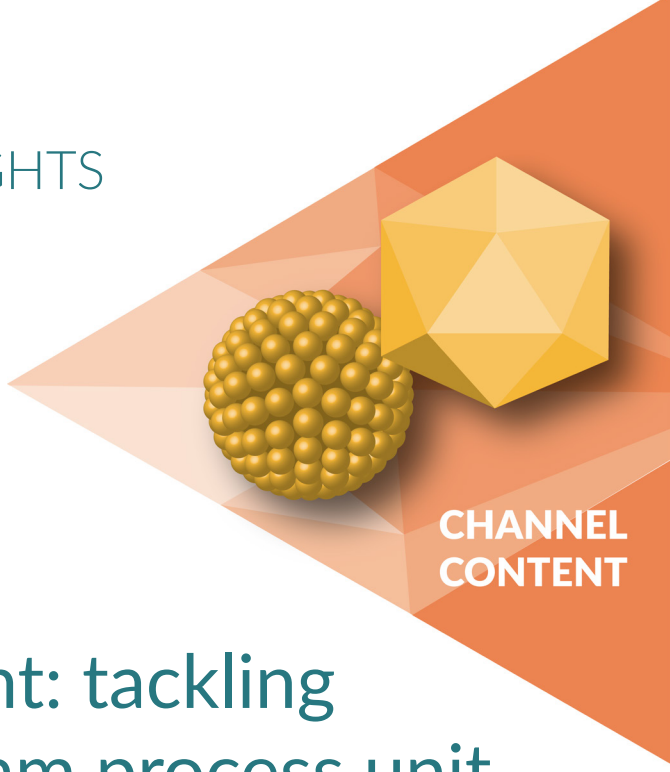
CELL & GENE THERAPY INSIGHTS



VECTOR CHANNEL:
PURIFICATION

PODCAST INTERVIEW

Room for improvement: tackling suboptimal downstream process unit operations for viral vectors

DR CLIVE GLOVER is Senior Global Marketing Manager, Cell & Gene Therapy, Pall Biotech, UK, leading their cell and gene therapy business. Previously he was responsible for driving product development efforts around cell therapy at GE Healthcare and has also held positions in marketing and product management at STEMCELL Technologies. Clive holds a PhD in Genetics from the University of British Columbia.

PROF. DANIEL SMITH is Chief Scientific Officer at Cobra Biologics. Prior to his appointment as CSO in June 2014, Daniel was a Knowledge Transfer Manager and Senior Technologist for four years within the bioProcessUK team at the HealthTech & Medicines Knowledge Transfer Network. Formerly, Daniel spent five years at Cobra in roles including Senior Scientist, QC Team Leader, Head of Process Technology Transfer and Commercial Scientific Development Manager. He has over eight years academic research experience, 20+ research publications to his name and a PhD in Molecular Cell Biology. In June 2018 he was nominated an Honorary Industry Professor in the School of Life Sciences at the University of Warwick.

DR DAMIAN MARSHALL is the director of new and enabling technologies at the Cell and Gene Therapy Catapult and has almost 20 years of industrial experience gained working for SME's and large companies. He is responsible for providing vision, expertise and leadership to a team of ~70 scientists working with a wide range of cell and gene therapy developers. Together they are addressing some of the barriers to growth within the industry, supporting the development of new technologies and helping create an advantageous environment that gives companies working in the UK a sustained competitive advantage.

Cell & Gene Therapy Insights 2019; 5(S2), 165–176

DOI: 10.18609/cgti.2019.024

Q Clive, could you frame for us the downstream bioprocessing challenges in viral vector production, and why do you feel innovation is so badly needed in this particular area?

CG: I think there are many challenges associated with manufacturing gene therapies overall. And the consequence of these challenges is an incredibly high cost of goods, and actually more important in many cases, an inability to produce enough virus to treat the patients that need it.

