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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:
Preclinical & Translational R&D

Guest Editor: Sven Kili

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CELL & GENE THERAPY INSIGHTS

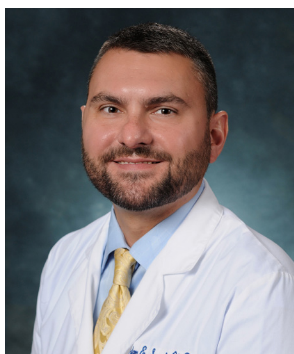
PRECLINICAL & TRANSLATIONAL R&D

SPOTLIGHT

INTERVIEW

Preclinical toolkit evolution for cancer vaccines & CAR-T cell therapy

David McCall, Commissioning Editor, BioInsights, speaks to **Adam Snook,** Associate Professor, Department of Pharmacology, Physiology & Cancer Biology, Thomas Jefferson University



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Q What are you working on right now?

AS: I am working on several translational projects around cancer vaccines and CAR-T cells. We have completed a Phase 1 trial and have an ongoing Phase 2 clinical trial looking at cancer vaccine to prevent the recurrence of gastrointestinal (GI) cancers. We have patients enrolled with colorectal, pancreatic, esophageal, and gastric cancers, and they are receiving this vaccine after standard adjuvant therapy in the hopes of preventing recurrence. We are working on CAR-T cell therapies to treat active metastatic disease. We are aiming to initiate a clinical trial of our lead CAR-T cell product around the middle of this year.

Q How would you broadly characterize the current nonclinical toolkit in the CAR-T space – the current state-of the art, as well as key remaining shortfalls and needs for innovation?

AS: We have come a long way, but we also have a long way to go. There are different problems associated with the different domains of cell and gene therapy. For example, much of the work done in CAR-T cells involves human cancers in immunodeficient mice treated with human CAR-T cells. The nuances of the context of the tumor microenvironment and its impact on CAR-T cell efficacy and detecting off-target toxicity cannot be understood fully in those model systems.

We are starting to make advances. There is an increasing number of humanized mouse systems out there, either through peripheral blood mononuclear cell (PBMC) transfer into mice, a reconstituted immune system, or even bone marrow transplants in humanized mice. There are more model systems being generated to do human gene knock-in into mouse models to recapture some of that on-target toxicity, or to investigate other impacts of interaction with the therapy and the on-target antigen.

However, when it comes to vaccines, we are largely stuck with using syngeneic mouse model systems. We generate mouse versions of the vaccines where they express the mouse version of the target, and learn as much as we can there. The problem is that when we pivot to the human versions of our vaccines, they have never gone into an animal model system for safety or efficacy testing as the antigens do not line up – therefore, we cannot learn anything about safety or efficacy with those human versions of those vaccines using syngeneic mouse models.

Q Where is innovation happening in the development of animal models to improve prediction of efficacy, especially in cellular immunotherapy?

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AS: The major innovations are around the humanizing model systems. Reconstituting mouse models through PBMC transfer and bone marrow transfer is by far our biggest innovation. Additionally, there is a growing use of larger animal systems. Some non-human primate work is being done around CAR-T cells, for example. The most applicable of these larger models are canine studies because there we get to conduct actual clinical trials where a companion dog, someone's pet, comes in with a cancer and investigators treat it with a specifically designed version of the therapy. This gives investigators a much better idea about safety and efficacy, and provides a new option to treat the pet. It is not broadly accessible yet. However, at the University of Pennsylvania, there are pioneers in CAR-T cell therapy alongside a fantastic veterinary school, and there has been some collaboration there to perform canine cell therapy trials. The National Cancer Institute (NCI) has also put together a consortium around testing and analyzing canine cancer therapies to advance therapies for pets and humans.

"The major innovations are around the humanizing model systems. Reconstituting mouse models through PBMC transfer and bone marrow transfer is by far our biggest innovation."

Q What is your view on the current utility of *in vitro* and *in silico* models as translational tools? How do you expect this area to evolve in the future?

AS: It is not an area in which I have found a whole lot of utility, although it is great for hypothesis generation. We find markers and use the cancer genome atlas (TCGA), but these tools are not particularly useful for giving us hints about safety and efficacy. This is because those systems rely a lot on expression. Some targets are present in a tumor and absent everywhere else, but many of the targets that we are working on do not fall into that paradigm. One target we are testing right now in patients is expressed throughout the entire intestine. Anything you do *in silico* or through databases is going to say that is probably a bad target, but we found that it is a favorable target due to other mechanisms that prevent toxicity. However, those mechanisms are not captured yet in *in silico* work.

In terms of *in vitro* models, we have made a great deal of progress in the field in the last decade around organoids and induced pluripotent stem cell (iPSC)-derived models. These have given us model systems to either model normal cells or potentially cancer cells, in certain circumstances. Colon organoids were among the first such models to be developed, and they provide a great tool for us. From a regulatory perspective, the US Food and Drug Administration (FDA) is starting to look for use of primary human-derived cells that are

normal or iPSC-derived cell systems for off-target toxicity screening. The field has seen so many severe toxicities with CAR-T cells in people where we could not test those toxicities in a mouse model due to species differences. In the past, investigators have gone into patients with much bigger unknowns around potential off-targets. But some of those newer *in vitro* model systems are now good at detecting them, and are going to be pushed by regulatory bodies as far as using them to de-risk CAR-T cells.

Q Could you elaborate on progress in development of models specifically related to dosing?

AS: Dosing is unusual – when it comes to CAR-T cells or vaccines, I have not yet found model systems to be good for telling us about dose levels. If you scale allometrically it does not end up panning out, so often we will use the *in vitro* or animal model dosing data, as well as patient data from similar products, to provide a rough guess about where to start for patients. We may go in a bit lower, then work our way up, knowing we are going to have to cover a wide dose response range before we land at a place that is potentially effective whilst still being safe.

Q What models can provide insight on the combinations side?

AS: Model systems for combinations are more useful. In the context of cytotoxic T-lymphocyte-associated protein (CTLA)-4 and programmed cell death protein (PD)-1, we have taken the human versions of those genes and put them in mice so that the mice express the human versions of those targets. This allows people to use the human versions of those drugs and test them in combinations with various different kinds of therapeutics. We are seeing this certainly around checkpoint inhibitors, which is a huge field for immuno-oncology, of course. Several combinations are being tested now, centered mostly around PD-1 as a part of the combination. We are also seeing it in CAR-T cells, through potentially adding checkpoint blocking drugs. Many researchers are doing gene edits in CAR-T cells and we are also looking at those kinds of combinations – PD-1 deletion or other kinds of gene deletion – and then testing those combinations in CAR-T cells in animal models. We have found that this method is good at predicting potential synergies in patients.

Q What for you is the latest progress with, and outstanding innovation need surrounding, the application of single cell sequencing technology in advanced therapies discovery and nonclinical R&D?

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"...there are people making advances around creating blood vessels with parenchymal cells and recapitulating tissue organization, for example. It will be interesting to see where they go and where they might be able to have an impact, especially in cell therapy."

AS: For me, the biggest impact for single-cell sequencing is reverse translation. This means taking materials from patients in trials, studying those, and potentially figuring out mechanisms of efficacy or resistance. We are also starting to see more applications of single cells on the chemistry, manufacturing and controls (CMC) side, through things like vector copy number analysis in CAR-T cell therapies, or looking at off-target edits made by CRISPR Cas9 editing in CAR-T cells. In fully syngeneic systems, people are using single-cell analysis for mouse cancers to look at subsets of immune cells and their impacts on efficacy. So far, though, I have not seen as much application of single-cell techniques in the standard human CAR-T cells in mouse models to tell us about efficacy.

Q What do we need to do as a community to move preclinical and translational R&D forward in the cell and gene therapy space?

AS: Regulation is among the most interesting and challenging areas right now. We are advancing cell and gene therapy so quickly that it is particularly challenging for regulations to keep up – especially around gene editing, where regulators' expectations around on- and off-target editing change very quickly. CRISPR Cas9 has been the focus for many gene editing approaches in trials to date, but there are several companies that have come up with their own, novel designer nucleases, which are going to be in trials very soon. They will potentially have different dynamics. Furthermore, figuring out what regulators' expectations will be around CMC for those kinds of therapies is a big unknown. The FDA for its part has recently released some draft guidance and captured feedback on those in order to move gene-edited cell therapies forward with greater confidence and speed.

Q You mentioned that organoids are effective compared to other preclinical tools. What do you see as the next steps towards that technology's advancement?

AS: One of the ways we can advance them would be through more sophisticated *ex vivo* systems, which for the most part are homogenous. A colon organoid would contain colon epithelial cells – they can differentiate into some epithelial subsets, but, overall, they are not very complex. It is hard to capture the nuances around the different kinds of cells as well as their spatial organization. I have seen work around tissue-on-a-chip-like technologies. They are still in their early days, but there are people making advances around creating blood vessels with parenchymal cells and recapitulating tissue organization, for example. It will be interesting to see where they go and where they might be able to have an impact, especially in cell therapy.

Q Lastly, can you pick out one or two key goals and priorities that you have for your work over the coming 12–24 months?

AS: Our key goals are moving some of our therapeutics from the end of preclinical development into first-in-human clinical trials. As I've mentioned, we have one CAR-T cell product that will begin a clinical trial this year, and a combination vaccine trial that will start up this year as well.

