matrix developed in the early 1990s, with a relatively poor rigidity and an average bead size of ~34 microns. The Cytiva custom resin team and Cobra decided to explore the option of developing a high-flow and high-capacity version of PlasmidSelect Xtra, based on a higher flow, agarose-based matrix. The two initial candidate base matrices were the Capto ImpRes and Capto ImpAct matrices, with average bead sizes of ~40 and 50 microns. The ImpRes base matrix has a smaller average pore structure as compared to the ImpAct base matrix. In total, 8 different prototypes were developed for testing and evaluation by Cobra, with ligand density as one of the key variables **(Figure 12)**.

The prototype based on the Capto ImpRes base matrix combined with a high ligand density offered the best overall performance. In a benchmarking study, the results were in favor of the new Capto PlasmidSelect resin, as opposed to the legacy PlasmidSelect Xtra resin. The combination of a more rigid base matrix, combined with a higher ligand density, allowed for 44% less resin, almost 50% lower consumption of buffer, and roughly 14% faster processing **(Figure 13)**. This case study illustrates the impact a modern, high-capacity chromatography resin can have on production capacity.

Q & A

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Q Has the recent success of mRNA vaccines had an impact on pDNA processing?

HI: Due to the pandemic and the resulting success of mRNA vaccines, we can definitely see that everything around mRNA has sparked an interest. Since you need plasmids to make the mRNAs, we see significant interest in making a variety of different plasmids at different volumes and for different purposes.

PG: For mRNA vaccines it is a template, so the volumes of plasmid needed for each batch are significantly different, compared to transfection in the production of viral vectors, for example.

HI: Each plasmid can give rise to several hundred – maybe even up to 500 – copies of the mRNA. So even if you need larger quantities of mRNA, the volumes for the plasmids will still be rather small.

Q Will there be one plasmid DNA process in the future that fits all needs, or do you think the process will depend on the application?

HI: I don't think so – as mentioned above, different plasmids could be used for different purposes.

To give an extreme example, if you want to have a plasmid that is going to be the active pharmaceutical component in a DNA vaccine, then it needs to be of GMP grade, whereas if you want to transfect cells, then perhaps the plasmid doesn't have to be very pure. Then again, for mRNA I think it is important to have reproducible results from one lot to another, and in different volumes.

If you are going to make an expression system, you essentially need minute quantities of the plasmids that can be made on the lab bench. Whereas if you need three, four, or five different plasmids for cell and gene therapy applications, the quantities could be rather significant.

We need to keep in mind that we need to develop different processes, with a focus on different volumes and different qualities, depending on what they target or what they are designed for.

PG: One thing I will mention here is the size of the plasmid, and requests for much larger plasmids than were used before. This adds another dimension to the processing.

HI: This is a good point from Peter, and I think everyone has seen the recent progress of self-amplifying mRNAs. If they make it to the market, the corresponding plasmids are significantly larger than the ones we have seen up until now.

Q Can the transient transfection technology also be used for commercial manufacturing?

ML: Yes, it is already used in commercial manufacturing. Here once again, I would focus on the scale that is required. If you need huge scales, transient transfection may not be the optimal technology from a cost-of-goods perspective.

Q Are there other alternatives to the described polishing strategy if there are residual HCPs that need to be reduced?

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ML: We have a product called Capto Core 400 which can be used for reduction of smaller host cell impurities, and also smaller DNA fragments and so on, that you can add in addition to the other steps if needed. What type of polishing strategy you choose also depends a lot on the serotype.

Q Is Fibro for AAV available to test?

PG: We had hoped that at least the smaller units would be launched by now, but the launch is delayed. We have several collaboration partners, and the aim is to make it available for others to test by the end of this year or early next year.

Q What is favorable with resins as compared to Fibro for AAV, in your opinion?

PG: The impurity reduction is similar as with Fibro, but I think overall you get better impurity reduction with a resin. The difference is quite minor, but it does make sense that it is usually a subsequent polishing step. If there is an advantage with resin, that may be the advantage. But it depends very much on your fill, your serotype, and the actual ligand employed.

Q Will the Fibro be applicable in GMP production environments?

PG: Yes – we aim to have these units available in sizes that are compatible with GMP manufacturing, and the sizes can process around 200 liters in one cycle.

The exact volume you can process in one cycle is of course very much dependent on the titer, but it is in that range, and the initial launch will include units of those sizes.

The Fibro technology will have larger titers launched, so there is an opportunity in the future to make even bigger units for AAV capture, possibly processing more than 1,000 liters in a cycle.

Q How does the yield performance compare for Capto PlasmidSelect Xtra versus legacy PlasmidSelect Xtra?

HI: Having a high throughput doesn't mean anything unless you have good recovery and yield. I would say that the yield is on par with the legacy PlasmidSelect Xtra resin, keeping in mind here that we have almost identical bead size. The legacy is about 34 microns, the new about 40 microns, but the pore size is the same. It is the rigidity that makes up most of the difference, but yield I would say almost identical.

Q For purification of AAV, have you found any difference between the serotypes? For example AAV2 versus AAV5, and in the propensity to aggregate?

ML: In our hands at least, AAV2 has been more prone to aggregation than AAV5. We have seen a little bit of aggregation when it comes to AAV2, and you have to monitor the salt concentration in the buffers and so on to avoid that. We also sometimes use various additives, such as sugars or pluronic.

Q How critical is the initial UFDF step for AAV capture column performance, and why?

ML: When you are working with resin columns, in most cases you need to reduce the volume, otherwise the loading time will be very long. But of course, the UFDF step will also reduce impurities to a large extent, so you can get quite efficient purification in that single step. However, if you are working with affinity resins, you usually have very good selectivity with those, so you can load relatively crude material on them.

Q Does the Fibro remove intermediate capsids and provide more full capsids in your experience, and also is it known to reduce packaged extraneous host cell DNA?

PG: This depends on the ligand. For a standard AAV affinity ligand, there is no discrimination between full and empty capsids. But we have also made anion exchange versions and so on, and can get some resolution on this kind of Fibro structure in between full and empty.

As to whether it is known to reduce packaged host cell DNA – yes, certainly. If you have a ligand that doesn't make the DNA co-elute, which most affinity ligands do, it is of course possible to remove this type of DNA.

Q Finally, do any of the presenters feel there will be a need for AAV production by continuous manufacturing process, inclusive of both upstream and downstream processes? If yes, would the Flex-ready platform provide a potential way to complete any continuous purification?

ML: This is a good question, and is debated quite a lot in the industry currently. The answer is yes, I think continuous manufacturing could have a place in these processes. Of course, the virus is relatively stable, so I don't think you necessarily have to have continuous due to the stability of the virus. But you can reduce the size of the bioreactor, so there could be an advantage here.

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PG: I think that continuous has potential. It may be other processes that are simpler to make in a continuous mode like lentiviruses that are being excreted, and also exosomes. Eventually, I think AAV may also move in that direction.

ML: Pete makes a good point – I think it is mainly the secreted serotypes that will be suitable for continuous manufacturing.

Some years ago, Cytiva's custom resin organization was contacted by a leading CMO within the field of plasmids, Cobra Biologics, with a request to co-develop a resin allowing for higher productivities. Under the terms of the collaboration, the Cytiva team would develop different prototype resins for testing based on existing base matrixes and ligands at Cytiva, and Cobra would give valuable input on how these prototypes worked in their processes, and what the specification could or should be to satisfy both parties.

BIOGRAPHY

Henrik Ihre, PhD
Director Strategic Technologies, Cytiva

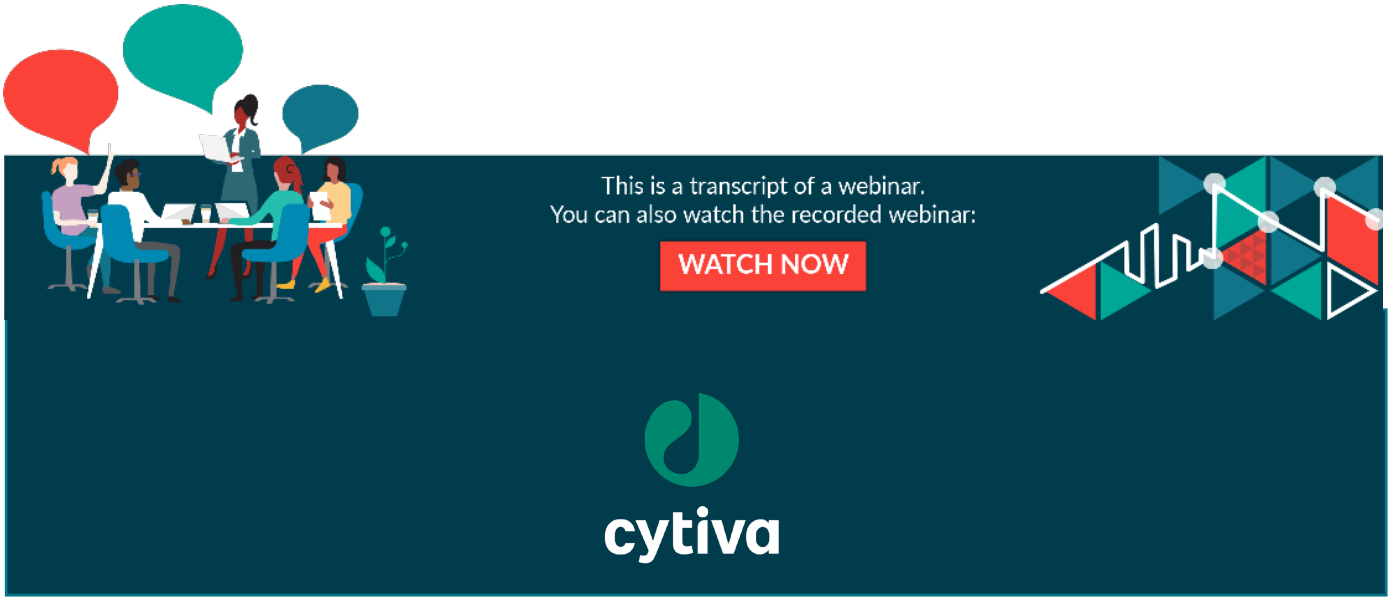
Henrik is Director of Strategic Technologies at Cytiva, with a specific focus on Downstream purification of biopharmaceuticals. He is based in Uppsala. Henrik earned his Ph.D. in synthetic organic polymer chemistry from the Royal Academy of Engineering (KTH), Sweden and post-doc training from UC Berkeley in California, USA. He joined Amersham Pharmacia Biotech in August 2000, which was acquired by by GE Healthcare in 2004. He has devoted his career to the design and development of several purification products enabling the manufacturing of life changing drugs on the market. Henrik started as a Senior Scientist in the R&D chromatography resin department and later transferred into the role of Product Manager for several key products such as the Protein A portfolio. For more than ten years, he headed and expanded the custom consumables organization offering customization within segments such as Chromatography resins, Ready To Process columns, Small scale pre-packed columns and Primer supports for oligo synthesis. During his time in the custom consumables organization, he made valuable contributions to product design and development, bringing more than 30 key products to the market, which now are a part of the Cytiva downstream offering.

Peter Guterstam, PhD
Product Manager, Next Generation Resins & Technologies, Cytiva

Peter is Global Product Manager at Cytiva with responsibility for products tailored for downstream processing of Advanced Therapy Medicinal Products. He is based in Uppsala, Sweden. Peter earned is PhD in Neurochemistry from Stockholm University in 2009. He has been with GE since 2003, primarily working in various roles associated with oligonucleotide synthesis and purification. Since GE's acquisition of Puridify, Peter has the business responsibility to develop products based on the Fibro technology to complement Cytiva's portfolio of chromatography resins and to develop it further to generate tailored solutions for the manufacture of Advanced Therapy Medicinal Products.