In particular, the challenges in downstream are such that at present time we're seeing losses of greater than 60% and sometimes up to 90% of the product. Given how much expense and time is spent on the upstream portion, not to mention the cost associated with the downstream portion, there's significant room for improvement here. What we really want to be doing is getting up to the 65–70% recovery from the downstream process, which is really what we see in the monoclonal antibody sector which we use as the benchmark here.

I'd say it's not really easy to point to one unit operation causing this loss. Rather it comes from the fact there are multiple suboptimal unit operations being stitched together. One of the reasons each of these unit operations is suboptimal is because the equipment has generally been borrowed from the monoclonal antibody word and has not been optimized for viral vector production. I think there's significant room for improvement, and I think it's across the whole board of the downstream processing unit operations.

Q Can you tell us about the Innovate UK Grant and the partnership formed around it for you three, and how it came about and how did each company get involved?

DS: The Innovation UK grant really came about through a discussion that I and my colleague Tony Hitchcock, Technical Director at Cobra, had with Clive and the people at his organisation about how best to really solve the challenge of these sub-optimal processes for AAV production and gene therapy applications.

This discussion led us to the question of: what could we do in this space? Tapping into the experience we have at Cobra as an end user from a CDMO perspective, a contractor development manufacturing organisation, and the challenges we faced; and Clive and his organisation, as a

DOI: DOI: 10.18609/cgti.2019.024

technology provider, what challenges could they see coming along the line, how could they respond to that to meet the need?

We had a few meetings around this and put together the framework for the potential project. We then realized one of the biggest areas needed and innovation is required in is the area of advanced analytics to support rapid process development and rapid manufacturing approaches.

From that point on we looked for a number of partners and really the Cell and Gene Therapy Catapult was an obvious partner as they have a lot of experience in upcoming technologies, de-risking upcoming technologies, and really understanding their use and application.

A discussion with all three partners ensued, we came together and came up with a plan, we then applied for Innovate UK funding through their medicines manufacturing stream. That competition was looking for a step change in the productivity and/or the cost of goods of manufacturing platforms. Viral vectors and their application in gene therapy is a major driver for UK government and as such Innovate UK fund a lot of projects in that area.

The partnership came together, and that's really how everyone got involved. I'll leave it to Clive and Damian to explain their aspects of better than I can. But from us as a CDMO we're really looking to work with these partners to bring synergies together in order for us to have a better commercial proposition for our customers.

CG: The story very much evolved as Dan described it. From an equipment provider point of view, what is fantastic about this kind of grant is enabling that really close partnership with the likes of Cobra, who have that real-world end user experience. For an equipment provider like us it really is significant to be able to get that kind of input into our product development process.

And then of course as Dan has already described, the analytics are key to all of this field, and getting a partner with great access to analytics who has really spent some time trying to develop these analytics just means we can all move faster in this particular field.

So a really good partnership and looking forward to the results coming out.

DM: As mentioned by Dan and Clive, the Cell and Gene Therapy Catapult has a long-standing relationship with both Cobra and Pall. This has involved discussions around the challenges for viral vector manufacturing, with some of these discussions actually helping guide the development of our analytical capabilities.

This is because analytics is often seen as more of a pre-competitive space and is therefore potentially more open to collaboration. I think when this project was being put together we were quite a natural fit to come in with both Cobra and Pall to look at this.

Q Why was chromatography in particular was chosen for this project?

CG: Obviously chromatography is one of several steps involved in downstream purification of virus. But our observation was that if you get the chromatography right, it really significantly enhances the overall productivity of the downstream processing.

Current chromatographic techniques for AAV in particular are very expensive and often require very harsh conditions that could actually destroy the infectivity of the virus. Of course we don't really know whether they do destroy the infectivity of the virus because the analytics are so poor as we've just discussed. So there's clearly room for improvement in this particular step.

"Current chromatographic techniques for AAV in particular are very expensive and often require very harsh conditions that could actually destroy the infectivity of the virus."