We are working on manufacturing a combination of adenovirus and listeria as two vaccine vectors. Nobody has tested these vectors in combination before, in either patients or mouse model systems. The nature of our work can be antigen-focused at times, which allows us to explore different kinds of vector systems. During my early days in this work, we tested adenovirus vector, vaccinia virus vector, and even a rabies virus vector as potential vaccine candidates because we were interested in the antigen and open to different possibilities for the carrier. Moving this new vaccine combination to a trial this year and seeing how that might impact GI cancer recurrence is probably the thing I am most interested in and focused on.

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PRECLINICAL & TRANSLATIONAL R&D

SPOTLIGHT

INTERVIEW

Navigating the path to clinic for ATMPs: a regulator's perspective

David McCall, Commissioning Editor, BioInsights, speaks to **James McBlane,** Preclinical Assessor in the Clinical Trials Unit, UK Medicines and Healthcare Products Regulatory Agency (MHRA)

JAMES MCBLANE completed BSc and PhD degrees in pharmacology and worked for the drug company, Wellcome, before moving to the Medicines Control Agency and then to a Japanese biopharmaceutical company, where he worked for 10 years. He returned to work at the Medicines and Healthcare products Regulatory Agency (MHRA) in 2005 and had a role as an assessor split between the Clinical Trial Unit and also the unit for Biological Products, where he is now based. He has worked on thousands of clinical trials and hundreds of marketing authorisation applications and given development advice to hundreds of companies. For 6 years from 2013, he was part of the European Medicines Agency's Committee for Advanced Therapies (the CAT) and until early 2019, he was also part of the EMA's Scientific Advice Working Party.

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Q What are you working on right now?

JM: There are two elements to my job. One is the assessment of applications, which, in the last 3 months, has taken up about 40% of my time. The other 60% is spent giving advice to companies about development of their products. The framework of the assessments includes both clinical trials and marketing authorization applications. The advice is currently taking up extra time due to material delays during COVID, which created a backlog.

I actually spend only a relatively small amount of my time on advanced therapy medicinal products (ATMPs). One reason for this is that if a company wishes to have an ATMP on the UK market, there are two components to consider: Northern Ireland and the rest of the United Kingdom (England, Scotland and Wales). If the company wants both markets, it must go to the European Medicines Agency (EMA) to gain access to the Northern Ireland market, and the MHRA for the rest of Great Britain. In that case, the companies could see that coming to the MHRA at the same time as the EMA puts them in some jeopardy, should review by either authority have a negative impact on that of the other. At any one time, they would rather do one or the other, and Northern Ireland represents the larger market because EMA approval also allows access to the other countries in the European Union.

The UK Government does not want to put UK patients at a disadvantage in accessing these ATMPs, and where the EMA has given a positive opinion, which includes for Northern Ireland, then the question is: how can it be different in the rest of Great Britain from Northern Ireland? This has led to the approach where if an ATMP has been licensed by the EMA, the MHRA will do a light-touch review, and only raise objections if there is a serious public health concern recognized: this seems unlikely to happen.

Therefore, as an MHRA assessor, we are not likely to see many ATMP products first because most come to the European regulators first, and MHRA will only see them if that review is positive. This happens specifically for ATMPs – it is not happening for most other products, partly because whereas all ATMPS are obliged to go through the centralized European system, this is not so for most products.

Q Nevertheless, you have amassed a tremendous amount of experience in the preclinical assessment of ATMPs over the past decade and more. Firstly, can you take us on a journey through the general trends and evolution in both regulators' and sponsors' expectations and approaches over this period?

JM: In the last 10–15 years, we have established a greater reliance on proof of concept in rodent-based studies. For instance, when CAR-T cell products first came onto the scene, they were considered amazing products with tremendous efficacy. They were

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initially developed by studies in mice and rats, and they were not studied in non-human primate species to obtain their initial clinical trial approvals. Instead, they went from proof of principle pharmacology studies involving experimental circumstances with a human tumor and immune system in an immunodeficient mouse, using that mouse as a living test tube to demonstrate proof of principle.

> "Regulators should be more comfortable now to accept that profile from industry rather than have an expectation that there should be additional separate studies for safety requirements."

10–15 years ago, there were more products tested on what might be considered unusual species, such as sheep, goats, and pigs. At the time, species were considered in terms of the nature of the use of the product. For instance, if a product was designed to be injected into the knee (there were some tissue engineered products around at that time), then a large animal of relatively similar physical size as a human and with weight-bearing joints was desirable to give proof of potential efficacy in humans.

In those circumstances, there was uncertainty as to what should be done for safety studies, though. One change over the last decade has been a move away from requiring separate GLP (Good Laboratory Practice) studies for these products, where *in vivo* pharmacology studies are carried out and the animal's vital functions are monitored. In that context, there is no need to do additional safety pharmacology studies or general-toxicity studies. Regulators should be more comfortable now to accept that profile from industry rather than have an expectation that there should be additional separate studies for safety requirements.

The other trend is an increased focus on *in vitro* methods to look at carcinogenic risk. Typically, we would see mice studies followed up for several months and demonstrating no tumors. (Of course, if there was a demonstration of new tumors in the mice, they would not come before the regulator in the first place). That is an uncertain scientific basis on which to make a judgment, though. Having a human cell product in an immunodeficient environment in a mouse is not an effective way to assess whether there is a carcinogenic risk. Instead, we would now look for *in vitro* methods demonstrating genetic stability and *in vitro* cell growth. That is one of the major changes.

Q Do you see evidence of a recent trend in sponsors once again looking to use more large animal models?

JM: I would not say so. I think the trend has been in the other direction, in fact, towards a greater reliance on rodent models. I do wonder if there is a perception currently that the US Food and Drug Administration (FDA) wants to see more industry studies done on non-human primates. However, I do not think this perception is necessarily true.

Again, I think it partly depends on the indication. For example, if you are going for a novel indication directly into the heart and injecting a cell product or virus into that area, then you could argue that if you use a small heart like a mouse heart, the dose will distribute over the small area involved and give a therapeutic effect; scaling up to a human volume, distribution may not suffice to give the therapeutic effect. It may be that in a mouse heart, that dose would give a much better effect than would be seen in a human heart. So, there is an argument to say that to investigate dose and assess efficacy, you should use a model that mimics human size. A rodent might overestimate the potential of the product. This could be one justification for using something other than a rodent.

From my perspective, the animal model selection should be case-by-case. But the general principle is that you should be using the lowest form of animal you can: in other words, you should not be using pigs, sheep, or primates if you can perform relevant studies in mice and rats.

Q As someone who provides advice on how to successfully navigate the preclinical-first-in-human clinical trial transition, what would you pick out as the key pitfalls that ATMP sponsors tend to struggle with at this stage?

JM: The biggest issues are not in the preclinical dossier. Proof of concepts and proof of preclinical principles tend to be reasonably robust, and we do not get people applying who have no idea of why a product would work. There is usually a reasonable rationale for believing the product might have efficacy. This is obviously important because these products are going straight into patients, and a patient must have a chance of getting an efficacious dose. Even from the very first dose, you should be trying to identify what your estimated best dose will be. Otherwise, you are not offering the patient any efficacy: you are asking them to take risks that are, in a formal sense, unknown, as the product has not been given to humans before, without any chance of benefit.

The problems I see tend to come when shifting over from academic development to industrial/commercial development, as there is often a major change in manufacturing. Consequently, it can become difficult to understand whether the product used in the initial studies, including the proof of principle studies, is the same as the product which is to eventually be put on the market.

One difficulty that regulators often have, both with companies and academics, is getting that perspective understood. During development, either for scale-up or practicality reasons, manufacture will often change. It is hard to tell with great accuracy that a single protein is similar to another single protein, and with cells, it is almost impossible to show by a series of tests that you have a similar product. The manufacturing controls the product profile – therefore, when manufacturing changes, it could cause problems that are not seen the same way on the developer's side as on the regulator's side.

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> "From the preclinical perspective, for ATMPs more than any other type of product, by the time you reach your first human dose, the likelihood is that you will have completed all of your preclinical development. That is unusual for most drugs, which may have ongoing reproductive toxicity or carcinogenic studies, for instance, well into clinical development."

The other thing I would highlight, which is more of a clinical issue, is the lack of robustness common in ATMP clinical testing. Many of these products are licensed on single trials which are not comparative – the comparators are done with historical controls or with a panel of selected patients, rather than as randomized control trials. The most common reason for this is because ATMPs are frequently developed for very rare indications.

However, even with more ATMPs now being developed for larger markets offering plenty of patients with whom to run randomized control trials, we still rarely see these comparative trials being conducted. I do not understand why this is.

I was on the EMA's Committee for Advanced Therapies (CAT) for many years, and it seemed almost every product would come with the same perspective: the product would be for a very rare indication, meaning it would be unfair on patients to run a placebo-controlled trial. Therefore, you give all the patients in the study the active product and compare them against a control group that the companies would devise with selected patients. That is not as good as running side-by-side, blinded trials, which is the best way to really show if something works.

The issue here is that once you have done the initial trial, if you get a large therapeutic response in these otherwise difficult to treat cancers, then in the future you cannot perform a randomized control trial, because nobody is going to volunteer knowingly to be the person who gets the control. If you do not do it at the beginning, it becomes difficult to do later.

So, these two elements are what I see as being the biggest difficulties: first, the consistency of manufacture and proving the product is the same, and secondly, the approach to clinical testing, which is partly due to the nature of the indications these products are aimed at.

Q What best practices should be employed to avoid these issues?