Mats Lundgren, PhD
Customer Applications Director, Life Sciences, GE Healthcare, Sweden

Mats has more than 25 years of experience in the field of biotechnology. He holds a PhD in Immunology, Cell and Molecular Biology from the Karolinska Institute, Stockholm, Sweden and extensive post-doc training at the MRC Clinical Sciences Centre, Imperial College School of Medicine in London, UK. In his industrial career Mats has had positions as scientist, team manager and VP at Pharmacia, AstraZeneca and smaller biotech companies. In his previous function, Mats was managing both the Cell line and Upstream Process Development teams at a major biotech company. In his current role, Mats works across different viral vector and vaccine application projects as well as general upstream topics, focusing on customer support, applicability of new technologies and manufacturing solutions.



AUTHORSHIP & CONFLICT OF INTEREST

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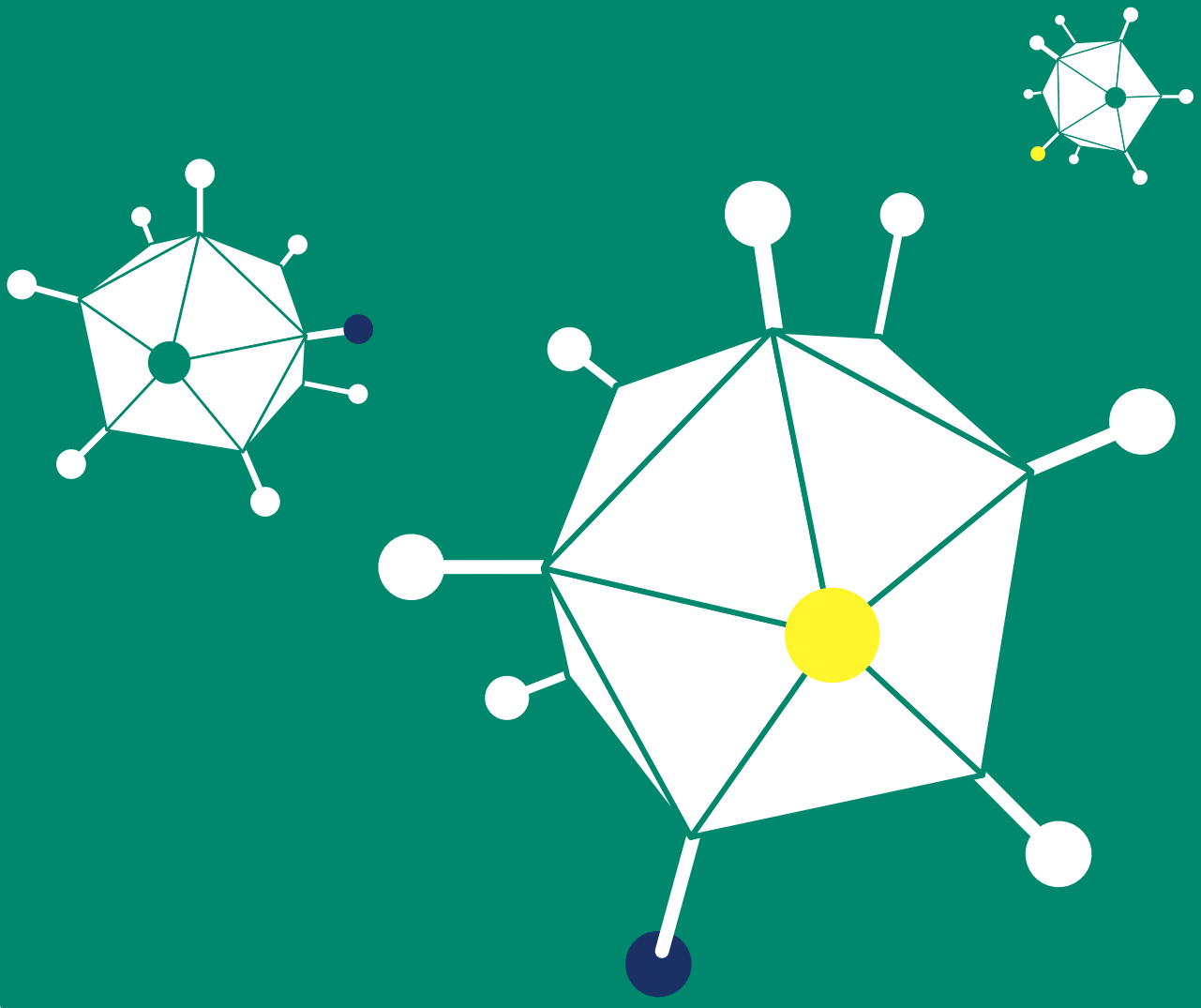

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MARCH 2021 Volume 7, Issue 3

CELL & GENE THERAPY INSIGHTS

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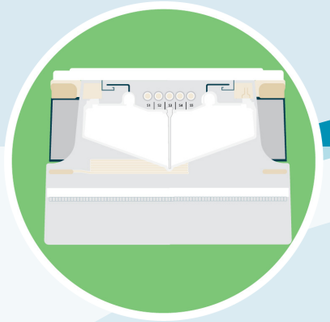
A demonstration of the Cocoon® platform: a bespoke solution to minimize manual touchpoints in cell therapy manufacturing

The Cocoon® Platform streamlines patient-scale cell therapy manufacturing by minimizing manual touchpoints. Highly automated while still maintaining flexibility during process development, the platform gives you increased control over your specific process, resulting in greater consistency, lower batch failure, and reduced costs, while providing higher quality product.

This demonstration shows you the handful of steps that require manual operations, although most of the process occurs automatically. You will see that even the manual touchpoints can be done quickly and effortlessly. To see the full video, please see our FastFacts demonstration here or to learn more about the Cocoon® Platform, please visit the website.

SAMPLE LOADING



Starting material & reagents

Suspension or adherent cells

Fresh or frozen leukopak

Preload reagents: media, cytokines, growth factors, virus, buffer, anti-bodies, activation reagents, other (as required)

ACTIVATION

Activation

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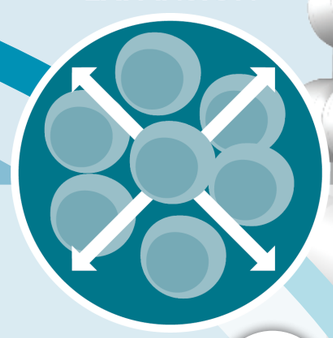
EXPANSION



Expansion

Suitable for T cells, dendritic cells, NK cells iPSC, HSC, MSC

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Active perfusion

Final formulation

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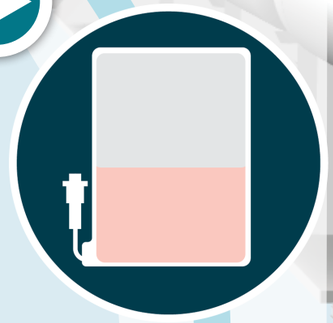
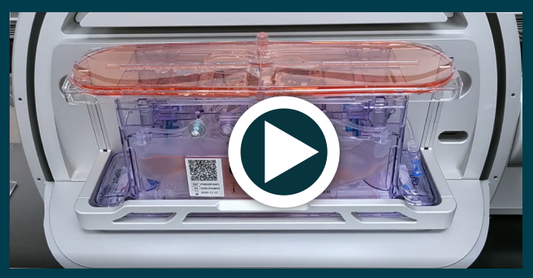
HARVEST



Watch the entire FastFacts demonstration of the Cocoon platform here:



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March 2021

Regulatory Insights

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March 2021
Volume 7, Issue 3

REGULATORY PERSPECTIVE

INTERVIEW

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Regulatory Insights

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REGULATORY PERSPECTIVE

Current status and future perspective of gene therapy products in Japan

Yoshiaki Maruyama, Akira Sakurai, Masaki Kasai, Shinichi Noda & Futaba Honda

Six years have passed since the Pharmaceutical Affairs Law was revised and renamed the Pharmaceuticals, Medical Devices and Other Therapeutic Products Act (PMD Act), which came into force in November 2014. As of January 2021, 10 regenerative medical products have been approved. Research and development of regenerative medical products (cell and gene therapy products) is accelerating, especially for gene therapy products. The development of *ex vivo* gene therapy products active in Japan and overseas, and commercialization has become a reality through products such as KYMRIAH® and YESCARTA®. In addition, the development of *in vivo* gene therapy products such as the viral vector product, ZOLGENSMA®, and plasmid vector product, COLLATEGENE®, has also become a reality. We believe that the development of gene therapy products will become more and more active. This review describes the current status and future perspective of gene therapy products from experiences of the Pharmaceuticals and Medical Devices Agency (PMDA).

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REGULATORY FRAMEWORK FOR GENE THERAPY PRODUCT UNDER PMD ACT

Regenerative medical products in PMD Act are defined as:

1. Processed (any processed cell or tissue, such as propagation and/or differentiation, production of a cell line, activation of a cell by pharmaceutical or chemical treatment, alteration of a biological characteristic, combination with a non-cellular component, and manipulation using genetic engineering, with the aim of preparing desired cell products to treat a patient or repair or regenerate tissue. Isolation of a tissue, homogenization of a tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by γ-irradiation or other methods, freezing, thawing, and other such procedures that are regarded as minimal manipulations are not considered "processed.") (more than minimal manipulation) live human/animal cells that are intended to be used for either:
   - The reconstruction, repair, or formation of structures or functions of the human body; or
   - The treatment or prevention of human disease
2. Gene therapy

'Gene therapy products' in this review mean '*in vivo* gene therapy products' (such as adeno-associated virus (AAV)-based products) and '*ex vivo* gene therapy products' (i.e. genetically modified human cell therapy products, such as chimeric antigen receptor [CAR]-engineered T cells) among regenerative medical products.

Consultation & clinical trials notification for gene therapy products

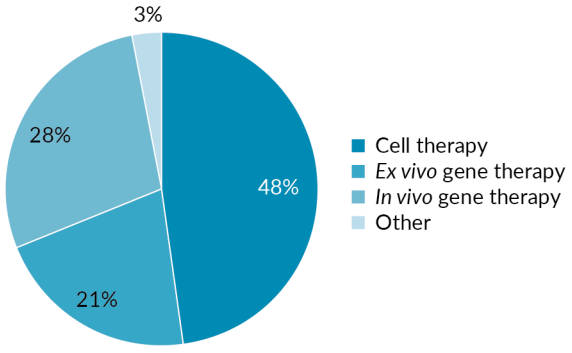
In order to achieve realization of innovative pharmaceuticals, medical devices, and regenerative medical products originating from Japan, PMDA launched the Regulatory Science

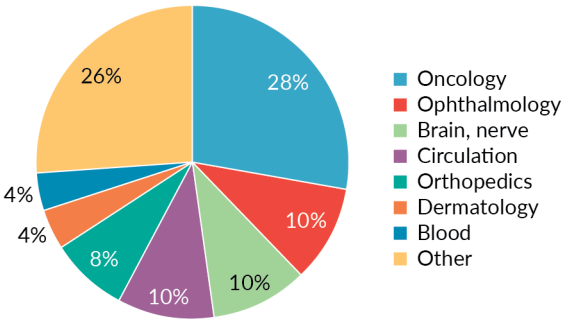
TABLE 1

Number of RS strategy consultations (R&D) for regenerative medical products.

	FY 2014	FY 2015	FY 2016	FY 2017	FY 2018	FY 2019	Total
Quality and safety	18 (44)	29 (55)	26 (64)	29 (71)	25 (54)	29 (53)	156 (341)
Clinical	2	11	14	13	5	11	56
Total	46	66	78	84	59	64	397

This consultation category includes consultations conducted as Pharmaceutical Affairs Consultations on R&D Strategy on and before November 24, 2014. Some consultations were divided into multiple sessions over several days to confirm the quality and safety of the relevant products before submission of clinical trial notifications. The tables in brackets indicate the total number of these sessions.