We also thought that Pall had some rather unique capabilities in the form of our Cadence™ BioSMB technology, which could really help to see whether we could make improvements, reduce the cost of this particular step, maybe in fact bring in new kinds of chromatographic techniques that haven't been able to be use before, and all in that context of better analytics where you can truly understand whether we are in fact optimising that particular step.

DS: From Cobra's perspective, a chromatography-based production system allows us to scale the process accordingly to meet the demand of our customers out there.

Current processes that are suboptimal really exist around lab-based processes. The best way at the moment really to get the quality of viral vector that a lot of our customers require from a regulatory point of view and from clinical and commercial use, uses very old fashioned non-scalable technology such as ultra-centrifugation and gradient centrifugation, and extraction of viruses and viral vectors from these centrifuge tubes, by very archaic means involving puncturing the tubes and drawing them out in needles. Obviously this isn't scalable, it's not cost effective, and also from a compliance point of view as we move to late phase and commercial production, the compliance aspects around scalability and reproducibility are really brought into question through this type of approach.

Therefore a chromatography-based system that has been used widely in classical biologics for a number of years is the best option, and we really need to understand better approaches to that to really make sure these clinical entities translate rapidly into a commercial environment.

DOI: DOI: 10.18609/cgti.2019.024

Q What are the key objectives for each of the groups involved in this?

DM: As has been mentioned, the Cell and Gene Therapy Catapult is responsible for delivering the analytical component for this project, and our activities fall into three main areas.

The first of these is the development of high throughput assays. As part of this project Pall are going to be looking to use a design of experiment approach to test and select suitable chromatography technologies. This can generate high sample volumes so it's important we can ensure the analytics don't become a bottleneck to innovation on this project.

To this end we're looking at implementing high throughput techniques but also investigating opportunities for assay automation.

Secondly we'll be looking at the development of rapid analytics that can measure viral vector quality during the downstream processing steps. The challenge is going to be to establish techniques which can provide data in a sufficient timeframe to support process decision making. But we also want to make sure we are developing techniques which can be routinely run within a QC laboratory environment.

The third area of activity is going to be the technology transfer. We want to develop a suite of assays in this project that we can then transfer to Cobra to support a proof of concept manufacturing run which will incorporate all the downstream processing steps developed by Pall.

CG: At a very high level what we're trying to do with this project is get proof of principle of how our Cadence™ BioSMB continuous chromatography system could work in this particular setting, for this particular application.

At first glance we expect there will be some utility for the machine as is. But then we also expect it to start giving us significant input into what are the improvements that could be made both in the chromatography technology as well as in the automation equipment to run it as well.

That's really the high level objective of what we're trying to get out of it. I think there are various steps to getting along to that. We'll initially be working with the partners to make sure we get a model system up and running. Obviously the output of the experiments are only as good as the model system we use, so we'll be working with Cobra to get particular strains of AAV up and running to do the experiment.

Ensuring we have sufficient quantities of the virus to actually run the experiment is not a trivial task. And then actually starting to work much more closely to screen through the possible chromatography techniques. And how it can apply in a continuous fashion. And that will be done in

collaboration with both parties. You know, using a lot of those analytics that Damien has just described.

DS: From a Cobra perspective there are three key, high-level objectives that we would like to see delivered or at least partially delivered throughout the collaboration.

The first one is an increase in productivity of viral vector purification. We need to get more purified material available for customers. That's a commercial driver.

The second one is we need to increase and speed up the approach to rapid process development leading into manufacturing. The timelines, the clinical timelines these products move through the development phases is very, very rapid. Unlike a traditional biologic which may take somewhere between 6 to 10 years to go from a Phase 1 status to commercial launch, we're seeing gene therapy vectors moving rapidly in timeframes of sort of 3 and a half to 4 and a half years to commercialization.

That puts an enormous burden on a manufacturer like ourselves to lock platforms and lock processes down early, and then that faster approach to PD and manufacturing is a real driver for us.