JM: From the preclinical perspective, for ATMPs more than any other type of product, by the time you reach your first human dose, the likelihood is that you will have completed all of your preclinical development. That is unusual for most drugs,

which may have ongoing reproductive toxicity or carcinogenic studies, for instance, well into clinical development.

On the other hand, neither academics nor fledgling ATMP biotechs can or will spend the money early in development to set up a manufacturing process that will be capable of one day treating 1000s of patients – not when they only want to do a first-in-human clinical trial treating a dozen patients. There is a trade-off between showing you have something that is worth investing in in the first place, and spending too much money initially only to find you do not have anything.

Nonetheless, I would advise developers to do their utmost to anticipate future needs. If you are going to do a toxicity study, or are evaluating a genotoxicity component *in vitro*, then the material you use for that should be representative of what you intend to move to the market, even if it is at an early stage. Product manufacture should be aligned to the eventual registered patent, or more preferably, it should actually be what is eventually registered.

Q What is your take on the continuing application of and reliance on preclinical *in vivo* models – and particularly small animal models – with their arguably limited value in areas such as immunotherapy? And how do you expect this area to evolve over the foreseeable future?

JM: When you first see a clinical trial application, it is at the stage where the sponsor believes the product in question is going to work. The regulator who looks at that clinical trial application must ask why someone thinks this product is going to work and be safe.

Sometimes, especially for ATMPs, you can see a reliance on work done with other products which removes the requirement to conduct some animal studies. For instance, for mesenchymal stem cells (MSCs), people may come up with their own version of an MSC which may not require any additional preclinical *in vivo* work. Instead, the sponsor can cross-refer to what is already publicly known and establish that their product is similar to the other MSC product's profile. The same may be true regarding the dosing methodology. You need to have a concept of patient treatment and how you are going to administer the product. While you probably do not need any direct *in vivo* data to justify if it is going to be given intravenously, if you are using an unusual route, such as injecting into the eye, you must have evidence to prove that this approach is necessary. Again, though, there may be another product of similar type that has previously been injected into the eye, for example, so you do not need to do that specific study because you can refer to publicly available information as justification.

My point is that while it may be possible to avoid conducting certain new animal studies today, preclinical *in vivo* data does remain of crucial importance. People often say that CAR-T cells were not reliant on animal studies to be developed, but it depends on where

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you draw the line. Clinical CAR-Ts were not given to animals, but there were 10 years of experiments before clinical testing started that used mice and CAR-T constructs of different kinds. The functional protein in the cells that did work in mice was then translated to humans.

> "Overall, I would say that today, regulators are in general much more willing to be flexible and partner with developers on unusual product development."

So, the question of whether we should expect the degree of reliance on animal models to change over the foreseeable future is a tricky one. It is hard to say we are still going to be doing things the same way in 100 years' time, as so much has changed in the last century. But for me, in terms of getting dosages, proof of principles, and safety, it is still difficult today to think that the answers are going to come from anything other than some sort of animal study.

In terms of specific areas where *in vitro* tools can supplant animal studies moving forward, the best example I see relates to the move away from *in vivo* carcinogenicity testing. Most of the western regulators, plus the likes of Japan and Korea, would mostly be aligned in not asking for *in vivo* studies for carcinogenicity for any ATMP.

For adeno-associated viruses (AAV), much of the work here has already been done. We know they do not integrate; they sit in the genome and do not do much harm. That kind of work does not need to be done again for a new product based on that technology.

In a cell therapy, we would be looking for genetic stability and *in vitro* cell growth. We would not be looking for *in vivo* work for that, because it is mostly not relevant. If you give the therapy to an animal and it does not cause cancer, it does not relate to not causing cancer in the human environment because there are so many other factors impacting the product *in vivo*. Those *in vivo* factors are not affecting the *in vitro* environment. The *in vivo* environment is a much tougher environment, as the immune system is basically missing in the *in vitro* assessment. If tumors cannot grow in vitro in the optimum environment for them, where there is nothing stopping them from growing, then you would expect there to be no problem *in vivo* in humans. There is no longer a need for proof of this to be derived from animal studies.

Overall, I would say that today, regulators are in general much more willing to be flexible and partner with developers on unusual product development. For instance, where a company can demonstrate that a product has no activity in any animal species and that it only works in humans, then it does not make sense for any work in animals to be done to characterize safety. 30 years ago, there was the mindset that animal studies should always be done under any circumstances, even if not relevant.

What do we need to do as a community to move preclinical and translational R&D for ATMPs forward?

JM: For me, this question seems to have a simple answer, which is that there must be better communication between developers and regulators so that there is a better understanding of each other's perspectives. This could happen in two ways.

One way would be for regulators to actively engage with the community, attending meetings and hearing other people's perspectives. Perspectives from other regulators can often be quite difficult to get hold of. For instance, I have personally never been to a US FDA company meeting. I have never had a chance to hear the FDA say something about a specific product. The EMA has provided engagement in the past, but now that the UK is no longer part of the EU, we no longer get that experience.

On the other hand, I would encourage both companies and academics in the early stages of R&D to consider getting scientific advice from regulators. Every regulator in the ICH sphere can offer scientific advice to companies about what they should be doing, or at least whether their plans would likely meet regulators' expectations of quality, safety, and efficacy.

Of course, even if you can do meet those expectations and get an ATMP to market, the other hurdle becomes whether someone will pay for it. In relation to that, if you are at the preclinical/translational stage, it is probably too early to come forward to the authorities. But you do need to consider the design of your Phase 3 and how you are going to demonstrate the benefit of the product, not just in terms of patient benefit but in pharmaco-economic terms. While your first trial is ongoing, then, you should be engaging with potential HTAs and payers. The MHRA offers combined advice with The National Institute for Health and Care Excellence (NICE) in the UK. You can get opinions on both what the regulator would feel you would need to do to get approval, and what NICE would feel you would need to do for them to offer a recommendation of reimbursement for your product. ATMPs are much more expensive than other biological products, so that kind of engagement becomes even more important. Scientific advice can be the answer to this problem.

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL R&D

SPOTLIGHT

Preclinical safety assessment in therapeutic genome editing

Roberto Nitsch
Director of the Gene Therapy Safety, AstraZeneca

"There is a clear need in this field for better and more translatable preclinical models. Using humanized models provides an environment in which human cells behave more like they would in the body…"

VIEWPOINT

On February 23rd 2023, David McCall, Editor, *Cell & Gene Therapy Insights*, talked to Roberto Nitsch, Director of the Gene Therapy Safety at AstraZeneca, about his work in the space of genome editing nonclinical safety assessment and model development. This Viewpoint article is based on that interview.

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My current work revolves around the application of a novel technology in the field of gene therapy called optical genome mapping. In this case, 'gene therapy' means gene editing for therapeutic purposes, using CRISPR/Cas9. Optical genome mapping allows the mapping of the entire genome of a cell in a single run and in a high-throughput manner. It is extremely specific and has a high detection limit. Currently, we are pioneering its use to study safety in the gene therapy field.

While CRISPR gene editing is more commonly used *ex vivo*, for example for cell-based therapies, my research is really focused on the use of *in vivo* gene therapy – and therefore, on studying the safety for *in vivo* applications.

Before gene therapies can become available to patients, it is essential that we assess any candidate gene therapy for off-target effects, its potential to cause chromosomal rearrangements, and any potential immunogenicity. Our gene therapy safety group is also continually working to identify any undiscovered issues, to ensure we ultimately deliver therapeutics that are optimized for both safety and efficacy.

There is a clear need in this field for better and more translatable preclinical models. Using humanized models provides an environment in which human cells behave more like they would in the body, generating data that are more relevant to patients than other, non-humanized models.

While there are a variety of *in vitro* and *in silico* models available, no preclinical model is perfect. Therefore, we are exploring alternatives to such models by using our existing knowledge base. For example, we have experience applying genome editing in patient-derived primary cells and developing better software and algorithms to predict some of the possible unwanted consequences of CRISPR. Nonetheless, this is becoming increasingly challenging due to the heterogeneity of the human genome and often, we must work with a reference genome that does not belong to our patient. Going forward, in order to design more effective medicines for all patients who need them, we must develop better software and more reliable algorithms that incorporate more of the single nucleotide polymorphism (SNP) variants and the heterogeneity of the different human genomes. In addition, the community is investigating the feasibility of using microfluidic systems, which are complex *in vitro* systems that nicely mimic the physiology of humans. However, there is still much more work to be done for their application towards regenerative medicine.

The need for better delivery of gene editing machinery is clear. Currently, we have several viral and nonviral delivery methods, each of which has its advantages and disadvantages. However, I believe that this delivery area will likely undergo an impressive transformation in the next couple of years.

The other field of significant innovation is gene editing applications. One particular area of excitement is gene editing without double-strand breaks. This could be in the form of alternatives to clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 that are based on CRISPR – base editors or prime editors, for example. I strongly believe that genome editing without double-strand breaks has significant potential to transform the field, especially when we want to precisely introduce a correction or a full gene into the genome, and not just disrupt the defective gene.

Gene therapy is already making a huge difference in people living with rare diseases. In the near future, based on current therapeutic trends, I expect to see gene therapy being used to treat patients living with non-rare, genetic disorders. Gene editing technologies such as CRISPR have the potential to enable treatment of numerous genetic diseases with high unmet medical need.

The ultimate goal of my team is to help bring gene therapies into the clinic. The efficacy of CRISPR technology has been widely studied, so myself along with my team devote specific attention to and focus on the

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safety profiles of gene therapy medicines. The evidence we have to date for the safety profiles of these therapies is compelling; I hope that we can be a part of bringing these medicines to patients.