*Areas of 3% or less were included in other.

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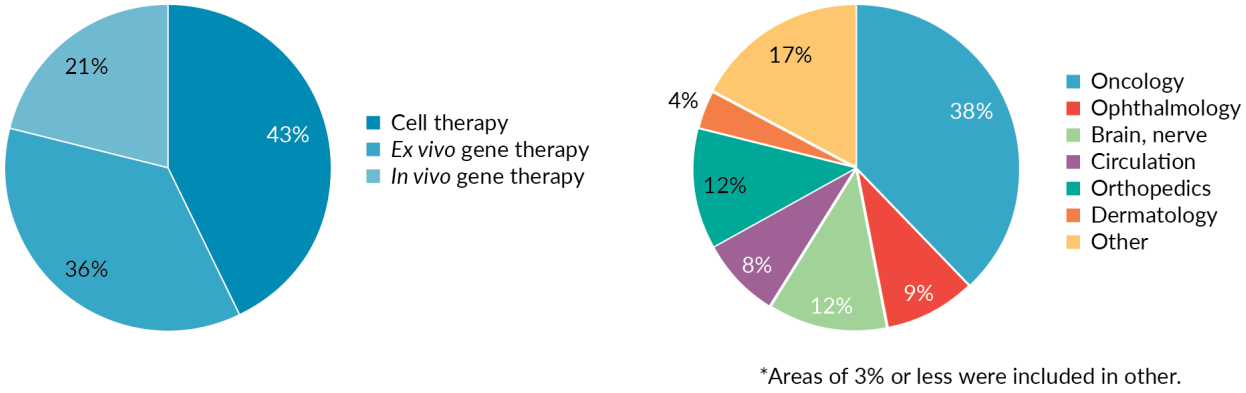
TABLE 2

Number of clinical trial notifications for regenerative medical products.

	FY 2014	FY 2015	FY 2016	FY 2017	FY 2018	FY 2019	Total
Initial clinical trial notification	3 (1)	10 (2)	16 (7)	13 (8)	18 (8)	13 (7)	73 (33)
After second time clinical trial notification	1 (1)	3 (2)	5 (0)	14 (10)	17 (3)	16 (7)	56 (23)
Protocol change notification	2	19	52	93	151	206	523

The table in brackets in parentheses indicate the number of notifications of 'investigator-initiated clinical trials'. The number within the bracket is included in the number outside the bracket.



*Areas of 3% or less were included in other.

(RS) Strategy Consultations (R&D – formerly Pharmaceutical Affairs Consultations on R&D Strategy), mainly for universities, research institutions, and venture companies that possess promising 'seed-stage' research or technologies. In such consultations, advice will be provided on the tests needed in the early development stage and the requirements for starting clinical trials. For regenerative medical products, RS strategy consultations (R&D) on quality (e.g. viral safety, microbial contamination, specification) and safety (e.g. general toxicity, tumorigenicity if applicable) is a mandatory consultation to initiate clinical trial because there are many items to be confirmed in the initial clinical trial notification. Table 1 shows the number of RS strategy consultations (R&D) since 2014. Approximately 40% of consultations are from universities, research institutions, and venture companies. Pie charts of the product categories (cell therapy products, *ex vivo* gene therapy products, *in vivo* gene therapy products and others [biologics raw and ancillary materials]) and area of disease in RS strategy consultation (R&D) are also shown in Table 1. 47% of the consultations are gene therapy products. The development status by disease tends to be largest in the oncology area.

Table 2 shows the number of clinical trials notifications since 2014 as well as pie charts of the product categories (cell therapy products, *ex vivo* gene therapy products and *in vivo* gene therapy products) and area of disease. 57% of initial clinical trials conducted in Japan are for gene therapy products. The development status by disease also tends to be largest in the oncology area. Approximately 80% of initial clinical notifications are with Japanese patients only, with the remaining initial clinical notifications being multiregional clinical trials also including Caucasian patients. Even for cell and gene therapy products, it is necessary to explain the efficacy and safety of the Japanese and the Japanese medical environment with reference to the intrinsic and extrinsic ethnic factors shown in "Ethnic factors in the acceptability of foreign clinical trial data (ICH-E5)" [1]. Information on clinical trials related to regenerative medical products

being conducted can be viewed on the clinical research information portal site [2,3]. The CAR-T/TCR-T cell products among *ex vivo* gene therapy products and *in vivo* gene therapy products registered in "Japic Clinical Trials Information" are shown in Tables 3 & 4, respectively.

Orphan designation

Regenerative medical products can be designated as orphan regenerative medical products based on the PMD Act if they are intended for use in less than 50,000 patients in Japan, and for which there is a high medical need. They are designated by the Minister of Health, Labour and Welfare (MHLW) based on the opinion of the Pharmaceutical Affairs and Food Sanitation Council (PAFSC). As of January 2021, 12 cell therapy products, 8 *ex vivo* gene therapy products and 4 *in vivo* gene therapy products have been assigned orphan regenerative medical products designation.

Review of gene therapy products

As of January 2021, 10 regenerative medical products have been approved in Japan. Four of 10 approved products are gene therapy products (Table 5). Their assessments are summarized in each review report published and freely available on the PMDA website [4].

In response to the "Basic Policy on Economic and Fiscal Management and Reform 2016" (adopted by the Cabinet on June 2, 2016) recommending the promotion of optimal use of innovative pharmaceuticals, the MHLW decided to develop product-specific Optimal Clinical Use Guidelines. PMDA cooperated with MHLW in developing the Optimal Clinical Use Guidelines. Optimal Clinical Use Guidelines for gene therapy products have been prepared for KYMRIAH® [5]. In addition, when it is necessary to determine the requirements of medical institutions and doctors necessary for using the product, the MHLW may request the related academic societies to create a guideline for proper use and publish it. Proper use guidelines for gene therapy product have been prepared for ZOLGENSMA® [6].

Regulation of LMOs

The Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No. 97 of 2003. The Cartagena Act) aims to ensure biodiversity in an international cooperation. The Cartagena Protocol on Biosafety to the Convention on Biological Diversity is an international agreement which aims to ensure the safe handling, transport and use of living modified organisms (LMOs) resulting from modern biotechnology that may have adverse effects on biological diversity, also taking into account risks to human health. It came into effect in 2014 as a law to properly operate the Cartagena Protocol on Biosafety to the Convention on Biological Diversity by taking measures to regulate the use of LMOs. The Cartagena Act is a law concerning the use of LMOs in Japan and covers the use of LMOs such as pharmaceuticals in Japan. 'Approval' by the competent ministers (MHLW and Minister of the Environment) is required when attempting Type-1 Use (no measures are taken to prevent the release of LMOs to the environment). In addition, 'confirmation' by the competent ministers (MHLW) is required when attempting Type-2 Use for industrial use (intend to use with taking the diffusion prevention of LMOs into the air, water, or soil outside facilities, equipment, and other structures indicated in Article 2, Paragraph 6 of the Act) (Table 6). When developing LMOs such as viral vectors, confirmation with the MHLW regarding the Type-2 Use is required before the start of the manufacturing. It is necessary to obtain the approval of the MHLW regarding the Type-1 Use at the medical institution before the start of the clinical trial.

PMDA conducted reviews of Type-1 Use and Type 2 Use under the Cartagena Act.

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TABLE 3

Clinical trials for CAR-T/TCR-T cell products in Japan.

Generic name, etc.	Title of the study	Target diseases	Primary sponsor	Identical number
CD19				
TBI-1501	A Multicenter Phase 1/2 Study for Relapsed or Refractory CD19+ B-acute Lymphoblastic Leukemia	A Multicenter Phase 1/2 Study for Relapsed or Refractory CD19+ B-acute Lymphoblastic Leukemia	Takara Bio Inc.	JapicCTI-173565 NCT03155191
Tisagenlecleucel	Phase 2 Open Label Trial to Determine Safety & Efficacy of Tisagenlecleucel in Pediatric Non-Hodgkin Lymphoma Patients (BIANCA)	Non-Hodgkin lymphoma	Novartis Pharmaceuticals	JapicCTI-194781 NCT03610724
	Phase 3 B in Acute Lymphoblastic Leukemia	Acute lymphoblastic leukemia		JapicCTI-184039 NCT03123939
	Efficacy and Safety of Tisagenlecleucel in Adult Patients With Refractory or Relapsed Follicular Lymphoma (ELARA)	Follicular lymphoma		JapicCTI-194610 NCT03568461
	Tisagenlecleucel vs Blinatumomab or Inotuzumab for Patients With Relapsed/Refractory B-cell Precursor Acute Lymphoblastic Leukemia (OBERON)	Acute lymphoblastic leukemia		JapicCTI-194622 NCT03628053
	Tisagenlecleucel in Adult Patients With Aggressive B-cell Non-Hodgkin Lymphoma (BELINDA)	Non-Hodgkin lymphoma		JapicCTI-194897 NCT03570892
	Study of Out of Specification for Tisagenlecleucel	B-cell acute lymphoblastic leukemia Diffuse large B-cell lymphoma		JapicCTI-194988 NCT04094311
	CAR-T Long-Term Follow Up (LTFU) Study (PAVO)	Long-term Safety of Patients Receiving CAR-T in an Eligible Clinical Trial or Managed Access Program		JapicCTI-205095 NCT02445222
S68587/UCART19	Phase 1, Open Label, Dose-escalation Study Followed by a Safety Expansion Part to Evaluate the Safety, Expansion and Persistence of a Single Dose of UCART19 (Allogeneic Engineered T-cells Expressing Anti-CD19 Chimeric Antigen Receptor), Administered Intravenously in Patients With Relapsed or Refractory CD19 Positive B-cell Acute Lymphoblastic Leukemia (B-ALL)	Adult relapsed/refractory B-ALL	Nihon Servier	JapicCTI-195059 NCT02746952
JCAR017	Trial to Determine the Efficacy and Safety of JCAR017 in Adult Subjects With Aggressive B-Cell Non-Hodgkin Lymphoma (TRANSCENDWORLD)	Lymphoma, Non-Hodgkin	Celgene	JapicCTI-184129 NCT03484702
	A Study to Compare the Efficacy and Safety of JCAR017 to Standard of Care in Adult Subjects With High-risk, Transplant-eligible Relapsed or Refractory Aggressive B-cell Non-Hodgkin Lymphomas (TRANSFORM)			JapicCTI-194718 NCT03575351
	A Study to Evaluate the Efficacy and Safety of JCAR017 in Adult Subjects With Relapsed or Refractory Indolent B-cell Non-Hodgkin Lymphoma (NHL) (TRANSCEND FL)			JapicCTI-205250 NCT04245839
Axicabtagene ciloleucel	A Phase 2 Multicenter, Open-label, Single-arm Study of KTE-C19 in Japanese Patients with Refractory or Relapsed Large B Cell Lymphoma	Refractory or relapsed (relapse after transplant or relapse after medication in patients ineligible for transplant) diffuse large B cell lymphoma (DLBCL), primary mediastinal B cell lymphoma (PMBCL), transformed follicular lymphoma (TFL) or High-grade B cell lymphoma	DAIICHI SANKYO Co.,Ltd.	JapicCTI-183914
CD20				
CART001	Phase 1 study of FCAR-CD20 therapy in patients with refractory CD20-positive B-cell non-Hodgkin lymphoma	CD20-positive B-cell non-Hodgkin lymphoma	National Cancer Center Hospital East	JapicCTI-194764
GPC3				
TAK-102	A Study of TAK-102 in Adult Patients With GPC3-Expressing Previously Treated Solid Tumors	Solid tumors	Takeda Pharmaceutical Company Limited	JapicCTI-205300 NCT04405778
BCMA				
JNJ-68284528	A Phase 1b-2, Open-Label Study of JNJ-68284528, A Chimeric Antigen Receptor T-Cell (CAR-T) Therapy Directed Against BCMA in Subjects with Relapsed or Refractory Multiple Myeloma	Multiple myeloma	Janssen Pharmaceutical K.K.	JapicCTI-195037 NCT03548207
	A Phase 3 Randomized Study Comparing JNJ-68284528, a Chimeric Antigen Receptor T cell (CAR-T) Therapy Directed Against BCMA, versus Pomalidomide, Bortezomib and Dexamethasone (PVd) or Daratumumab, Pomalidomide and Dexamethasone (DPd) in Subjects with Relapsed and Lenalidomide-Refractory Multiple Myeloma			JapicCTI-205280 NCT04181827
bb2121	Efficacy and Safety Study of bb2121 in Subjects With Relapsed and Refractory Multiple Myeloma (KarMMa)	Multiple myeloma	Celgene	JapicCTI-184195 NCT03361748
	Efficacy and Safety Study of bb2121 Versus Standard Regimens in Subjects With Relapsed and Refractory Multiple Myeloma (RRMM) (KarMMa-3)			JapicCTI-194719 NCT03651128
NY-ESO-1				
TBI-1301	Study of TBI-1301 (NY-ESO-1 T Cell Receptor Gene Transduced Autologous T Lymphocytes) in Patients With Synovial Sarcoma	Synovial sarcoma	Takara Bio Inc.	JapicCTI-173514 NCT03250325

Source: [12].
Investigational material; Regenerative Medical Products.