And then the third high-level objective is the reduction of the cost of goods. These things are exceedingly expensive to make and purify, and to remain commercial in the market place and competitive in that marketplace we need a cheaper way to do it. The healthcare providers need us to make a cheaper way of dogging it, for reimbursing strategies.

There's a massive macroeconomic driver here for the whole of this project. If I bring it right down to really what we're trying to achieve its knowledge transfer from technology providers like Pall into Cobra, but also from the Catapult into Cobra, exactly how the other two have described their technology drivers and push for that as well.

Q Something we're hearing about in this sector is the concept of continuous manufacturing. What is this and how could it benefit cell and gene therapy manufacture?.

CG: At its heart continuous manufacturing is pretty much what it sounds like. It's a method of manufacturing where all processing takes place without stopping from beginning to end.

Probably the image people connect with is the pictures you see on TV of car manufacturing plants, where there's a conveyor belt continually moving along where people and robots steadily add things to cars. Rather than the car sitting, and then everyone assembling it all at once and then it moves

DOI: DOI: 10.18609/cgti.2019.024

onto the next thing there. Continuous manufacturing is that conveyor belt in a sense. Within the bioprocessing industry we very much manufacture things generally in batch mode at the moment. It's fine, it gets the job done, but it creates a lot of waste in the process. Most other large industrial settings like petrochemicals and those sorts of things continuous manufacturing is the norm, and has been shown to be much more efficient.

At Pall we've been working on the concept of continuous manufacturing for monoclonal antibodies for quite a while now. And we believe it can bring many benefits to that process of monoclonal antibody manufacturing. For example we think the factory footprint for a monoclonal antibody factory can be reduced 40–90%. The subsequent costs to build the facility can be reduced by 25–60%. And the operating costs can be reduced by 25–60%.

Now those are numbers associated with a full continuous manufacturing suite. We're just obviously focusing on one particular unit operation here. But that should give you a flavour of why adoption continuous manufacturing can help in this particular seating.

DS: The continuous manufacturing approach in this area is going to really help from the point of view we can reduce that production footprint. There's a lot of investment, in the UK and globally at the moment, in the production and increased capacity for viral vector manufacturing.

Now people are building reasonably large state of the art clean rooms to do this. And as the market increases and more demand is coming through, more and more capacity will be needed.

However this changes it on its head. If we can have continuous process working on a smaller footprint, and a flexible approach to that, and even a step further and potentially close that process so it's completely self-contained and running on its own in a manufacturing suite, we should be able to get more and more of these things in parallel next to each other. Which will really open up the whole capacity issue to the marketplace, and ultimately as Clive has alluded to, reduce that cost of goods for both an investment point of view and process point of view.

> "...there may be a particular advantage that lentivirus has over AAV if you're thinking about continuous manufacturing..."

It's all about flexible manufacturing approaches as we move from early 21st century through this to really realize the potential these types of revolutionary therapies could have.

Q What are the particular challenges on the analytics development side?

DM: The challenges on the analytical development side are the challenges that are quite common across the gene therapy space. We're going to be looking at the development of high resolution and more accurate measurement techniques to support advanced bioprocessing.

On the higher accuracy side we're going to be looking at techniques such as digital PCR for the measurement of viral titre. This technology has the advantage that it provides absolute quantification of viral titer but it's also less prone to PCR inhibition. This is important when measuring downstream processing samples that could have a high salt concentration. In this case these high resolution techniques could be less prone to the detrimental effects that high salt can have.

We're also going to be looking at rapid techniques such as multi-angle dynamic light scattering. The great thing about this technique is it can provide a robust measure of viral concentration, particle size distribution, and aggregation, all in under 3 minutes form just a 30 microliter sample. So you're not using a lot of your precious material when you're looking at providing your analytical readout.

Another challenge we're going to look at in this project is the development of techniques that allow us to make reliable and meaningful measurements of empty versus full capsid ratios, which is a real challenge for the field.