BIOGRAPHY

ROBERTO NITSCH graduated in Medical Biotechnology from the University of Naples (Italy) where he also obtained his PhD in Molecular Genetics. He later moved to Vienna where he focused on mouse genetics and cancer biology, and lately recessive genetics. He then shifted his research topic to genome engineering with CRISPR/Cas9 and joined AstraZeneca in 2014 where he was responsible of the CRISPR mouse models for drug discovery and oncology. Since 2017, he is Associate Director in the Clinical Pharmacology and Safety Sciences pioneering safety assessments for Therapeutic Genome Editing. Today Roberto is the Director of the Gene Therapy safety group at AstraZeneca, and he is supporting the generation of CRISPR medicines.

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL R&D

SPOTLIGHT

Addressing gene therapy translational R&D bottlenecks in the CNS

Anindya Sen
Executive Director, R&D, Prevail Therapeutics

"Two major current bottlenecks in central nervous system (CNS)-directed gene therapy are finding the right capsid and identifying the right route of administration."

VIEWPOINT

On February 2nd 2023, David McCall, Editor, *Cell & Gene Therapy Insights*, spoke to Anindya Sen, Executive Director, R&D at Prevail Therapeutics, about novel AAV capsid innovation and preclinical-clinical translation challenges in the CNS gene therapy space. This viewpoint has been written based on that interview.

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As a scientist, genetic medicine is exciting because these new modalities, which include siRNA and gene therapies, target disease spaces where developing clinical candidates with intended therapeutic potential was a significant challenge for decades. Gene therapy is the most fantastic way to introduce a gene that is missing individually in patients. The technique is not new – many academic groups have worked with gene therapy. But the main issue in the space is that the work has been isolated, without any systematic effort on moving therapies toward the clinic. For this reason, and due to the concerns surrounding the death of Jesse Gelsinger in 1999 after treatment with an adenovirus-based gene therapy, industry has been hesitant to deeply delve into gene therapy.

When I moved to Prevail Therapeutics, a small gene therapy startup, in 2019, I was provided with the opportunity to build up their pre-clinical pipeline with multiple indications, including amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Parkinson's disease. Gene therapy is not one-size-fits-all. We must be cognizant of the disease space and how we approach it.

Prevail Therapeutics has two programs in the clinic, with four clinical trials between them. From being a start-up of 20 people when I joined, Prevail Therapeutics has grown to 100 people and is now a wholly-owned subsidiary of Eli Lilly and Company. We are one of the primary gene therapy units of Eli Lilly. My team does a lot of work driving discovery research through preclinical work to IND filing and interaction with regulatory agencies to support patient dosing.

A TRANSITION FROM SMALL TO BIG PHARMA

The transition into a large organization has been highly advantageous. When working within a small biotech, the focus is often on one or two programs, and the opportunities for platform development are minimal. Gene therapy itself requires a lot of platform development that could drive innovative medicines in a complex disease space.

We are an independent subsidiary of Eli Lilly, so we are doing what we do best, but with all the additional resources of a large organization to bring effective therapies to the patients.

Recently, there has been a push from the National Institute for Health and Care Excellence (NICE) and regulatory agencies to raise awareness for rare diseases. We have been focusing on investigating the rare disease space and seeing how our technology and modality can benefit these small patient populations.

NAVIGATING REGULATORY UNCERTAINTY

A strength of gene therapy is that it is a one-and-done therapy, but this can also be a weakness: once done, there is no going back. Once in a patient, the therapy stays there, unlike with small molecules or biologics. This means that the regulatory bar is high for us to ensure only effective and safe therapies are entering patients.

Regulatory interactions have been positive because regulators, both inside and outside of

the USA, are ready to learn from the sponsors and understand the sponsors' viewpoints. As a sponsor for a particular program, you must set the bar for yourself. You must ensure the candidate you are taking to the patient is going to be effective and safe.

When I started working in gene therapy, there were no clear guidelines at all. The first set of robust and concise guidelines appeared only recently. Two major current bottlenecks in central nervous system (CNS)-directed gene therapy are finding the right capsid and identifying the right route of administration. In addition, regulation of gene therapy will be a much larger space in the coming decade. Figuring out how to regulate or even shut down a gene therapy that has already entered the patient is key.

FINDING THE RIGHT CAPSID

Historically in CNS gene therapy, researchers have chosen to use the low-hanging, universal capsid fruit, which is often AAV9. People have chosen this due to its ability to cross the blood-brain barrier and because of the success of Zolgensma™, an approved gene therapy for spinal muscular atrophy. However, there is a need for new capsids within the field.

What works for gene therapy or a drug *in vitro* is often not translatable when you take it to a system like mice, non-human primates, or large animals. In an *in vitro* system, the system is usually overloaded with many adeno associated viruses (AAVs), so that AAV reaches most cells. When you are delivering AAV in mice or non-human primates, though, not all the cells are going to be affected. Thus, we must be careful when using *in vitro* systems. They are useful for initial screens – for developing an understanding of a candidate, or providing some *in vitro* proof of efficacy – but this is often not translatable. The main reason for this lack of translatability is the capsid.

We need to find a capsid that targets the maximum number of disease-relevant cells. If you want to hit neurons, you need to hit as many neurons as possible, without hitting other cell types. It is important to understand what the specificity is, the disease space you are going into, and the disease-relevant cell types.

There are many efforts underway by both smaller biotech and large pharma companies to find new capsids. One specific need is to find new capsids that target the cell type of interest, but also finding promotors that will specifically express in those cells. It has become clear that universal promotors do not express in all cell types with equal efficiency. If a gene is not efficiently expressed in your cell type of interest, even having a great capsid will not solve the problem.

In the CNS gene therapy space, one current issue is that the capsids for serotypes like AAV9 target dorsal root ganglia (DRG). There is no clear clinical consequence to this yet, but it may cause DRG toxicity with increased mono-nuclear cell infiltration. A novel capsid must de-target the DRG and the liver. For an optimal second-generation gene therapy, scientists are investigating tailor-made capsids for particular disease spaces. Such new capsids will take us closer to precision medicine in gene therapy.

FINDING THE RIGHT ROUTE OF ADMINISTRATION

With Zolgensma, patients are often dosed within the first six months of their life utilizing systemic delivery. At that time, the therapy crosses the blood–brain barrier relatively easily and so has good exposure to the CNS.

However, that is not the case when treating an adult. In adults, what has been shown to be effective in multiple therapeutic programs is intra-cisterna magna (ICM) delivery, where gene therapy is injected into the cerebrospinal fluid (CSF) providing reasonably good brain exposure.

Many programs that involve a secreted protein are at an advantage, because they

do not need to hit every single cell. For cell autonomous indications, where protein is not secreted, the viral vector must hit all the target cells, so a safe and effective route of administration becomes important.

We can explore many different kinds of administration in preclinical testing. We can directly dose into the brain through the parenchyma, perform ventricular dosing, or directly dose into the affected site in the brain. However, much of this work is not clinically translatable because of an unfavorable risk–benefit profile.

The risk-benefit is important to consider. For example, with aromatic l-amino acid decarboxylase (AADC), a disease that affects young children, the risk is acceptable because if patients are not dosed, they will die. On the other hand, adult indications which are often most prevalent in the elderly, such as Parkinson's disease and Alzheimer's disease, are not immediately life-threatening and so the bar is higher.

Overall, it is key to bear in mind that not all potential routes of administration are clinically translatable. The future will be about having both a great capsid and a functional route of administration. Initially, researchers performed systemic delivery and were worried about the liver, as that is where a large part of the therapy ends up. There is current work in the space towards discovering a capsid that is systemically delivered but de-targets the liver. That way, the therapy will go into the brain and have good CNS exposure, but any liver toxicity associated with systemic delivery will be minimized.

THE CHALLENGE OF DOSE TRANSLATION

In gene therapy, it is challenging to perform a dose translation from preclinical models to the clinic. For small molecules or even large biologics, there are pharmacokinetics (PK) and pharmacodynamics (PD) datasets available, and you can use PK/PD parameters to model the patient dose. For gene therapy, this is much harder, as there is no good method for accurately measuring PK. In terms of PD, while it is the case that the US Food and Drug Administration and other regulatory agencies have asked sponsors to look at biodistribution, modeling is hard. Therefore, sponsors must work closely with regulatory agencies to understand and reach a conclusion about how a dose translation from mice to a non-human primate to a patient can be performed.

THE FUTURE OF GENE THERAPY

Right now, all gene therapy is AAV-based. Future technologies may benefit from being delivered without the use of AAV, as the virus has drawbacks. AAV is safe, but it is still a virus. The next generation of gene therapies will deliver a payload of interest without having an AAV capsid. Instead, a nanoparticle or another sort of inert vehicle could be used that does not carry the same baggage as a AAV.

The other future area of interest is regulation. That is key because we need to know that once a therapy goes into the system, we still have a level of control over it. The space needs to investigate how to regulate once a gene therapy has been given.

As someone who has been in the preclinical development space for a long time, one piece of advice is to be careful when choosing a preclinical model. Understand that some of the endpoints you look at in the preclinical model are probably not going to be translatable in the clinic. That is fine, as long as you know what you are looking for and how you are going to interpret that preclinical model. In precision medicine, you need to know what you are looking for and design a drug based exactly on the needs of that particular disease space – again, this is not a 'one-size-fits-all' field.