TABLE 4

Clinical trials for *in vivo* gene therapy products in Japan.

Generic name etc.	Title of the study	Target diseases	Primary sponsor	Identical number
Ad-SGE-REIC	A Phase 2 study of Ad-SGE-REIC	Malignant pleural mesothelioma	Kyorin Pharmaceutical Co.,LTD	JapicCTI-184040
TBI-1401(HF10)	A Study of Combination With TBI-1401(HF10) and Ipilimumab in Japanese Patients With Unresectable or Metastatic Melanoma	Patients with Stage IIIB, IIIC or IV unresectable or metastatic malignant melanoma	Takara Bio Inc.	JapicCTI-173591 NCT03153085
	Phase 1 Study of TBI-1401(HF10) Plus Chemotherapy in Patient with Unresectable Pancreatic Cancer	Patients with Stage III or IV unresectable pancreatic cancer		JapicCTI-173671 NCT03252808
Beperminogen perplasmid	A Phase 3 study of AMG0001 in Fontaine grade III patients for PAD	Chronic arterial obstruction	AnGes, Inc.	JapicCTI-195088
OBP-301	Phase 1 Study of Combination Therapy with OBP-301 and Chemoradiotherapy in Patients with Locally Advanced Esophageal Cancer	Esophageal cancer	Chugai Pharmaceutical Co., Ltd.	JapicCTI-205294
	Phase 2 Study of Combination Therapy with OBP-301 and Radiotherapy in Patients with Locally Advanced Esophageal Cancer			JapicCTI-205125
Onasemnogene abeparvovec-xioi	Pre-Symptomatic Study of Intravenous Onasemnogene Abeparvovec-xioi in Spinal Muscular Atrophy (SMA) for Patients With Multiple Copies of SMN2 (SPR1NT)	Spinal Muscular Atrophy	Novartis Pharmaceuticals	JapicCTI-184203 NCT03505099
	Single-Dose Gene Replacement Therapy Using for Patients With Spinal Muscular Atrophy Type 1 With One or Two SMN2 Copies	Spinal Muscular Atrophy Type I		JapicCTI-194664 NCT03837184
	Long-term Follow-up Study of Patients Receiving Onasemnogene Abeparvovec-xioi	Spinal Muscular Atrophy Type I Spinal Muscular Atrophy Type II Spinal Muscular Atrophy Type III SMA		JapicCTI-205305 NCT04042025
Fidanacogene elaparvovec	A Study to Evaluate the Efficacy and Safety of Factor IX Gene Therapy With PF-06838435 in Adult Males With Moderately Severe to Severe Hemophilia B (BENEGENE-2)	Hemophilia B	Pfizer	JapicCTI-205228 NCT03861273
Voretigene neparvovec	Study of Efficacy and Safety of Voretigene Neparvovec in Japanese Patients With Biallelic RPE65 Mutation-associated Retinal Dystrophy	Biallelic RPE65 Mutation-associated Retinal Dystrophy	Novartis Pharmaceuticals	JapicCTI-205455 NCT04516369

Source: [12].
Investigational material; Regenerative Medical Products.

Table 7 shows the review status since 2014. The target regulatory review times were 6 months for approval of Type-1 Use and 2 months for confirmation of Type-2 Use, with the goal of achieving 50% (median) of applications for each type.

There was a request from developers to review process, such as clarifying the procedure

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TABLE 5

Approved gene therapy products (as of January 2021).

#	Brand name	Non-proprietary name/	Indication or performance	Sponsor and website	Approval date
1	KYMRIAH®	Tisagenlecleucel (CD19 -directed genetically modified human [autologous] T cell)	▶ Patients up to 25 years of age with B-cell precursor acute lymphoblasticleukemia (ALL) that is refractory or in second or later relapse ▶ Adult patients with relapsed or refractory (r/r) large B-cell lymphomaafter two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high grade B-cell lymphoma and DLBCL arising from follicular lymphoma	Novartis Pharma K.K. [13]	26/03/2019
2	COLLATEGENE® Intramuscular Injection 4 mg	Beperminogene perplasmid (Hepatocyte growth factor (HGF) plasmid vector)	The treatment of ulcers in patients with chronic arterial occlusion (arteriosclerosis obliterans and Burger's disease) who have not responded sufficiently to the standard drug therapy and are unable to undergo revascularization	AnGes, Inc. [14]	26/03/2019
3	ZOLGENSMA®	Onasemnogene abeparvovec (recombinant adeno-associated virus serotype 9 [AAV] capsid shell containing the human survival motor neuron [SMN] gene)	Treatment of patients with spinal muscular atrophy (including those with genetically diagnosed presymptomatic SMA) who have negative for anti-AAV9 antibodies	Novartis Pharma K.K. [13]	19/03/2020
4	YESCARTA®	axicabtagene-ciloleucel (CD19 -directed genetically modified human [autologous] T cell)	Adult patients with relapsed or refractory (r/r) large B-cell lymphomaafter two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high grade B-cell lymphoma and DLBCL arising from follicular lymphoma	Daiichi Sankyo Co., Ltd. [15]	22/01/2021

and shortening the time from application preparation to approval or confirmation. In response to this, PMDA has set a new consultation menu related to the Cartagena Act. This consultation is conducted prior to submission of application for Type-1 Use, or submission of application for confirmation of Type-2 Use under the Cartagena Act. The consultation intends to provide guidance and advice regarding sufficiency of data for submission and the appropriateness of description for each individual LMO and for matters specified in regulation on Type-1 Use or Type 2-Use.

FUTURE PERSPECTIVE OF GENE THERAPY PRODUCTS IN JAPAN

Regenerative medical products, which are expected to overcome intractable and serious diseases, are expected to play a role in conventional medicine all over the world. In Japan, although basic research has produced excellent results, it has been said that there are many problems in realization. Against this background, the inconvenience caused by applying the conventional regulations on pharmaceuticals and medical devices to products

TABLE 6

The Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No. 97 of 2003).

Type	How to use	Points for review	Examples
Type-1 (Approval)	Deliberate release: The use of LMO without preventive measures against their dispersal into environment	▶ Environmental risk assessment ▶ Risk assessment for third party	Gene-expression virus vector for human use
Type-2 (Confirmation)	Containment use: The use of LMO while taking preventive measures against their dispersal into environment	Reasonable system according to risks for using of LMO	Use virus vector for gene modification of the cells in manufacturing process

such as regenerative medical products as they are has been eliminated, and a regulatory framework has been established for regenerative medical products. As mentioned in the current status of regenerative medical products, the development of *ex vivo* gene therapy products is active in Japan and overseas. To lay down basic technicalities required to ensure the quality and safety of *in vivo* gene therapy products and gene therapy products among regenerative medical products, related guidelines for gene therapy products have been issued by MHLW/PMDA (Table 8).

To facilitate development of innovative pharmaceuticals, medical devices, and regenerative medical products, MHLW worked with academic and research institutions studying and developing state-of-the-art techniques to develop guidelines that aim to establish procedures for quality and safety assessment based on regulatory science, and also conducted a project on personnel exchange between those institutions and the PMDA or the National Institute of Health Sciences (NIHS) from the 2012 fiscal year (FY) to the 2016 FY. As a result of the project on personnel exchange, various guidelines have been published. On July 2019, "Ensuring the quality and safety of gene therapy products" (PSEHB/MDED Notification No.0709-2, July 9 2019) was issued [7]. This notice is a complete revision of the "Ensuring the Quality and Safety of Gene Therapy Drugs" (PFSB/ELD Notification No. 0701-4, by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated July 1, 2013; hereinafter, the "old notification issued by the ELD Director").In addition, On March 2019, "Guidelines on Cancer Immunotherapy Development" (PSEHB/MDED Notification No.0308-1, March 8 2019) was issued [8].

In addition, PMDA established the Scientific Committee in 2012 as an organization to deliberate on matters related to the scientific aspects of operations such as the examination of pharmaceuticals and medical devices, which has been discussing and compiling opinions on products such as regenerative medical products. The development of

TABLE 7

Review under the Cartagena Act (Median Regulatory Review Time).

	FY 2014	FY 2015	FY 2016	FY 2017	FY 2018	FY 2019
No. of preliminary reviews for Type 1 use	3	2	3	1	7	8
Median review time (months)	0.8	0.9	2.9	2.9	6.0	4.7
No. of preliminary reviews for Type 2 use	25	21	23	17	30	29
Median review time (months)	1.3	1.0	1.3	1.3	1.1	0.9

Note 1: 'Type 1 use' refers to cases where no measures are taken to prevent the release to the environment. 'Type 2 use' refers to cases where such measures are taken.
Note 2: The review time in FY 2014 through FY 2016 represents the time spent for review at PMDA, while that in FY2017 and FY 2018 represents the sum of times spent for review at PMDA and MHLW.

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TABLE 8

Related guidelines for gene therapy products in Japan.

Guidelines	Issued date	References
Ensuring the quality and safety of gene therapy products	July 9, 2019	[7]
Guidelines on Cancer Immunotherapy Development ▶ General principles on late-phase clinical studies ▶ Basic principles in quality, non-clinical and clinical studies of cellular products used for cancer immunotherapy	March 8, 2019	[8]
General principles on late-phase clinical studies	March 8, 2019	[8]
ICH Considerations: General Principles to Address Virus and Vector Shedding	June 23, 2015	[16]
ICH Considerations: Oncolytic Viruses	June 23, 2015	[17]
General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors	June 23, 2015	[18]
Report on Consideration on Quality and Safety of Gene Therapy Products Using Genome Editing Technology (PMDA Scientific Committee)	February 7, 2020	[10]

genome editing technology is energetically promoted as an epoch-making technology that can specifically cleave, modify, and edit a specific gene, and its practical application is expected as a new gene therapy method [9]. Based on the above, on February 2020, the PMDA Scientific Committee published the "Report on Consideration on Quality and Safety of Gene Therapy Products Using Genome Editing Technology" (dated February 7, 2020) [10,11].

In conclusion, gene therapy products account for 47% of RS Strategy Consultations (R&D) which is mandatory consultation for starting clinical trials and 57% of initial clinical trial notifications. In anticipation of active development of gene therapy products in Japan, the regulatory system for the use of LMOs has being strengthened and various guidelines related gene therapy products are being issued.