Current approaches such as transmission electro microscopy can give a really good measure of empty to full capsid ratios but the turnaround time for the assays can be long, and the equipment required can be prohibitively expensive. So we're going to be looking at cheaper and faster alternatives but we don't to compromise on accuracy or robustness.

Finally we're going to be looking at opportunities to multiplex assays. An example we will be investigating in this project is the application of multiplexed ELISA for the measurement of purity and impurities throughout the downstream processing steps.

> "...we're in a unique position here in viral vector manufacturing because the standard is not yet really established."

Importantly, all of these assays are going to be developed with the view they have to be performed in a QC laboratory. So we will be looking for simple technologies that provide really meaningful measurements that can be transferred between laboratories relatively easily.

DOI: DOI: 10.18609/cgti.2019.024

Q Looking ahead to a potential future focus specifically on lentiviral vectors, what are the particular challenges unique to that virus that you anticipate encountering?

DS: Lentivirus is obviously used a lot at the moment with numerous clinical trials utilizing this virus for the modification of T cells in the CAR-T and immunotherapy space. Lentivirus itself is a relatively simple virus to make. It can be made in a similar way to AAV. However, there are certain differences between AAV and lentivirus. One being size and the other being stability. Lentivirus is a fragile virus. It's more prone to conformational changes and disruption of that confirmation throughout the bioprocess. So the process conditions we may encounter and optimize throughout the project that are relevant for an AAV vector may not be applicable to a lentiviral vector.

The other real challenge we have with lentivirus at the moment from a manufacturing point of view is that because of its size, and because of some surface properties around the electrostatic charge on the surface, it seems to stick to filters very tightly. As Clive has alluded to we can get roundabout 60–90% loss of material for a bioprocess at the moment. It's not quite the same for a lentivirus, we can get a bit more recovery for lentivirus, but what we get at the back end of the process, when we try to sterile filter lentivirus, you can lose something up to 80–90% of the product in that single unit operation filtration step. That is a real challenge and barrier for commercialisation for a lot of these projects as we go up in scale.

One thing specifically for lentivirus is the ability to understand that filtration challenge. How do we make it better? How do we optimize it? How do we get to a 50/50 relationship with lentivirus going through the filter, if not trying to get a lot more for it?

Those are the real problems I see we're going to encounter from a lentiviral point of view in a similar approach to continuous manufacturing.

CG: What Dan has pointed out are certainly significant challenges for lentivirus. I also think there may be a particular advantage that lentivirus has over AAV if you're thinking about continuous manufacturing, and that is that lentivirus is a secreted virus as opposed to most serotypes of AAV which are intracellular and therefore require some of lisal step.

In the case of lentivirus they're all secreted, which can then actually lend itself quite nicely to continuous processing. You can imagine some kind of upstream profusion process that could then flow seamlessly into the downstream processing.

Again I think that's quite a long way away and we need to do a lot of optimization of each of the other unit operations, but there may be a way to

make that link between the upstream and downstream easier in lentivirus than it is in AAV.

DM: The challenge in the analytical area is hopefully lower, because the assays we're developing for AAV should mostly be capable of being modified to measure lentiviral vectors. For example, using digital PCR you'd need to change the primers and probes but the overall technique would remain the same.

Therefore we're actually hoping we'll be able to have a versatile toolkit for monitoring a range of different viral vectors that not specific to AAV or lentivirus.

Q Beyond exploring other vectors, what would be the next step in terms of future projects you would each like to see to follow this one, to benefit viral vector bioprocessing as a whole?

DS: I think there are a lot of unanswered questions still in the whole area of viral vector bioprocessing. One of the key things is the underpinning science around the bioprocess and the conditions of the bioprocess, and the effect it's having on the viral vector itself.

Little is known really about structure function relationships of these viral vectors when we put them in a flow condition, when we're flowing around tubing, flowing across membranes, looking at pressure differentials across those membranes, binding to column chromatography, stripping off of column chromatography, makes even harsh environments.

Clive alluded to it earlier on. We really don't know what's going on to the biology of that vector, and therefore the function of that vector. A lot more needs to be done in that space.