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BIOGRAPHY

ANINDYA SEN who is a geneticist/neurobiologist by training and is a pharmaceutical professional with experience in driving high impact, modality agnostic research groups with development experience in small molecules, gene therapy, and antisense oligonucleotides. His interest in drug development was fostered by postdoctoral training at Harvard Medical School, followed by preclinical research at Biogen in the Neurodegeneration/Neurology team. At present, he is a key member of genetic medicine efforts at Prevail Therapeutics, a wholly owned subsidiary of Eli Lilly and Company, focusing on AAV-based gene-therapy, dedicated to driving innovative treatments for patients with neurological disorders.

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL R&D

SPOTLIGHT

INTERVIEW

Assessing the evolving AAV preclinical safety landscape in 2023 & beyond

Abi Pinchbeck, Assistant Editor, BioInsights, spoke to
Basel T Assaf, Distinguished Scientist, Preclinical Safety, Sanofi

BASEL T ASSAF is a board-certified veterinary pathologist and a distinguished scientist in the Preclinical Safety Department at Sanofi. In his current role, Dr Assaf oversees the preclinical safety support for the Genomic Medicine Unit and serves as a preclinical safety representative on governance committees for the review and management of the gene therapy portfolio. Prior to joining Sanofi, Dr Assaf held several positions with increasing responsibilities in toxicology and pathology, including FDA Commissioner's fellowship in the Office of Tissues and Advanced Therapies of US FDA, Investigative Pathologist at the Oregon National Primate Research Center and the Vaccine and Gene Therapy Institute of Oregon Health and Science University, and Senior Principal Scientist - Veterinary Pathologist in the Drug Safety Research and Development of Pfizer Inc. Dr Assaf has a PhD in Comparative Pathology from University of California – Davis, and a post-doctoral research and anatomic pathology residency training from the New England Primate Research Center of Harvard Medical School.

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Q What are you working on right now?

BA: We are working on a diverse platform of cell and gene therapies and applying viral and non-viral gene therapy to different formats to treat a wide range of disease indications.

Q What for you are the main challenges in getting therapies to the clinic in the cell and gene therapy space?

BA: Simply put, you want to eliminate as many variables as possible in your drug development process. This is primarily achieved by creating consistency within the program as early as possible and ensuring minimal changes in the middle of the drug development – especially in the manufacturing process. The earlier you can minimize or eliminate any potential changes, the faster you can move to the clinic. But currently, particularly for chemistry, manufacturing, and control (CMC) processes end up being changed during clinical trial phases, which leads to the need for bridging and comparability studies. For example, a change in CMC may lead to a change in pharmacological or toxicological properties of the candidate product, which may consequently necessitate a repeat to some of your pharmacology or toxicology studies, which will likely delay the product development timeline.

Q As both a former FDA employee and an industry preclinical R&D expert, how has the preclinical safety landscape for advanced biologics evolved over recent years?

BA: It has changed drastically, particularly for Adeno-associated virus (AAV)-based gene therapy products, which holds a major market share in the field of gene therapy. AAV was discovered and utilized at the turn of the century in the wake of severe adverse findings that were seen with other viral vectors such as earlier versions of adenovirus and retrovirus vectors. One notable example is the case of Jesse Gelsinger in 1999, which halted the field of gene therapy and prompted the need to find safer alternatives. AAV emerged as a viral vector safer than earlier versions of viral vectors for several reasons. It is replication defective as it does not contain the complete machinery to replicate on its own, and it is non-pathogenic to humans. Many humans are naturally infected with AAV, but they do not get sick. It has a lower capacity to activate the immune system than adenovirus. Thus, AAV is believed to be a safer viral vector compared with adenovirus and retroviruses. However, with the expanded use of AAV, the use of AAV at high dose levels, and the advancement of genomics and sequencing

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> "Nonclinical programs are complex. The key considerations prior to running an investigational new drug (IND)-enabling Good Laboratory Practice (GLP) toxicology study consist of five pillars that allow for a successful program progression to the clinic."

technology in gene therapy in general, safety signals have started to appear. On the upside, in many of these findings, toxicity and safety concerns continue to be animal-specific and have not been seen in humans.

The safety concerns that are discussed most often in the field of AAV gene therapy of potential relevance to human patients include the immune responses against AAV the could occur under certain circumstances and with the potential for toxicity associated with that immunogenicity. Hepatotoxicity is another concern, typically associated with immune responses as mentioned earlier, and typically happens around four weeks post-vector administration. Another concern is dorsal root ganglion toxicity that has been seen in non-human primates (NHP) and some rodent species, particularly rats. This has not been reported in humans as much, despite the thousands of patients treated with AAV. The final main concern is thrombotic microangiopathy, occurring when AAV is injected intravenously at high doses. This is almost exclusively seen in humans and has not been reported in animals. There are safety concerns that so far have only been reported in nonclinical species and hence only pose a theoretical risk to humans. These include the concern about the integration of AAV and the potential for developing hepatocellular carcinoma, which to date is only reproducible in mice due to a unique species-specific insertion site, and acute liver failure uniquely reported in NHPs.

Despite only thrombotic microangiopathy and the immunotoxicity associated with AAV being seen in humans, there are good safety measures and mitigation strategies required during clinical trials to monitor and understand these outcomes as well as ensure that the other animal-specific risks and not translatable to humans. Developing biomarker, monitoring, and mitigating strategies to minimize or reverse such safety concerns in humans is important to reap the great benefits of gene therapy for patients with unmet medical needs. When you perform risk assessments and have good monitoring and mitigation strategies for these potential safety concerns, the benefits significantly outweigh the safety concerns.

Q What would you pick out as the key considerations in designing preclinical R&D programs specifically for AAV gene therapies?

BA: Nonclinical programs are complex. The key considerations prior to running an investigational new drug (IND)-enabling Good Laboratory Practice (GLP) toxicology study consist of five pillars that allow for a successful program progression to the clinic. The IND-enabling GLP toxicology study should be viewed as a steppingstone from earlier pharmacology studies to enabling clinical trials.

The first pillar is to identify the dose-responsive relationship and understand the efficacious dose range. This information will be used for two main purposes: initially, to identify the dose range to test in the IND-enabling GLP toxicology study, and to define the first safe and efficacious dose to test in clinical trials.

The second pillar is understanding the pharmacokinetics of the therapy you have. Within the field of gene therapy, this is typically called a biodistribution assessment. In US Food and Drug Administration and European Medicines Agency guidance, the need to understand the gene therapy product kinetics is described, specifically the peak and persistent exposures within the animal, to permit the assessment of safety at these exposure levels. This pillar is essential to understanding the dose-toxicity profile of your test article at efficacious exposure levels.

This is a good segue to the third element, which is the exploratory toxicity assessments. You do not want to discover major red flags during your IND-enabling GLP toxicity study. Identifying these potential red flags in earlier exploratory toxicity studies will also permit adequate time to develop mitigation strategies for successful program progression. This includes, for example, modifying the study protocol to include immunosuppression in the study design if immunogenicity of the test article is predicted to be animal-specific and may not be translatable to humans.

The fourth pillar is understanding the relationship of your test article to your route of administration. Programs typically start with predefined route of administration based on the characteristics of the candidate gene therapy product and the target disease indication, such as the intravascular route for systemic muscular dystrophies or going into the cerebrospinal fluid compartment such lumbar intrathecal route, the cisterna magna route, or the intracerebroventricular route for neurodegenerative and neuro-inflammatory diseases. However, changes in the route of administration during program development could change the biological characteristics of the biodistribution and the exposure of the test article. Understanding the relationship of the test article to the route of administration and avoiding a change in route of administration during the product development is favored to avoid creating gaps that will likely require additional bridging work and potential delays in program progression.

The fifth and last element is critical – the consistency within CMC processes. Changing the CMC parameters may result in changing the characteristics of the test article. While small changes are sometimes not meaningful, other times can create a negative or positive impact on the critical attributes of the test article. A change in the CMC in a way that changes the critical product attributes will create gaps in the preclinical program that will necessitate additional bridging or comparability studies. Keeping the CMC consistent and understanding the characteristics of the test article, the impurity profile, the potency, and – in the case of AAV – the level of empty capsids, is very important.

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Nailing these five pillars down as early as possible, minimizing or avoiding changes, and understanding how they interplay together to support a seamless IND-enabling GLP-compliant toxicity study is key to a successful program for AAV gene therapy product.

Q How and where is innovation in animal models enabling better prediction of efficacy and safety in the cell and gene therapy field?

BA: It is important to remember that non-human animal models are just models and may not be completely predictive of humans. There is a significant biological overlap between different species, but they are not 100% identical, and even within animal species there is diversity. Hence, the gap will always continue to exist, as much as we try to close it to improve the predictability of testing in animal studies. With that being said, tremendous efforts have been taken to improve the utilization of animal models to better predict the efficacy and safety in gene and cell therapy product development.

Innovations we are seeing more often stem from how animal models, particularly mouse models of disease, were originally generated. The better an animal model is at recapitulating and mimicking the human condition, the better the predictability of that model. Historically, animal models were generated by knocking a gene in, out, or down to result in a certain phenotype that you try to reverse and correct by your drug candidate. In the field of gene therapy, many of the diseases we treat stem from a problem in a single gene. In the past, due to limitations in technology, the practices of knocking in, out, or down a gene involved some level of randomness. With newer technologies such as CRISPR-Cas technology, this can be done with significantly higher precision to allow for better models closer to the human condition of disease. At Sanofi for example, we generated a phenylketonuria disease mouse model using such approach [1].