TRANSLATION INSIGHT

This review has introduced the development trends of gene therapy products after the enforcement of the PMD Act. Regenerative medical products provide innovative medical care centered on products. In order to deliver safer and higher quality of regenerative medical products to the medical field more quickly and contribute to the improvement of medical standards, we would like to work on solving problems through cooperation between patients, industry, government, and academia. We hope that this information will help you to develop products quickly.

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Regulatory Insights

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INTERVIEW

Examining Health Canada's agile new approach to regulating Advanced Therapeutic Products

ELIZABETH TOLLER is an experienced policy specialist and public sector leader with over eleven years' experience working in the Government of Canada. Her passion for health issues and social policy has seen Elizabeth split her time in government between Health Canada, Immigration, Refugees, and Citizenship Canada, and the Privy Council Office. Elizabeth currently serves as the Executive Director for Regulatory Innovation in Health Canada's Health Products and Food Branch, driving the modernisation of Canada's food and drug regulations in support of innovation. Outside of work, Elizabeth is a mother of two and garden enthusiast.

LIZ ANNE GILLHAM-EISEN is the Director of the Office of Policy and International Collaboration. As Director, Liz Anne oversees regulatory policy development and stakeholder engagement for Biologics and Radiopharmaceutical Drugs Directorate. She is also responsible for the coordination of international collaboration and capacity building activities. Liz Anne led the policy development for: the *Safety of Human Cells, Tissues and Organs for Transplantation Regulations* (2007); the *Blood Regulations* (2014); the regulations under the *Assisted Human Reproduction Act* (2019); and the *Interim Order Respecting the Importation, Sale and Advertising of Drugs for Use in Relation to COVID-19* (2020). Liz

Anne has published several articles on the organ and tissue donation process as well as on safe donor screening and has co-authored book chapters on the regulation of cells, tissues and organs and advanced cellular therapies.

NADINE KOLAS is a senior policy analyst and former researcher with expertise in reproductive biology and early mechanisms of cancer development using mouse models and human cell genomics approaches. After completing her PhD in molecular genetics from Albert Einstein College of Medicine in New York City, she conducted post-doctoral work at the Lunenfeld-Tanenbaum Research Institute in Toronto where she discovered novel genes that modulate DNA repair. Her work has been published in journals including Science and Cell, she has held fellowships from CIHR and the Terry Fox Research Institute, and won the 2008 Polanyi Award for medicine/physiology from the Ontario government. Shifting her focus from research to knowledge translation, she turned down an offer to start her own lab in favour of policy with Health Canada and has been the senior policy analyst for advanced cell therapies in Health Canada's Health Products and Foods Branch since the beginning of January 2017. Prior to joining the Branch she worked with an expert panel, led by David Naylor, to review the federal ecosystem supporting fundamental research in Canada; and developed policy for knowledge translation and research governance for management of Health Canada's regulatory research programs including chemicals, nanomaterials, Northern contaminants and air pollution.

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Q What are you each working on right now?

ET: The Health Products and Food Branch (HPFB) is the regulator of health products and food within Health Canada, and we are also the largest Branch in Health Canada with over 2,000 employees. We cover a wide area – we are the group that reviews and approves drugs and medical devices, including those for COVID-19, but we also look at the vitamins available at local pharmacies in Canada and develop nutrition-related guidance.

Our current regulations have served us well for many years, but the Branch has done a lot of work over the years on developing foresight, to ensure we're focused on the key change drivers on the horizon. We've also done extensive review of regulations to see what the bottlenecks and challenges to innovation are within our regulatory system and find ways in which we can modernize. Based on this work, we launched an ambitious Regulatory Innovation Agenda in 2019, focused on making our regulations even more agile and responsive to innovation without compromising safety, efficacy, and quality.

My principal role is to shepherd the overall implementation of that initiative, which is Branch-wide, and involves 14 different projects across health products and also on the food side. For the first 6 months after the pandemic hit, I was 100% dealing with stakeholder

communications and engagement for COVID-19. However, since the summer, I have been wearing two hats, going back to my regulatory innovation role while also continuing to support COVID-19 engagement.

LG: The Office of Policy and International Collaboration, within the Biologic and Radiopharmaceutical Drugs Directorate (BRDD), sits within the Health Products and Food Branch. We specifically regulate biologics and radiopharmaceuticals, which of course includes cell and gene therapies. We are responsible for developing the policy, working with stakeholders, and also the guidance documents and interpretation of regulations specifically around biologics and radiopharmaceuticals.

We are heavily involved in working with Elizabeth's team. One of the pillars that Elizabeth will be talking about is the Advanced Therapeutic Product (ATP) pathway. I have a unit within my group working to help implement that pathway. It is worth noting that it is not restricted solely to biologics, but rather it is a pathway for any innovative product that cannot be regulated effectively through our current set of regulations.

We also regulate vaccines, so similarly to Elizabeth, since COVID-19 appeared we have been very much entrenched in working on the regulation of vaccines, as well as treatments such as biologic monoclonal antibodies and a convalescent plasmid for COVID-19.

At the very beginning of the COVID-19 pandemic, we took on some of the initial policy leadership and communications for a number of months, and then pivoted almost entirely to working on supporting the COVID-19 response. We developed the Interim Order to ensure Canadians could have access to treatments and vaccines for COVID-19 as early as possible. At the moment, we are mainly focusing on the vaccine submissions within BRDD, but we have been able to move back to some of these really important priority files, such as the Regulatory Innovation Agenda and the ATP pathway. We have teams that are concentrating on moving those pathways and projects forward.

NK: I work under Liz Anne in her policy shop, with expertise in cell and gene therapies.

I am essentially the eyes and ears on the ground for these products. I keep abreast of issues facing cell and gene therapies so that I can make recommendations on potential approaches that we might take to reduce challenges to innovation and patient access to safe and effective products in this area. I hold the pen on writing the cell and gene therapy related policy and guidance that Liz Anne mentioned, often by convening panels of other internal experts. I also support product classification by working with the Therapeutic Product Classification Committee where we discuss products that often sit at the intersection between two or more sets of regulations. It's often in dealing with complex classification issues where we see some of the regulatory challenges facing certain innovative products. When we see challenges, we identify options to address them. We frequently use policy-based approaches until regulatory changes become necessary.

I also do a lot of outreach with our various stakeholders to stay on top of their issues. Many of our stakeholders tend to be academics, as a lot of innovation for cell and gene therapies comes out of the academic space, and they tend to be new to the regulatory sphere. We maintain strong connections with our stakeholders – I tend to give a lot of talks at conferences and workshops, for example. People are often looking for some guidance because they are trying to navigate the

regulations, but they are not large pharmaceutical companies that have entire regulatory affairs departments. As academics, they often struggle with how to bring their products out of the lab and into the regulatory sphere, so we work to support that. I also work with our US FDA, EMA, and Japanese PMDA colleagues on different regulatory harmonization initiatives around cell and gene therapies as another mechanism to reduce regulatory challenges to innovation.

Q How would you describe the evolution of Health Canada's regulatory framework for Advanced Therapeutic Products over recent years – what have been the key drivers or points of focus?

ET: Our Department has gone through several iterations of modernization over the years, but one of our most important regulatory change initiatives has been the Regulatory Review of Drugs and Devices, which started around 2017. This is focused on enabling better access to health products through improved alignment of the partners in our own health care system, and through collaboration with our international partners.

Since 2017, our Department has done a lot of foresight work. What I mean by that is horizon scanning, and talking to different companies and stakeholders, both traditional and non-traditional – our innovation hubs, for example – to find out what is on the horizon over the next 5–10 years. What do we need to be paying attention to? At the same time, our Government launched a significant regulatory review of the health and biosciences sector and the agri-food sector to understand the roadblocks to innovation in each one.

Based on the foresight exercise and on that regulatory review, we heard loud and clear from stakeholders that we needed more agility in our regulations to be able to stay on pace with the innovation that is occurring, particularly as medicines are becoming more complex, more precise, and more personalized. We can't have regulations that are a one-size-fits-all approach, and which are overly prescriptive. They need to be more flexible and more internationally aligned. They also need to have the ability to be a bit more future-proofed because regulations take years to update, generally speaking.

It was those forces, those exercises that set the course for our latest vision for modernization, which is the aforementioned Regulatory Innovation Agenda. This is comprised of several distinct projects, all of which are aimed at making regulations more agile across the lifecycle of the products we regulate, starting from modernizing the way we regulate clinical trials, all the way through to how we license them. It includes the new pathway for ATPs.

Part of our vision is to create a 'regulatory sandbox'. This is for products that are so unique and complex, such as ATPs, that they don't fit into our system and we can't regulate them effectively through our regular pathways. This notion of regulatory sandboxing is, in the simplest terms, an ability to set tailored oversight requirements in order to be able to address the unique characteristics of these products. For example, perhaps we have a cell and gene therapy that is manufactured at the point-of-care, which is very different to what we are traditionally used to with something made in a normal manufacturing company setting. Or in the AI space, we may have something AI-enabled that uses machine learning and the algorithms are constantly changing. That is very difficult for us to regulate within our existing regulations.

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"...things are changing. The ATP pathway and regulatory sandboxing represents a very collaborative approach where we would be working not only with our internal experts, but also going out externally, looking at those who have developed the technology, and gaining an understanding of how it is regulated internationally."

LG: In terms of evolution of our drug regulations, we have had some changes over the years, for example the new provisions under Vanessa's Law to protect Canadians from unsafe drugs, and significant undertakings such as the Regulatory Review of Drugs and Devices. However, the sandbox is a really important tool we have developed – it is an innovative tool to deal with very innovative products. We are very excited to be able to offer this as a potential pathway.

We have been regulating cell and gene therapy products for a number of years. Our first gene therapy clinical trial was authorized back in 1994 under our *Food and Drug Regulations*, and we have approved a number since. But as time goes on, things are changing. The ATP pathway and regulatory sandboxing represent a very collaborative approach where we would be working not only with our internal experts, but also going out externally, looking at those who have developed the technology, and gaining an understanding of how it is regulated internationally.

We do of course require evidence that any given product is safe and efficacious and for some ATPs, there may not be a clear clinical trial pathway. In those instances, we have several options available to us within this particular pathway. We can look at a market authorization or an individual license, or there is also an order of permission, which means we would set the standards to which users would have to adhere to be able to use these products. There may be an attestation that they are adhering to them, or it might be much more like a typical establishment license. These are some of the types of things we are looking at. But again, it would only be for those products that cannot be regulated through the current regulatory pathways.

ET: To build on what regulatory sandbox means, we call it a sandbox because the intent is to bring all the right people together – not only in our own regulatory sphere, but with our health technology assessors as well – to think about what the right requirements are, and to consider what evidence they might need to make downstream decisions.

It also involves the users and payers, as we really want it to be a network of actors that come together from the get-go to figure out the requirements and work things out in a very collaborative way. This can make the regulatory, reimbursement, and health system integration processes

"...particularly for some therapies, such as ones manufactured at the bedside, it will be more difficult to use the traditional pathway."

more efficient for sponsors, thus supporting faster medicines access for Canadians. Then, we can essentially validate the regulation of these complex novel products and adjust as we go. We can apply terms and conditions on the authorizations we issue through this pathway, allowing us the leeway to adjust the licensing requirements as we learn from real-world experience.

The intention is to learn from the sandbox experience over time, amend or create new regulations based on what you have learned, and then remove the given product from the sandbox environment. It is an iterative, collaborative experience of regulating, and learning as we adapt our regulations.

LG: When it comes to cell and gene therapies specifically, we are looking at an inventory of therapies down the road that could be candidates. And particularly for some therapies, such as ones manufactured at the bedside, it will be more difficult to use the traditional pathway.