Another thing I think needs to be linked to this is the bioprocess itself is one part of the puzzle. The downstream challenge is one part of the puzzle. Hopefully through this type of project we can get a lot more understanding of how to increase the productivity and reduce the losses across a downstream process.

There still remains a challenge of what can we do in the upstream process as well? Can we make more virus within the same footprint? Can we make better quality virus within the same footprint? An example here is AAV. We touched on this difference between empty and full capsids. Depending on the serotype you're using of AAV you might get as little as 1% actually encapsulated with the right material of interests, compared to 99% empties or containing other things. how do we find that 1% in that big pool? Can we change the biology? Can we change the upstream process so 1% now becomes 90%? And it's a much easier job in downstream purification to remove the 10% wastage opposed to 99% wastage.

DOI: DOI: 10.18609/cgti.2019.024

I think those types of things are where I'd like to see it, and to support all of that, I'm sure Damian can touch on this, is far more near-time, real-time analytics that allows us to get a handle on these things very early on within a bioprocess environment. Not having to wait until the backend of the process to analyze a near pure product at that point.

DM: I couldn't agree more with what Dan has just said there, because a really big area of interest for the Cell and Gene Therapy Catapult is the application of process analytical technologies or PAT.

PAT is a mechanism for designing, analysing and controlling the manufacturing process through the measurement of critical process parameters and critical quality attributes with a view to ensuring your final product quality.

With this in mind, PAT opens up an opportunity to look at sensor technologies which can be embedded into the downstream process to make real time, or at least near real time measurements of viral quality. We think this could be a significant enabler for technology development and advanced manufacture in the future.

CG: Clearly any sophisticated bioprocess you put together is only going to be as good as the basic biology you know about the system and analytics you're able to deploy to control the system.

So assuming both those things are done, and I should say that's a big assumption because that's not a trivial piece of work by any stretch of the imagination. But once we've done those sorts of things I see us coming together with a much more sophisticated manufacturing process for all of these viruses.

So bear in mind here we're just focusing on the chromatography step, whereas we need to look across the entire process from end to end, looking to optimize all those unit operations, ensure all those unit operations fit together, and then if we find that a continuous manufacturing process is in fact beneficial for viral vector manufacturing overall, put those together in a entirely continuous process.

I think we're in a unique position here in viral vector manufacturing because the standard is not yet really established. If you look over at monoclonal antibodies where there is a very clearly established process, there is a lot of hesitance around adopting continuous manufacturing techniques despite the potential gain they can bring to it.

In the field of viral vector manufacturing I think we really have that opportunity to come to the table with a much more sophisticated manufacturing operation that ultimately brings that flexibility we need in order to get the virus at a cost-effective price to the patients that really need it.

So I'm really looking forward to this and hope this will be the first of many steps as we go along our way to making that better viral vector manufacturing.

AFFILIATIONS

Clive Glover[1], Daniel Smith[2] & Damien Marshall[3]