The other helpful innovation is not directly related to the animal models, but to the tools used in animals to mimic the clinical scenario. This becomes particularly important for neurodegenerative diseases. For example, there are practices of real-time MRI-guided intracranial dosing of patients. Subsequently, having similar MRI-guided dosing of animals becomes of high value. The higher the precision of delivery of these test articles with low levels of variations in the dosing procedure, the tighter the dataset to inform and support clinical trials.

Q How could existing knowledge gaps caused by insufficiently predictive animal models be resolved moving forward? Where do you see the greatest innovation – and need for further innovation – in this regard?

> "To summarise, the key areas of innovation lie in creating more predictive in *vitro* systems by using more human cells in the settings of *in vitro* testing and using 3-dimentional cultures of human and animal cells to predict the complex biology within these species."

BA: Gene therapy in general is remarkably diverse. Looking at gene-modified cell therapy specifically, there can be issues when developing human cells and injecting them into animals, as this can lead to graft-vs-host rejection of transplanted human cells. You may need to use animal models that are immunosuppressed, which means you will not be assessing the potential impact of the immune response on these cells, and hence you do not know what the human immune system may respond to these cells once injected in human patients. These cells are beyond minimally manipulated, so the process of generating these gene-modified cells may render them immunogenic in humans.

If you are using an AAV-based *in vivo* gene therapy, then the challenges in developing non-clinical animal models also vary. One challenge is the lack of strong predictive value and translatability of *in vitro* cell-based assays to *in vivo* animal-based testing, and again from animals to humans. This problem is most apparent in dose translation for example.

I am excited to see improvements in the value of *in vitro* testing. Newer technologies allow utilization of human cells to create *in vitro* disease models in human cells are valuable to build more confidence in the test article's ability to create pharmacological effects. Another improvement is redefining dose translation parameters between animals and humans, such as the gene efficacy factor that may improve on the predictivity in dose selection [2]. The concept of gene efficacy factor, in conjunction with 2-dimentional and 3-dimentional in vitro systems may play a significant role in dose prediction and success of gene therapy programs.

To summarize, the key areas of innovation lie in creating more predictive *in vitro* systems by using more human cells in the settings of *in vitro* testing and using 3-dimentional cultures of human and animal cells to predict the complex biology within these species. These are valuable preclinical innovations and novel strategies have the potential to help bridge any potential gaps in improving the predictability of preclinical testing.

Q As we begin 2023, what are your main priorities for your work over the coming years?

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BA: We have a great pipeline, and I am looking forward to many regulatory submissions that will help us to get into the clinic sooner and help to bring medicine to patients faster and help improve the quality of their lives. This is a sincere priority for my 2023, and I hope we will be able to successfully accomplish that.

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CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL R&D

SPOTLIGHT

Key considerations when translating a cell therapy product into the clinic

Anthony Ting
Principal, BRL C> Consulting

"The development of a cell therapy product involves conducting extensive preclinical testing to demonstrate the safety of the product, and that it is likely to be effective in treating the targeted disease or condition."

VIEWPOINT

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The translation of a cell therapy product into a clinical trial involves a complex and multi-faceted approach. There are many challenges that must be overcome in order to successfully bring a cell therapy from the laboratory to the clinic. Unlike small molecules or biologics, cell therapies are highly complex and involve the use of living cells, which can

be complicated to control and manipulate. Therefore, one of the major challenges is how to create a therapeutic product that provides consistent results not only in the lab but with patients as well.

It is difficult to predict the clinical benefit of a cell therapy based on non-clinical studies and therefore, multiple assays should be performed that characterize the cell in identity, composition, and potency. These data are not only used for release testing and a potency assay: they are also important for identifying critical process parameters and the critical quality attributes (CQAs) for the cell therapy product. For the developer, this requires not only developing assays that measure the mechanism(s) of action, but also utilizing analytical tools (e.g. single cell imaging analysis, mass spectroscopy, and various omics) to further characterize the product. With the accumulation of more quantitative data, additional tools such as artificial intelligence and machine learning can be implemented to further understand what makes a cell therapy product successful.

The development of a cell therapy product involves conducting extensive preclinical testing to demonstrate the safety of the product, and that it is likely to be effective in treating the targeted disease or condition. This testing typically includes good laboratory practice (GLP) studies in animal models. However, more recently, the US Food and Drug Administration has announced that animal testing will no longer be a requirement before entering clinical trials. This should enable more companies to develop products such as organ-on-a-chip and organoid models, which will in turn allow for more expedited and hopefully, more predictive testing. Also, while difficult to achieve in the early stages of development, the developer should consider implementing good manufacturing practice (GMP)-grade cell therapy products for the GLP studies.

Cell therapies can be expensive to develop and manufacture. Furthermore, in most cases, the initial manufacturing process will most likely not be the final process used for the commercial product. This can make it difficult for companies to invest early in the manufacturing development of cell therapies, which can slow down the pace of research. Nonetheless, developers need to implement a manufacturing road map that considers the requirements for changes in the manufacturing process, and development analytical tools that can establish CQAs to demonstrate comparability of the cell product whenever changes are made. Additionally, the road map should include scaling up the production of the cell therapy. Cell therapies are often produced in small quantities, which can make it difficult to meet the demand for the therapy if it is successful in clinical trials, and can further delay availability of the product to patients (as has been observed in the CAR-T space).

Overall, translating a preclinical cell therapy into a clinical trial is a complex and challenging process that requires a significant investment of time, money, and expertise. However, the potential benefits of cell therapies, including the ability to treat patients with previously untreatable diseases and conditions, are limitless.

BIOGRAPHY

DR ANTHONY (TONY) TING has over 30 years of academic and industry experience in translational science and global regulatory filing with over 20 years in the cell therapy field. He is the Chief Commercialization Officer and on the Board of Directors for the International Society for Cell and Gene Therapy (ISCT) and serves on committees for the Alliance for Regenerative Medicine (ARM) and the Health and Environmental Sciences Institute (HESI). He is currently the Principal for BRL C> Consulting. Most recently, he served as the Chief Scientific Officer for Bone Therapeutics, a publicly traded company in Brussels, where he developed a novel induced-pluripotent stem cell platform for genetically engineered mesenchymal stem cells. Dr Ting also served on

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the senior management team of Athersys, a Nasdaq-listed clinical-stage cell therapy company, as Vice President of Regenerative Medicine and Head of Cardiopulmonary Programs where he was responsible for all stages of development, from the bench to the bedside for the cardiovascular and pulmonary programs with Athersys' most advanced cell therapy product MultiStem®, an allogeneic adult bone marrow-derived stem cell product. Prior to joining Athersys, he was a Principal Investigator and Head of the Novel Inhibitors Screening Group at the Institute of Molecular and Cell Biology (IMCB) at the National University of Singapore, which identified new therapeutic targets through high-throughput screening. Dr Ting did his post-doctoral fellowship in the lab of Richard Scheller at Stanford University in Molecular and Cellular Physiology, received his PhD in Cell Biology from Johns Hopkins University and his BA in Biology from Amherst College.

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CELL & GENE THERAPY INSIGHTS

Scalability

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VECTOR CHANNEL: SCALABILITY

February 2023
Volume 9, Issue 1

INTERVIEW
Maximizing efficiency & flexibility in viral vector manufacturing scale-up
Denis Kole, Jon Petrone, Marc Bisschops & Nathan Hazi

INTERVIEW
Sharing best practices in AAV vector scale-up
Seth Levy

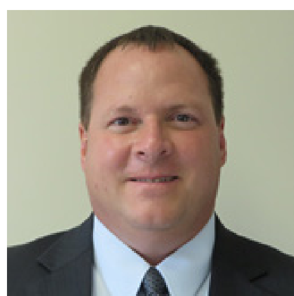
CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL: SCALABILITY

INTERVIEW

Maximizing efficiency & flexibility in viral vector manufacturing scale-up

Elisa Manzotti, CEO and Founder of BioInsights, speaks to four of Pall's biotechnology experts from across the field of gene therapy manufacturing – (pictured left to right) **Denis Kole, Jon Petrone, Marc Bisschops** & **Nathan Hazi** – about how to optimize and scale viral vector production by utilizing efficient technologies and platform processes.



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Q Let's begin with the key challenges in viral vector processing and in particular, those relating to scalability – what would you each pick out as the most significant pain points for the gene therapy sector at present, in your various specific areas of expertise?

MB: In the regulatory part of the drug discovery and development program, there is a challenge in the industry to find a clear definition of the so-called quality target product profile (QTPP). This is essential to defining the critical quality attributes (CQAs) and the critical process parameters (CPPs) later on and needs to be well described in the chemistry, manufacturing and controls (CMC) section of your regulatory filing.

The analytical support for process development manufacturing is being established. However, there is still a lot of work to be done before we can have the proper characterization of these therapeutic entities, which is essential for adequate process development.

DK: There are a few challenges faced during viral vector processing, which vary in significance depending on the type of viral vector. Many of these challenges are related to the complexity and diversity of advanced therapeutic medicinal products (ATMPs). Solutions and alternatives need to be considered to achieve the required productivity and CQAs.

For example, for adeno-associated viral (AAV) vectors, process reproducibility and scalability of the triple transient transfection process, in order to produce a product with consistent quality attributes, is a challenge that needs significant development work at bench-scale. A well-considered strategy for scaling up to commercial manufacturing would allow us to produce robust process control and batch-to-batch consistency.