Q You mentioned the recent need to prioritize COVID-19 vaccines and therapeutics. How has this impacted your Advanced Therapeutic Product-related activities, specifically, and what is the outlook and approach from your point of view in terms of ensuring a minimal amount of momentum is lost for the cell and gene therapy field as a whole during the pandemic?

ET: From the broadest perspective, COVID-19 obviously affected our Department in terms of how it organized itself. We needed to make sure we were doing everything we possibly could to prioritize the review of COVID-19-related submissions of all types, such as vaccines, therapeutic drugs, testing devices and PPE, as well as sanitizers and disinfectants. We put in place a range of agile measures that helped us to speed up our review process without compromising our high standards of safety.

On the vaccine and treatment front we put in place an emergency order, known as an Interim Order, that allowed our reviewers to perform what we call a rolling review. This allows them to take information from companies as it becomes available, as opposed to waiting for the information to arrive at the end. We set ourselves up so that we had several dedicated teams of reviewers all looking at that information in real time, with enough capacity to review many vaccine submissions at once. That is just one example of the many measures that we took to facilitate the various different product lines we regulate.

It is important to emphasize that while we have prioritized COVID-19 submissions, we have simultaneously worked to maintain our performance standards to the best of our abilities so that Canadians maintain their access to the other types of important medication they need.

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It is also important to mention that although this will have had an impact on the timing of our modernization work, it also served as a pilot project that let us 'test drive' many of the agile ways of regulating that we are trying to push forward in our modernization. For example, using agile tools like terms and conditions is a common theme in nearly every project that we are doing, whether it be clinical trial modernization and ATP pathways, or the new licensing products we are advancing.

The COVID-19 experience really confirmed for us the importance of proactive and accessible outreach to and engagement with stakeholders, particularly as we were dealing with so many new and different kinds of stakeholders who weren't used to meeting our regulations. For example, companies re-tooling to create personal protective equipment; distilleries making hand sanitizers. There was a significant effort by our Branch to get out there proactively and talk to any company interested in making a submission, and to make sure they understood the Canadian regulatory conditions we had put in place. It was crucial that we understood what they were doing and what their problems were, so we could provide advice, connect them to other federal partners where necessary, and make sure we were supporting them as much as possible. All this effort was to help make sure Canadians were getting access to the products at the same time as, or even before, other countries that have much bigger market shares than us.

So in a way, everything we have done during COVID-19 has allowed us to maintain a lot of momentum on our modernization journey.

LG: We have met all performance standards for cell and gene therapies in 2020. The pandemic hit us in March, but we still authorized 27 clinical trials for cell and gene therapies in that calendar year, all within our regulated time frame of 30 days or less, compared to 30 in 2019. Interestingly enough, we have approved 3 clinical trial applications (CTAs) for cell therapies for treatment of symptoms related to COVID-19, so it has actually brought in additional types of cell therapy CTAs spurred by the need for COVID-19 treatments.

It hasn't been without a great deal of effort. We have had to move people off other projects, and an extreme number of hours and a lot of overtime have been put in to ensure we are meeting our performance standards, as well as addressing the COVID-19 pandemic.

From the policy point of view, we were hoping to pilot a product through the ATP pathway a little sooner and because we had to move away from that work to some extent, we have yet to determine the product we are piloting. But again, we have a team in place now and with the support of Elizabeth's team, we are once again looking to identify a pilot product.

Q Looking to the future, how will Health Canada look to continue to evolve its framework for and approach to regulation of Advanced Therapeutic Products?

"...while we have prioritized COVID-19 submissions, we have simultaneously worked to maintain our performance standards to the best of our abilities."

ET: One observation we have made in reflecting on COVID-19 relates to the response to proactive engagement and outreach with stakeholders. There is a nice synergy here with an objective we have in our modernization vision, which is to create a concierge service for stakeholders to help them navigate the complex regulatory system – particularly those who might be eligible to go through the ATP pathway.

We recognize this could likely be SMEs or physician entrepreneurs; innovators that are not equipped with the same strong regulatory affairs departments as traditional bigger pharma companies. We want to have a sort of single-window, helpful service that will allow us to be accessible and available, and to communicate in more simple language.

LG: There are a lot of academic groups out there who are less familiar with the level of evidence needed to bring a product to market, or to bring a product to Canadians and have them be able to access it. Some of the initial workshops or engagements we are planning will be more like information sessions to provide an understanding of how the regulatory system works, what evidence is needed, and how we can move forward together in bringing some of these promising products to market. For example, we are working with those in the autologous cell therapy community, including the particular stakeholder groups wanting to use those products, to provide them a better understanding of how such products are regulated.

A good example of this is that many countries have been concerned with the autologous stem cell products that have been made available. We have clarified that these products are drugs and regulated under the *Food and Drugs Act* and *Regulations*, and as such need evidence of safety and efficacy. We are looking at how we can perhaps work together, and where the evidence threshold should be. Perhaps a future pathway for them could be the ATP pathway, once that evidence threshold has been met.

NK: Just further on the point regarding autologous therapy products: as we were doing the analysis and setting up our position paper, we did of course realize that there would be certain barriers to market access for these products, should they be proven to be safe and effective.

The ATP pathway is very exciting to us because it is going to open up the ability for us to tailor our requirements and overcome barriers in our existing regulations provided products prove to be safe and effective. The goal of good regulation is not to keep good products from patients – it is about trying to make sure that we are dealing with products and their risks appropriately.

Q Can you expand on which specific aspects of cell and gene therapy regulation require a more bespoke, less 'one-size-fits-all' approach, in your view?

LG: A few years back, we recognized that particularly for cell therapies, there were some unique requirements for clinical trials. The sponsors coming forward in this field are not your typical large pharma sponsors. Several years ago, we released a guidance document specific to clinical trials for cell therapies, which has been used quite a bit by our stakeholder groups and has made things a little easier. And as has been mentioned, autologous stem

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cells that are manufactured at the bedside are going to be a challenge, so those will potentially benefit from the ATP pathway.

But again, this will not be for all of these products. For many cell and gene therapies, the current pathway is flexible enough – particularly our clinical trial pathway, which is really well suited to gathering evidence for a number of these particular products in Canada, especially in light of the future amendments we plan to make to it.

> "The ATP pathway ... is going to open up the ability for us to tailor our requirements and overcome barriers in our existing regulations provided products prove to be safe and effective."

NK: There were a few things we anticipated might have been problems, such as highly individualized therapies. With those, we worked with some of the flexibilities we have in the existing *Food and Drug Regulations*, which were built with the intention of being technology agnostic. We have managed to use some of those flexibilities to keep on top of a lot of the innovation – we were able to adapt our lot release program to the highly individualized nature of autologous CAR T therapies, for instance. So although we anticipated problems, by working with the US FDA, EMA, and Health Canada, we now have very harmonized approaches in place for these sorts of issues.

However, we are expecting that there might be some problems when we start looking at things like 3D bio-printers, and some of the new CAR T manufacturing equipment that can be used at the point-of-care. Some of the responsibility for manufacturing these drugs using this decentralized model is going to lie in the hands of the manufacturing equipment provider, but certain equipment will have sufficient flexibility for users to make adaptations that will create different products each with unique risk profiles. How do we distribute the regulation of the risks associated with that? What does it look like? Is it different for each point-of-care manufacturing device? How do we address a machine learning component, if one exists, that can be updated by a third party? The ATP pathway is so incredibly exciting to us because we can see these questions coming, but previously, we were having a difficult time understanding how we were going to address the risks in a reasonable way under our existing frameworks that was not going to impede access to these products.

ET: Within the ATP context, it is also important to recognize that we might have to start working with different players than we are used to – like hospitals, for example – where a lot of these products might actually be made and administered. While we do that already for radiopharmaceuticals, the breadth of products is rapidly changing. That is an important point to stress.

As Liz Anne was saying, our existing regulations are generally well enough adapted to deal with some cell and gene therapies, but we are looking at ways to make those traditional licensing systems we have more flexible and agile. Those tools will go a long way toward supporting products that have some uncertainty around them. This is especially important for questions around efficacy. Terms and conditions are a tool that has been a common feature in all our

interim COVID-19 measures. They are also an agile measure that play a role in most of our longer-term modernization projects. Terms and conditions allow us to be agile and authorize a drug for market while attaching additional conditions or limitations on the authorization, and in so doing help us to regulate more effectively across the product's lifecycle.

LG: Another key element is the use of real-world evidence, both for upfront decision-making (as in some cases, structured clinical trials may not be possible for some of these products) and perhaps even more so, in the medical device area. We have very much been looking at how real-world evidence can be gathered, submitted, and reviewed in the post-market surveillance setting as well. It is certainly an important element we have been talking about for a while and will be looking into further as we move forward.

NK: A lot of issues with cell and gene therapies also overlap with those of rare diseases, so terms and conditions are incredibly valuable to us in that setting, too. It may be completely unrealistic to wait to have something available on the market for broad distribution, if having a large enough dataset will take another 50 years, provided there is sufficient evidence of benefit.

Coming down the pipeline, we have things like software updates and machine learning, and we have to consider how we continue to regulate those without needing a new product submission each time something comes along. Gene editing is another example of this, which is going to be very patient-specific. The ability to keep an eye on these things helps us distribute risk across the product's lifecycle and be more comfortable with emerging products, so that we can allow them out into the market, but keep the ability to introduce more requirements for them as needed.

Q What can you tell us about Health Canada's plans and priorities relating to international regulatory harmonization for the cell and gene therapy space? Are there any key points of regulatory convergence, or divergence, that you have in your sights?

ET: International harmonization is a key tenet of everything we do in our modernization objectives. We recognize the importance of creating conditions here in Canada that make us as competitive as other jurisdictions that have much larger market shares. We represent a small part of the global market, but we still want to make sure our population gets access to the latest and greatest medications that are out there.

To do that, our rules and requirements need to be aligned to avoid situations where there might be undue burden to make a submission here in Canada. We want that

"It may be completely unrealistic to wait to have something available on the market for broad distribution, if having a large enough dataset will take another 50 years."

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convergence to be there as much as possible, while recognizing that we have a unique population and that we are an independent regulator. To this end, we are actively involved in sharing information and resources with the global regulatory community. For example, we are members of several international consortia with other regulators, such as the Access Consortium. Greater convergence with like-minded regulatory partners helps foster synergy to address scientific and regulatory issues, align on regulatory requirements to reduce undue burden, and provide opportunities to implement work-sharing initiatives among regulators. These efforts support our ultimate goal of enhancing Canadians' access to health products by making Canada a more accessible jurisdiction to file applications.

"...we are actively involved in sharing information and resources with the global regulatory community."

LG: The international approach has been a cornerstone for us for a very long time as far as trying to harmonize is concerned. We are very involved in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). We also are very active in the International Pharmaceutical Regulators Forum (IPRP), which has both a gene therapy and a cell therapy working group. And within those subgroups, there are a number of projects we have been working on.

Additionally, COVID-19 has really underscored that we need to work globally to move forward; we cannot work in isolation. The International Coalition of Medicines Regulatory Authorities (ICMRA) has been meeting every two weeks during the pandemic, and the top-level members of each agency hold discussions on how we are moving forward, with the aim of harmonizing and exchanging information.

Within ICMRA there is also an innovation working group and within that, we have done a lot of work on looking at the different pathways that each regulatory authority has for their ATPs. For instance, there was a 3D printing case study that we worked through. So again, trying to learn from each other and harmonize where possible as we move forward at the international level is crucial.

NK: The importance of harmonization is really solidified by the fact we have the heads of agencies meeting and prioritizing this high-level focus on innovation and harmonization, and then we also have the IPRP and ICH, which enable regulators at the working level to get together, talk about some of the practical issues we are encountering, and harmonize our thinking.