[1]Senior Global Marketing Manager for Cell and Gene Therapy, Pall Biotech

[2]Chief Scientific Officer, Cobra Biologics

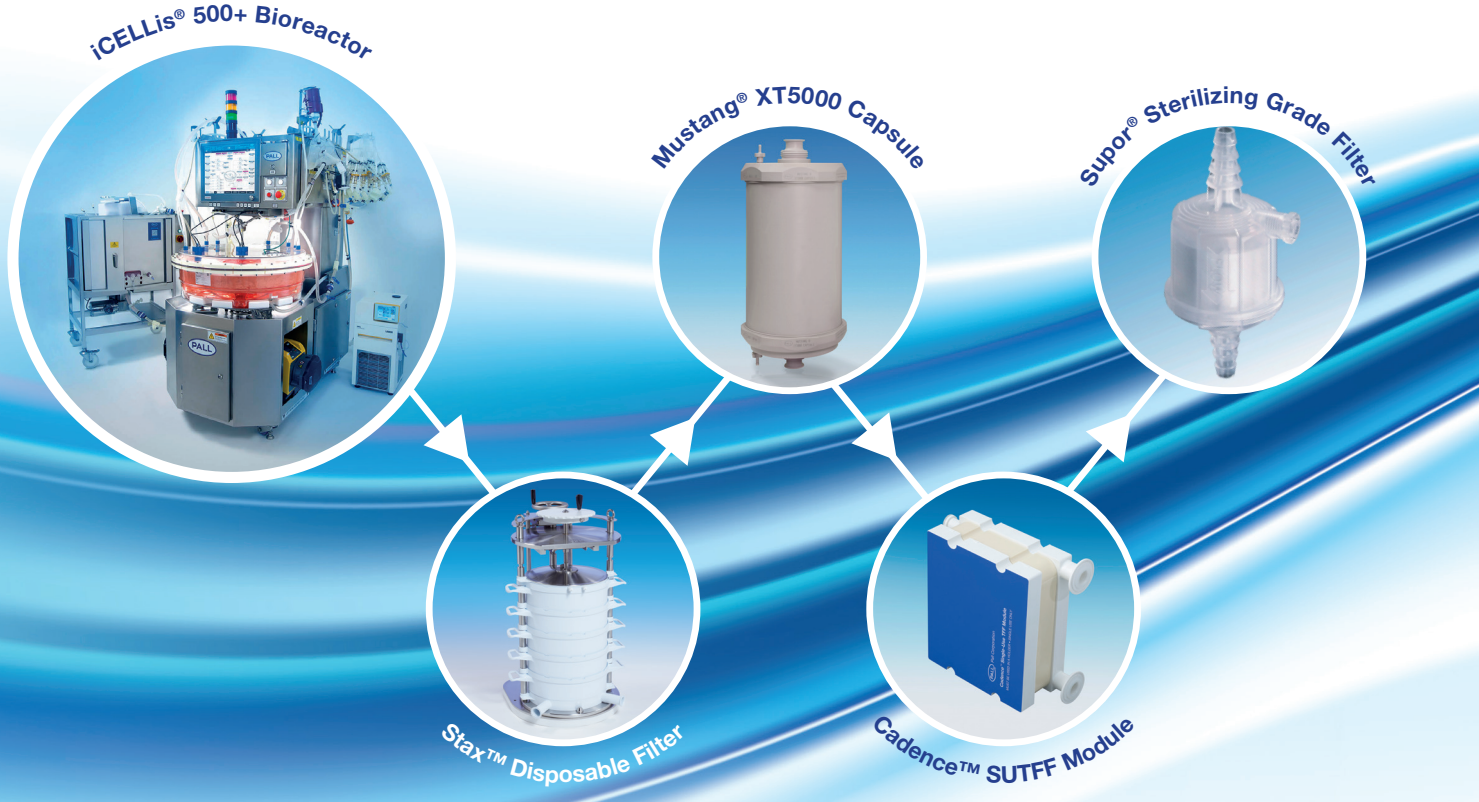
[3]Director of New and Enabling Technologies, Cell and Gene Therapy Catapult

DOI: DOI: 10.18609/cgti.2019.024

PALL Biotech

An End-to-End, Integrated Gene Therapy Solution

- **Accelerate Development**
- **Simplify Your Process**
- **Reduce Risk**



Pall Biotech can provide a fully integrated, scalable, and disposable gene therapy solution from development through to commercialization, from upstream and downstream processing through to formulation and filling. Multiple, standard unit operations can be combined into a single, automated process.

- Simplify and streamline your process while reducing risk for operator error
- Reduce time to market
- Reduce overall cost of goods

Learn more about the benefits of moving to an integrated solution in this case study, *An End-to-End Solution for Large Scale Adenovirus Manufacturing*.

Watch today at www.genetherapywebinar.com

Continuously Improving Bioprocesses

The freedom to choose your gene therapy journey