A low percentage of full-versus-empty capsids, and overall heterogeneity in product and product-related impurities pro-duced within the bioreactor, are other challenges that can impact commercial manufacturing process design and scale. The separation of impurities and the enrichment of full-from-empty capsids are critical for successful AAV production, as empty capsids are considered as process-related impurities that need to be removed (or reduced in level) during the purification process. This becomes more complicated for viral vectors where current processes result in inconsistent impurity profiles. Extensive process development efforts are required to establish a process control strategy that ensures we can consistently achieve the target quality attributes of the product.

> "In the regulatory part of the drug discovery and development program, there is a challenge in the industry to find a clear definition of the so-called quality target product profile (QTPP)."
>
> **– Marc Bisschops**

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In addition, we cannot ignore the high cost of goods related to producing viral vector therapies. This is especially true for triple transfection processes, where the cost related to securing GMP-grade plasmid DNA and transfection reagents becomes a significant burden.

Lentivirus (LV) processes typically suffer from low viral yield during upstream processing and poor recovery rates during downstream process steps. This is mainly due to viral stability and the sensitivity of viral particles to physical and chemical stress.

Finally, there is a lack of availability of robust, accurate, and rapid-turnaround analytical assays. This complicates in-process sample testing and batch release testing as existing analytical assays require extensive development efforts, are typically time-consuming, and may result in delayed timelines.

NH: From an upstream perspective, one of the first challenges of scaling up a viral vector process is deciding what platform you want to use at your production scale. Ultimately, you need to choose a platform that meets your commercial yield requirements, CQAs, and company timelines, all while giving you an economical process yield.

From these high-level requirements, you must then start narrowing down your options. Should you use an adherent or suspension platform? What production scale bioreactor size will you need? What production mode will you need? Are there promising new technologies or techniques available? Some of these decisions will likely be made for you based on your intellectual property and what you have demonstrated at the research and discovery phase. Once you start to formulate your vision of what your process will look like at the production scale, it is a matter of making a plan to reach that final production process. This might seem like a daunting task, and it is where a knowledgeable industry partner can be helpful.

Q Focusing on the upstream process, what have been the most significant technological advancements that are delivering the cost-effective yield and titer improvements required by industry?

NH: We are seeing improvements in several areas. At the bioreactor level, adherent bioreactor options are expanding in terms of the diversity of capabilities, features, and sizes. For suspension bioreactors, there is a growing interest in a variety of automated tangential flow filtration (TFF) based perfusion systems that allow higher density cultures. Early results with some of these devices look extremely promising for intensifying viral vector processes.

At the process analytical technology (PAT) level, we are seeing increasing interest in $dC0_2$ probes for better process control, and wider utilization of capacitance-based biomass probes for monitoring biomass and informing real-time process decisions. We are even seeing this sort of approach for real-time monitoring of envelope virus production in the bioreactor, which is a cutting-edge technique.

At the reagent level, there is a growing field of novel transfection reagents, beyond polyethylenimine (PEI), that offer improvement in particular applications. There are also a variety of small molecules that enhance transfection or infection efficiency and productivity. Cell-line development continues to improve suspension-adapted cell-line productivity, and stable cell lines will continue to increase in popularity. Innovation in viral vector and capsid engineering is allowing for higher empty-to-full ratios and improved viral tropism and specificity. Whilst not strictly an improvement to the yield, this does reduce the required dose and batch sizes.

DK: The field of ATMPs, even though it is young relative to more traditional therapeutic modalities, has made some significant advances driven by the need to tailor solutions to the unique requirements of viral vector process development and manufacturing. The development of serum-free and animal component-free media, and the offerings by several vendors targeting the development of custom media for viral vector applications, can have a positive impact on minimizing lot-to-lot variability, improving the safety of viral vector therapies, and achieving better productivity.

In addition to this, smart process development approaches using a design of experiment (DoE) approach combined with scale-down modeling, as well as the advancements in the field of digital twin utilization, will continue to offer opportunities for further process improvements, and carry the potential to reduce overall cost for viral vector-based therapies.

Q What is the availability of genuinely scalable upstream process machinery such as bioreactors in the therapy space, and what can be done to ensure optimal consistency through scale-up/out into and through bioreactors of differing volume?

NH: I do not think it is possible to have a perfectly scalable bioreactor family. It is hard to make scalable equipment that does not have to abide by some constraints of physics, engineering, or manufacturing. This does not mean that scalability is all guesswork, though. A well-designed bioreactor family has adequately wide design space at the production scale, which is matched on the smaller scale systems.

It is important to understand the limitations of your production-scale equipment and to design a robust bench-scale process that resides within the boundaries of the production-scale. Technical support scientists from the equipment manufacturers are a great resource for providing a treasure-trove of experience and knowledge, which when harnessed, can shorten development times significantly.

Regarding the question of scalability from the smallest flasks to the production scale: I often see that viral vector proof-of-concept processes are hastily developed in unrefined, uncontrolled T-flasks or spinner flasks, and there are frequently hurdles in going from this proof-of-concept scale to the first benchtop bioreactor. Once the process is in that first controlled bioreactor, scaling up to larger sizes is much more easily managed.

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> "I often see that viral vector proof-of-concept processes are hastily developed in unrefined, uncontrolled T-flasks or spinner flasks, and there are frequently hurdles in going from this proof-of-concept scale to the first benchtop bioreactor."
>
> **– Nathan Hazi**

Overall, I would say that this issue is becoming less of a problem as the viral vector industry matures, but it is still a potential pitfall to be aware of. I would recommend starting to use a bioreactor as soon as possible.

MB: A quality by design (QbD) approach can be helpful in this endeavor, as it allows you to focus on quality during the technology transfer and scale-up of these processes. A perfect scale-up does not exist, but focusing on what matters in your upstream process can help reduce variability and build a more consistent process as you scale up and tech transfer to other facilities.

QbD has often been used for recombinant proteins and monoclonal antibodies (mAbs), and more recently, for viral vector downstream processing. It is good to see that more effort has recently been made to define the QbD space for upstream process cell cultures, both adherent and suspension. This will help considerably in building more consistent processes and delivering more consistent product quality moving forward.

Q What is next for transient transfection given the potential challenges that may arise at larger scales?

NH: Transient transfection has traditionally been the workhorse of the viral vector industry, and for good reason. As the industry emerged, scientists needed a system of vector production that was fast, nimble, and flexible, which is exactly what transient transfection offers. It can be used with adherent or suspension cultures, it allows for easy swapping of a gene of interest or capsid plasmid, and it is much more scalable than other transfection methods that are limited to small scales. If time to market is a primary objective in upstream development, transient transfection is there to answer the call.

However, there are some limitations. The challenges of transient transfection are well understood – mainly cost, operational complexity, reproducibility challenges, and a lack of institutional knowledge. Plasmids and transfection reagents are expensive. It is difficult to handle large volumes of reagents during the complexation and transfection steps within appropriate

timeframes. Deviation from consistent execution of those steps can lead to poor reproducibility between batches. Additionally, due to the multitude of parameters involved in transfection, it can be hard to predict the optimal transfection parameters for a new process. This often requires complex DoEs, which can be laborious and are not high-throughput in those cases where scale-down models are not fully representative of the production scale system.

We will see improvement in transient transfection, however; I am encouraged by the growing availability of new transfection reagents, which are specialized for certain applications. There are also novel transfection-enhancing small molecules starting to appear on the market that look promising. There is a growing knowledge base about ideal complex sizes and how to routinely achieve them. I am hopeful that we will soon see some products that will allow us to consistently perform transfections at large-scale.

The challenges of transient transfection do create some pressure to move beyond it. Stable cell lines will have a growing presence in viral vector production, because the technology offers improved simplicity, reproducibility, and hopefully, productivity. One such example is CEVEC's inducible stable cell line for AAV, which accommodates the project-specific transgene and capsid gene and delivers a top monoclonal producer single-cell clone. There is a lot more information available on this topic, and I would recommend listening to the *Cell and Gene Therapy Insights* interview with CEVEC, published in 2021.

Transient transfection has a firm footing in the viral vector manufacturing space, and we will continue to rely on it in the future. But there will be instances where it makes sense to switch to a stable cell line, particularly for the larger clinical indications that require the large yields.

Q Enabling viral vector process intensification is a particular point of focus for the field at present – can you provide some pointers to an optimal approach in this regard? For instance, how to enable viral vector process intensification at smaller scales?

MB: Process intensification has multiple dimensions to it. Often, we look at the specific productivity of each of the primary unit operations, which is expressed as the amount of product produced per unit volume per unit of time. But this approach sometimes ignores the other dimensions involved in biomanufacturing, such as the impact of buffers and consumables, which can add to both the complexity and the footprint of the total operations.

Furthermore, once at the manufacturing scale, other bottlenecks appear. One of those bottlenecks, which I refer to as 'the invisible elephant in the cleanroom' is that the amount of data being produced often becomes a burden because it is scattered across many systems and sources. What is more, these varied data come at a relatively high speed. We often see that manufacturers can produce a batch of drug substance in a few weeks, but then the release of the batch report can take multiple months because all that information needs to be populated in a single document. You cannot release the drug substance without that batch report, so that becomes a challenge that we cannot ignore in the field of process intensification.

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Last but not least, the journey from discovery to development to clinical or commercial manufacturing comes with certain risks. This is where people can lose a lot of time. A holistic approach to process intensification covers all of these aspects, while at the same time reducing complexity and with that, risk of operator error.