As an example of the benefit of these sorts of exercises, Liz Anne, together with my predecessor in my own role, developed a summary document of all the different regulatory thinking around cell therapies in 2013 as part of the IPRP Cell Therapy Working Group. That exercise helped harmonize a lot of the thinking around how we would be addressing the risks associated with cell therapies. Such harmonization reduces regulatory barriers to patient access for innovative products. This activity in turn helped drive the formation of a feeder group into the ICH, which is another harmonization vehicle enabling the regulators and industry to work

together on different guidance documents related to different types of risk – safety, manufacturing, efficacy – and areas of convergence.

In addition to the harmonization efforts happening at all levels that I've just mentioned, we have another one at the working level with the EMA, the US FDA, and Japanese PMDA to further keep on top of the cell and gene therapy field. As an emerging area, there are constant challenges around how we deal with issues that continue to arise, and how we converge our requirements for what we might expect from sponsors, to the extent that we can within our different regulatory frameworks. There is real buy-in to ensure harmonization of how we regulate these products, even on the part of larger regulators.

Q Are there are any areas of divergence you see emerging? And as Health Canada takes a more bespoke approach, is there a risk of that divergence happening naturally?

ET: Our new pathway for ATPs is unique – there is nothing out there quite like it. We have an opportunity to be a world leader by having this in place. Is there a risk of divergence? Potentially, but that is not our intention. We are looking to have flexibility: if our regulations are blocking access to an innovative product, but the US or another country has requirements already in place that are more flexible, we can leverage those requirements in the sandbox and use them as an example to work from. Or, if the developers of a product were to choose Canada as their first country of entry, we would have the ability to accommodate them through the ATP pathway. In other words, we have a unique tool that allows us to converge where it makes sense to do so.

LG: Within the cell and gene therapy area, there is a little bit of divergence – for instance, looking at the position we have taken on some products in some cases and how they are being used, particularly with the idea of hospital exemption. I think some regulators are going back and looking at whether or not amendments are needed for that pathway, because it is being used in a way that they didn't expect or want. On our side, we do not have a hospital exemption provision – drugs are regulated as drugs within Canada and must adhere to the requirements as they are set out.

NK: Within the cell and gene therapy field, we have had sufficient flexibility with our current regulations to remain broadly harmonized with other major regulators. That said, and as Liz Anne mentioned, there are a few areas where we diverge a bit. Health Canada's ATP pathway has the potential to leverage other regulators' approaches in setting tailored requirements for cell and gene therapies that may be eligible for the pathway.

At the end of the day, everyone has the same goal to balance the need to support innovation while ensuring patients are not exposed to products with an inappropriate level of risk compared to the benefit. We are always trying to strike that balance, so in that way, on a global level, we are harmonized, albeit with our own legal and regulatory frameworks.

Q Finally, can you each summarize your chief goals and priorities in your respective roles over the coming 12–24 months?

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ET: My goal will continue to be advancing our Regulatory Innovation Agenda by building on the insights we have learned through our regulatory response to COVID-19. While a considerable challenge for us all, the pandemic has served as an opportunity to 'test drive' key agile measures we had already planned as part of our regulatory modernization work. We will continue to leverage these insights as we move forward, to inform future agile approaches to regulation. This includes working alongside Liz Anne and her team on both the ATP framework and concierge service.

LG: Working specifically in the biologics area, we can see that cell and gene therapies and personalized medicines will be a big part of the future and save many lives. They've always been among the most innovative and exciting products.

I am excited to be in this regulatory area and want to be able to provide opportunities, not impose barriers. Being able to address the fact that there will be access to these products, but that it will be done safely and under proper regulatory authority, is my priority. This will involve working together with the community to do this very collaboratively.

I am also looking forward to piloting the first product through the ATP pathway, learning from it, and moving forward to see many products use this pathway in the future.

NK: We have great jobs; it is a fantastic area to be in. I cannot believe I have been able to utilize my scientific training in a job where I actually get to help some of these innovations reach patients.

Like Liz Anne, I always like to highlight the fact that regulations should not be a barrier to innovation but should rather enable it. I am very excited about the ATP pathway, considering some of the challenges we recognize the *Food and Drug Regulations* may pose with certain new innovations on the horizon. It will be interesting to re-examine the landscape, knowing we have this tool in our belt. I look forward to continuing to work with stakeholders to help support them in their activities. It is vitally important to get products out of the labs and into first-in-human trials, and then help them progress beyond that.

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INTERVIEW

Stem cell therapy: current obstacles and innovations

JOSHUA HARE is Chief Sciences Officer, Senior Associate Dean for Experimental and Cellular Therapeutics, Director of the Interdisciplinary Stem Cell Institute (ISCI), and Louis Lemberg Professor of Medicine at the University of Miami Miller School of Medicine. Dr Hare is a practicing cardiologist and an expert in cardiovascular medicine, specializing in heart failure, myocardial infarction, inflammatory diseases of the heart, and heart transplantation. He is an internationally acknowledged pioneer in the field of stem cell therapeutics for human heart disease, and evaluates patients from all over the world for participation in clinical trials of this new experimental therapy.

Dr Hare is the director of the Interdisciplinary Stem Cell Institute, an Institute devoted to basic scientific and translational work in the field of stem cell therapy and regenerative medicine. The Institute houses 70 faculty members and 15 independent research groups. Dr Hare led the first randomized allogeneic mesenchymal stromal cell (MSC) clinical trial for patients with myocardial infarction and has served as Principal Investigator of three major NHLBI programs that advance cell-based therapy. He has pioneered the use of allogenic MSCs for multiple disease areas. Under his leadership and in partnership with many faculty across the University of Miami campus, ISCI now has active programs in cancer biology, cardiology, aging, neonatology, skin diseases, bone diseases, neurologic diseases, ophthalmology, and a program devoted to the ethics of stem cell therapy.

Dr Hare has published more than 350 original research articles, editorials, and review articles, and is the recipient of four active grants from the National Institutes of Health, three of which are active R01's. He holds 27 active Investigational New Drug applications for cell-based therapy in patients with heart disease, including the first in the United States for Idiopathic Pulmonary Fibrosis and Aging Frailty. Dr Hare is the PI of the UM National Heart Lung and Blood Institute Cardiac Cell Therapy Research Network (CCTRN) center; together these awards fund ~$3M in basic and translational research annually. Under his leadership,

ISCI has been awarded $10M from The Starr Foundation and a $26M award from The Soffer Family. Dr Hare has served in the past as chair of the Cardiac Contractility and Heart Failure study section of the National Institute of Health (NIH) and as a permanent member of the CICS study section, chaired the Stem Cell Working Group of the American Heart Association (AHA), and served as Chair of the AHA Basic Cardiovascular Science Council.

Educated at the University of Pennsylvania (1984), Johns Hopkins University School of Medicine (1988), The Brigham and Women's Hospital (1994), and Harvard Medical School, Dr Hare spent 12 years on the faculty at Johns Hopkins University School of Medicine where he rose to the rank of Professor of Medicine and Biomedical Engineering, and Director of the Cardiac Transplant and Heart Failure program in 2004 before joining the faculty at the University of Miami Miller School of Medicine in 2007. Dr Hare is an elected member of the American Association of Physicians (2011) and the Association of University Cardiologists (2007). Dr Hare is the inventor of 15 United States patents, and his research discoveries have led to the founding of four biotechnology companies.

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Q What are you working on right now?

JH: Thank you for that question; we are working on everything broadly in the field! The main area of focus in the lab right now is on induced pluripotent stem (iPS) cell biology. We have always had a great interest in iPS biology, but it hasn't been front and center because we have been so involved with NIH funded mesenchymal stem cell (MSC) clinical trials. We are at a wonderful point right now where we have pivoted to look at some basic, mechanistic biology that has a translational outlook. One of the key areas of my work right now is to refine processes for making pure cardiomyocyte progenitors from iPS cells, and we have some very provocative results.

I have always said that success in this field requires investigators to be equal opportunity employers as far as cell-based strategy is concerned. That pertains to the use of the cell and to having an open mind about mechanism of action. At the end of the day, a cell is a cell is a cell. We are all dealing with a class here. The thing that iPS cell biology teaches us more than anything else is plasticity – in other words, cells can transition from one state to another, so you can make mesenchymal stem cells from iPS cells. We have completed probably more MSC trials than anyone, but we have completed them, and now we have got lots of other really exciting stuff in the laboratory.

One of our big discoveries with cell therapy is what we call cell combination therapy (or CCT), i.e. that you should mix MSCs with cardiac cells. And now, we are making those cardiac cells from iPS cells, so this really isn't a far cry from what our interests have always been.

Q The big story of the last year for stem cell therapy has been its role in countering COVID-19. Could you tell us about the promise of

stem cell therapy and its progress in that particular area to date, and any efforts in which you have been directly involved?

JH: It is such an obvious thing to try, but the unfortunate part of it is that it never got adequate funding to get a meaningful answer, akin to what other trials got. One of the things that we articulated very early on in the process was that this is a great way to have an immune-balancing therapy that doesn't have substantial side effects, or doesn't offset viral clearance. Corticosteroids went through a massive multi-thousand patient trial in the UK that showed there was significance, but nobody looked at the side effect profile. With infusions of corticosteroids, we know the side effect profile there in terms of infections, diabetes, physical feature changes such as steroid facies, osteopenia and so on. MSCs can, we think, have the same positive effects but without the side effects. Right now, those trials are still being conducted, and we have done a lot of open access treatment.

Q Are any other approaches in terms of addressing COVID that have caught your eye from the cell and gene therapy space?

JH: The huge story here is that the current novel vaccine strategy is a gene therapy. You have got two strategies; one is a viral vector, and the other is just naked RNA. This is, to my knowledge, the first use of pure gene therapy as a vaccine, and it works beautifully. That is the headline.

Talking about cell and gene therapy, another question is whether or not you could use a combination. I know there is one group trying to use cells as the vector for the vaccine. But I think infusions of MSCs or MSC exosomes are a very legitimate way to try and treat COVID-related ARDS.

Q What challenges are you and other developers of cell therapy interventions against ARDS facing, and what challenges will you face moving forwards?

JH: The biggest problem is getting sufficient funding to do trials. There are almost no Phase 3 clinical trials of MSCs for any indication – basically you can count them on one hand. The ones that have passed Phase 3 in a way that has led to regulatory approval have actually been small trials. For some reason, cell therapy is not attracting the funding that other approaches are. It is not attracting the funding that gene therapy is, or the funding that CAR T therapy attracted.

"...success in this field requires investigators to be equal opportunity employers as far as cell-based strategy is concerned."

> "We do a lot of sophisticated imaging with MRI and CT scanning, and PET scanning, but there aren't readily available and readily accessible tracers to look at the fate of cells when you put them into the patient. Moreover, what you would really want to be able to do is look at secondary mediators … If a cell goes into the body and releases exosomes, it would be amazing to be able to track the exosomes to see where they go, and what they stimulate."

We can't advance cell therapy, whether it is with MSCs or iPSCs, without that kind of funding. We have to have that funding, but it hasn't gelled yet for the sources of money that are needed to really move these trials.

I heard a beautiful talk today on immunotherapy – monoclonal antibodies also went through a difficult period. In 2017, there was a very famous study published in *New England Journal of Medicine* using canakinumab, an anti-IL-1β monoclonal antibody in atherosclerosis. I can't quote the exact numbers without looking it up, but more than 10,000-patients were randomized in the study. So they studied 10,000 patients with a monoclonal antibody in a placebo-controlled, randomized trial and the effect was positive, lowering cardiovascular events in patients with high C-reactive protein levels. It proved the point that atherosclerosis is an inflammatory condition. But who is going to pay for a 10,000-patient trial to use MSCs for any kind of inflammatory condition? There is no entity out there putting up that kind of money, but without that kind of resource, how are we ever going to get the decisive answers that we need? The funding is the biggest challenge, and hopefully one day those kinds of studies will be done.

Q Is there anything on the scientific side in terms of additional obstacles?

JH: No, I think this is a beautiful scientific area. That is why we have changed our focus to iPS cells. It is truly something you can study in a dish. You can engineer what you want, and you can test specific targeted hypotheses about signaling checkpoints, and how well we can make a cell differentiate down this pathway rather than that pathway. Then you can make more targeted cell-based therapeutics. There is an Australian company that is making MSCs

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from iPS cells (Cynata Therapeutics) and I think that is actually the way forward for the field, to have a more specific and targeted entity.

There is also the regulatory challenge – regulators have complained about cell therapy in terms of lacking critical quality attributes and potency assays. The biggest challenge there is coming up with a compelling potency assay that clearly correlates with the outcome in your disease.

Q Where in particular have you seen recent progress in terms of new innovation, or repurposed innovation, for the enabling technologies that support translational R&D in your field?

JH: One of the things I love to do, and have the privilege of being able to do based on the facility I have at my lab, is the creation of large animal models.

In the heart failure field, there is an entity that is increasingly recognized as critically important: heart failure with preserved ejection fraction, or HFpEF. Most heart failure was initially attributed to heart failure with a reduced ejection fraction. It is increasingly appreciated that you can have heart failure with preserved EF, or HEF EF, but there has been no animal model for this, creating a big obstacle.

We created a very robust animal model, which we have now deployed to study regenerative medicine and cell-based treatments, so we are very excited about HFpEF.

Q And what would be on your wish list in terms of future areas for innovation? What are the greatest needs?

JH: *In vivo* tracking. We do a lot of sophisticated imaging with MRI and CT scanning, and PET scanning, but there aren't readily available and readily accessible tracers to look at the fate of cells when you put them into the patient. Moreover, what you would really want to be able to do is look at secondary mediators.

If a cell goes into the body and releases exosomes, it would be amazing to be able to track the exosomes to see where they go, and what they stimulate. I know the NIH has been interested in funding that for over 15 years, but not a lot has happened. We need to see more of that.

So, *in vivo* tracking of the fate not just of cells, but genes too, would be amazing. If we could do that it would really move the field forward.

Q What are the chief goals and priorities for your work, over the next 12 to 24 months?

JH: We are optimizing iPS cell-derived cardiomyocytes, so we will be ready to translate that into animal models to see if we can create a safer, more effective way to remuscularize the heart. We are going to pursue HFpEF more rigorously.

We are also going to be focusing on *in vivo* imaging to see if we can look at the fate of cells, exosomes, and genes that are injected into the body. And of course, we continue to collaborate broadly across scientific disciplines!

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CELL & GENE THERAPY INSIGHTS

Innovation Insights

SHELFIE

On my bookshelf...

Robert Deans

In CGTI Shelfies, we ask experts within cell and gene therapy to pick the publications that helped shape their field, and their own careers.

This week, we take a look into the bookshelf of Bob Deans, CSO at Syngetho.

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The Lac Operon (1970)

Edited by Beckwith JR and Zipser D. Cold Spring Harbor Press. 1970

For most of my early career I chased puzzles without much attention for big picture focus or value, which began with an exposure to molecular genetics as an undergrad at MIT. I was failing badly at aeronautics and physics when the purity of logic from the lac operon struck a chord, and motivated me greatly. The intuitive control to sense and respond to the environment was an epiphany at a time when I was looking for existential logic, and my decision to pursue molecular genetics was locked in.

Rogers J et al. (1980)

Two mRNAs with different 3′ ends encode membrane bound and secreted forms of immunoglobulin mu chain. *Cell* 20: 303–312.

Early molecular genetics succeeded in simple haploid organisms, while solutions to the physical structure of genes was solved. The finding of intron/exon gene structure in mammalian cells created new networks for genetic diversity. I was selecting model systems to study and build towards human disease, and was pointed towards blood cells for their accessibility. At the time, immunoglobulin genes were most valuable as a model for structural genetics, in which protein structure/function relationships could be created by gene rearrangements at both the DNA and mRNA level. The intuitive response to class switch and diversify a B cell undergoes in reaction to antigen binding was a strong hook for my career, and molecular immunology and hematopoiesis have been my core interest and career theme.

Shi J *et al.* (2014)

Engineered red blood cells as carriers for systemic delivery of a wide array of functional probes *PNAS* 111: 10131–10136.

Harvey Lodish published a transformative paper in PNAS involving hematopoietic stem cell gene targeting with subsequent derivation of erythroid enucleated lineages. This platform fully embraced the concept of de novo synthetic cell bodies suited for systemic circulation as metabolic factors. The ability to perform complicated genetics for function, while subsequently discarding the nucleus and template, was a radical concept for synthetic biology.

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Steinbeck J *et al.* (2015)

Optogenetics enables functional analysis of human embryonic stem cell derived grafts in a Parkinson's disease model.

Throughout my career, there has been a requirement to build a bioprocess platform in order to translate cell and gene therapies to the clinic. This has given gave me experience in adult and pluripotent stem cell biology and GMP processes, and accumulated experience in eight IND filings. One constant argument with regulatory agencies has been around the complex modeling of a cell therapeutic, where redundant pathways often exist to maintain homeostasis, and a reductionist approach to link potency with hypothesis causes difficulties. This inability for strong potency linkage has weakened translational efforts, particularly in incremental study findings. The lack of clear surrogates for potency has prevented accurate clinical data and stratification.

In building a cell therapeutic for Parkinson's disease, Lorenz Studer published an elegant work describing the use of optogenetics in vivo to ascribe donor cell function and control of locomotor function. A strong flag in the sand for rationale development modeling, and some excellent science.

Wang R *et al.* (2021)

Genetic screens identify host factors for SARS-CoV-2 and common cold coronaviruses *Cell* 184: 106–119.

With the advent of CRISPR editing tools, genetic diversity has been linked to structural biology with disruptive consequence. As demonstrated in this publication from Nevan Krogan at UCSF, using 'omics and machine learning tools, a broad systems biology map of disease can be constructed for rapid knowledge sharing and design of intervention strategies. In less than nine months, a systems biology map for COVID/human protein interactions was validated using CRISPR screening. From this, in less than nine months, drugs have been designed and advanced into phase III studies. And so, it seems it is time to appreciate engineering after all!

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CELL & GENE THERAPY INSIGHTS

Innovation Insights

SHELFIE

On my bookshelf...

Qasim Rafiq

In CGTI Shelfies, we ask experts within cell and gene therapy to pick the publications that helped shape their field, and their own careers.

This week, we take a look into the bookshelf of Qasim Rafiq, Associate Professor in Cell and Gene Therapy Bioprocess Engineering at University College London.

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Bioprocess Engineering Principles

Pauline Doran. Academic Press. 2nd Edition, 2013

This book has been with me since the first day of my undergraduate degree program in biochemical engineering, and for me it is one of the fundamental texts.

Now in its second edition, it is an absolutely fantastic book. It breaks down the fundamental engineering concepts that are critical for biochemical engineers to be familiar with, but connects everything to the underlying biology. A critical book for anyone looking at biomanufacturing, bioprocess engineering, biochemical engineering, upstream production, fermentation, or downstream purification.

This is one of the books that without doubt significantly impacted the work I do. It was a seminal text during my undergraduate program, and it is a seminal text for me now. I still go back to it, reference it, and learn from it, and it is something I am teaching from. When the second edition was released, I even preordered my copy six months in advance!

I teach biochemical engineering, introduction to biochemical engineering, and cell and gene therapy bioprocessing to students at UCL, and it is the number one book that I recommend to my students – even if they are not in engineering – so that they can appreciate engineering concepts and challenges.

Thomson JA *et al.* (1998)

Embryonic stem cell lines derived from human blastocysts. *Science* 282(5391): 1145–1147.

I studied biochemical engineering at UCL, and it was a four-year masters and engineering program. By the end of my third year, I was still undecided about what I wanted to do. I wasn't hugely connected to the biotechnology industry at this point, and I felt the gains we could get in biopharmaceutical manufacture were minimal.

In my fourth year I had the opportunity and pleasure of being taught by Professor Chris Mason. He introduced me and my fellow students to the world of stem cells, regenerative medicine, and cell therapy. I became fascinated. This was back in 2008, ten years after the release of this key paper demonstrating and describing the process of deriving and subsequently characterizing human embryonic stem cells. This paper transformed the sector – it is fascinating biology, and beautifully written.

It is only a few pages long, but when I go through this with my students, we can spend up to 45 minutes just on the abstract, going through each and every word. The paper in itself is the absolute proof that biologists needed

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to demonstrate that they have been able to isolate, derive, and characterize their embryonic stem cells. This was a paper that I went through with Chris Mason, and it changed my whole outlook, and effectively decided my career path. I could see the potential of stem cells, and what we could do moving forward, and the need for biochemical engineers and biomanufacturing to realize the potential of these cells in a clinical and commercial setting.

Takahashi K *et al.* (2007)

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* 131(5): 861–872.

This paper has gone down in history, and quite rightly so. It was a contributing factor to Yamanaka receiving the Nobel prize, and it changed many people's perceptions on cell lineage and cell differentiation, and the opportunities this would provide for providing a cell source for almost every other cell type. Now, when we look at the impact of induced pluripotent stem cells, we can see the global impact they are having, whether it is CAR T cells, NK cells, blood cells, or whatever other cell type. For me, iPSCs have dramatically changed the way we look at cell development, and the opportunity that cell therapies present.

Getting Things Done

David Allen. Piatkus. 2015

I am sure many people will be familiar with David Allen's seminal book Getting Things Done, and the GTD Method. I found this transformational – I was one of those individuals who struggled to keep up with not just deadlines, but even the day-to-day rigor of emails, meetings, and endless to-do lists of tasks.

David Allen's book demystifies how we approach things, and how we work – and perhaps the ways we should not work – and ultimately provides a system. A system that needs constant maintenance and work, but a system which has proven to be hugely successful for many within management, across a range of industries. It has worked for me, to the point where I am more productive, and also more organized and stress-free.

Perhaps the most critical thing I took away from Getting Things Done, is that it is not just about focusing on immediate tasks. Often, we have a tendency to focus on the here and now. Tomorrow I need to send emails, get a report done, meet a deadline – but as a result, we can lose sight of important things such as personal development, or long-term career plans. We constantly put these things off because we are focused on firefighting, rather than thinking strategically and long-term.

Toyota Production System: Beyond Large-Scale Production

Taiichi Ohno. Productivity Press. 1st edition 1988

My final pick has had a significant impact on my perspective of manufacturing as a whole. This book introduces the concept of lean manufacturing, which was pioneered by Toyota and Taiichi Ohno.

Coming from a biopharmaceutical perspective, you don't necessarily think about what is required for manufacturing, and the precision that is needed, particularly at large scale. You may not have an understanding of how it works in other industries, and how manufacturing has evolved. Taiichi Ono's book epitomizes the beauty of manufacture, the focus of manufacture, and what I believe we should implement and strive for in our work in cell and gene therapy.

There are a lot of examples in early automotive manufacture where they would have a whole production line to produce cars, but at the end you would have someone with a hammer and chisel knocking in the doors because they didn't quite fit. With cell and gene therapy manufacture and biopharmaceutical manufacture, we are often doing something similar: we do our end-stage release testing and realize it is just out of spec, and we are often not answering the fundamental question of why that is, and what the causes are.

This is where philosophies like lean manufacture come in to change that perspective. There is a huge amount that those of us in the biotech field can and should learn from other fields, such as the automotive industry. Bringing those philosophies into the work that we do can help us minimize and eliminate waste, and ensure we deliver safe, efficacious, and cost-effective products to the patients that need them.

AUTHORSHIP & CONFLICT OF INTEREST

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