> "From the downstream perspective, there are opportunities to link unit operations, reduce process volumes, and shorten process times."
>
> **– John Petrone**

It has been said that continuous bioprocessing could be a way to enhance process intensification, including for viral vectors. Although I am a great fan of process intensification through continuous bioprocessing, it would require analytical support and robust product characterization to be in place before you can embark on that journey. I think it will take a bit more time before we reach that point for viral vector manufacturing. Having some further progress on that end will enhance process intensification. For now, we should look at making it easy, simple, and robust.

JP: From the downstream perspective, there are opportunities to link unit operations, reduce process volumes, and shorten process times. An in-line concentrator or a single-pass TFF device can be linked to the clarification, depth filtration, operation to reduce the volumes and the required liquid storage tankage.

The implementation of Mustang Q membrane adsorbers with their large open pore structure relative to chromatographic resins allows higher binding capacities of large viral vectors. The Mustang Q also operates at flow rates of 5–10 membrane volumes per minute, which is about 40 times the linear velocity of what can be achieved with a column operating at a four-minute residence time.

In process development, we can run experiments in 10–15 minutes. This means you can run 20–30 runs per day to enhance your knowledge base. At manufacturing scale, the reduced processing time of Mustang Q can minimize damage to labile vectors such as LV, whilst reducing buffer and waste volumes.

Q How well are the current range of purification technologies performing in terms of streamlining viral vector downstream processes whilst improving recovery? And how/where can we do better?

MB: The viral vector manufacturing industry has inherited many of its technologies from the manufacturing of recombinant proteins and mAbs. That has been the name of the game, and it has been quite successful.

However, it is necessary to consider that these technologies are not tailored to the specific needs of vector manufacturing. That relates to both the scale of the equipment and the performance of materials and absorbers used. Ignoring the specific attributes of viral vectors such as the sensitivity of lentiviruses or the sheer size of some viral vectors, leads us to slightly less efficient processes than we would want. There is room for more robust downstream processing if we can develop more appropriate filters and absorbers to handle these viral vectors.

Development in analytical support will help us to achieve more robust downstream processing, too. In order to develop a purification process, you firstly need to understand what you are doing, which requires fast and robust analytics. The accuracy and speed are not there yet – there is some room for improvement on this end.

JP: With labile vectors such as LV, traditional TFF operations can degrade vector quality over time. For stable productions like mAbs, the CPPs for TFF such as crossflow rate and transmembrane pressure are scouted in a single experiment performed in total recycle with the retentate and permeate streams directed back to a well-mixed feed tank. However, with labile products, this CPP scouting process may need to be conducted in a series of full TFF runs at the desired process conditions in a DoE fashion.

Another challenge is the variability in assay results – for example, +/- 10–20% is not uncommon for ELISA and quantitative PCR assays. This can affect the number of necessary replication runs to judge the performance of the different operating conditions. Another option is to run with single-pass TFF, which operates at reduced feed flux to minimize shear on the labile product, and with a single pump pass. This can allow the viral vector concentration to be achieved with high yields. If diafiltration (buffer exchange) is required, an in-line diafiltration device or a hybrid single-pass TFF approach can be implemented to achieve the high product concentration goals, perform the desired buffer exchange, and achieve high yields in the process step.

Q No discussion of AAV vector manufacture feels complete without some coverage of empty/full/partially full capsid ratio and its impact on gene therapy safety and efficacy – what is your take on how best to improve this ratio through process innovation and optimization?

DK: Achieving poor and varying levels of full-versus-empty capsids during upstream process production creates bottlenecks that complicate the AAV manufacturing process. A lot of great work has been done to improve upstream processes and increase full capsid production. However, for existing processes, separation and enrichment of full-from-empty capsids needs to be achieved during downstream processing, as empty capsids are considered impurities that can affect the efficacy and safety of therapies.

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While affinity chromatography is widely used and can generate high yields of recovered AAV, it lacks the ability to separate empty from full capsids. Ion exchange chromatography (IEX) is currently being used as the method of choice to separate full from empty viral capsids. Any processes that need to go to commercial manufacturing scale typically rely on anion exchange chromatography (AEC). However, significant development work is required at the bench scale before advancing these processes to a larger scale and commercial manufacturing. In most cases, these types of approaches do result in low full capsid recovery, at the expense of removing empty capsids.

There are a few new approaches that are utilizing the use of IEX absorbance, such as the Mustang Q and Capto Q, which resulted in a few improvements in full capsid enrichment. Some great work has been published in the last few years by Pall's Applications R&D team, who have described the separation of full-versus-empty capsids using an approach where a 1 mS/cm conductivity step gradient is used to allow for high-level enrichment of full capsids. The Cytiva team has also published data on the use of Capto Q Impress resin with dextran surface extenders, and the use of magnesium chloride and elution salt to significantly improve capsid recovery and purity.

However, considering the need for further process improvements, it is important to explore innovations that target upstream processing as well as downstream process steps. It would be possible to start as early as the capsid engineering stage by looking into cell line selection to improve full-to-empty ratios, and exploring opportunities for using producer cell lines.

Improvement in the upstream process holds the key to reducing operating scales and costs, thus enhancing productivity and safety. These improvements will open doors to the development of more viral vector therapies for ultra-rare diseases, which currently can be cost-prohibitive. Incremental improvements of existing downstream processes, as well as research into new materials and single-use solutions, are needed to achieve the productivity, product quality, and cost reductions required to make viral vector therapies more cost-effective and available to wider patient populations.

Q Standardized vector platform processes are another red-hot topic at present – can you tell us about Pall's approach in this regard and what differentiates it?

DK: Looking at the approaches that are used to manufacture more traditional biologics such as mAbs, we can quickly identify many benefits that a platform solution offers. These include standardization of the manufacturing process, improving timelines, process streamlining, increasing operational flexibility, and reducing process risk whilst also improving supply chain and overall plant flexibility. It makes sense to think along similar lines for the production of viral vector therapies.

> "To build a platform viral vector process, it is important to have a complete understanding and control of the CPPs that affect the CQAs of the product, and a good understanding of the flexibility as well as the limitations of the selected equipment."
>
> **– Denis Kole**

As a leader in bioprocess solution offerings with a wide range of technologies available for upstream as well as downstream production, Pall is perfectly positioned to support platform solutions for viral vector processes. On the upstream side, we offer automated bioreactors for adherent as well as suspension processes, which cover a wide range of scales from benchtop systems for process development approaches up to and including commercial manufacturing. On the downstream side, there is an extensive portfolio of single-use, modular technologies, many of which have been designed and developed specifically for viral vector processes. In addition to this, we have a widely established knowledgebase across many viral vector types and modalities, through hands-on internal R&D applications work that the process development services team has conducted in collaboration with our customers, and the field application support teams that work on customer processes at their sites. This places our teams in a unique position to offer both a wide range of equipment and consumables to support these platform solutions, and also have the experience of working directly with therapy developers and contract development and manufacturing organizations to optimize platform solutions for specific applications.

Q What are the minimum process parameters needed to build a platform viral vector process?

DK: To build a platform viral vector process, it is important to have a complete understanding and control of the CPPs that affect the CQAs of the product, and a good understanding of the flexibility as well as the limitations of the selected equipment.

In general, that includes determining the process parameters that control upstream culture conditions, such as the transfection or transduction efficiency, to ensure that the viral vector quality, impurity profile, and viral titer are consistent between batches. Determining and controlling the process parameters that ensure you are operating within the design space required to achieve viral vector quality, purity, recovery, and potency should be applied to all unit operations on both the upstream and the downstream steps.

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Finally, the second 'elephant in the room' is developing robust analytical assays that allow us to determine the quality profile of the product (including the purity, potency, stability, and aggregation of viral vectors) throughout the production system and in real-time.

Overall, it is extremely important to marry together process development at the bench with the process design for manufacturing-scale operations, as well as the selection of the equipment and solutions to be used for the process. This approach then allows us to design a robust process control strategy that we can scale up and down, which will result in minimal batch-to-batch variability and ensure the product's CQAs can be achieved.

MB: A focus on the concept of QbD is something I strongly support. It is extremely important to adhere to the philosophy of QbD and look at CQAs to design your process around CPPs.

To address manufacturability challenges, such as pressure drops and yield loss, there is a set of key process parameters that do not necessarily affect the CQAs, but which are still extremely important for developing a scalable and robust manufacturing process that delivers the required yield. As we scale up, this is something that should be included in the parameters that need to be characterized and controlled.

Q What does it mean to scale-up to products destined to only treat dozens of patients rather than hundreds or thousands? Can you automate cost effectively in that scenario, and if so, how?

DK: The commercialization of viral vector therapies and ATMPs in general has changed how we think about commercial-scale manufacturing for these therapies. This is mainly because manufacturing capacities and capabilities required to produce these classes of therapies are different from the traditional biopharma facilities, built to accommodate processes for thousands of liters of feed streams that need to be executed downstream.

Many cell and gene therapies target smaller numbers of patients and as a result, they require operations to be executed at much smaller scales. This, in turn, can be challenging as many of the available equipment, solutions, and automation, needs to be repurposed and may not be an optimal fit. However, the field is considering these challenges. We have already seen many solutions developed in the last few years, specifically for viral vector processes, that are now being used for the manufacture of commercialized viral vector-based therapies.

A great example is the use of automated, single-use fixed-bed bioreactor systems like the iCELLis, which offers opportunities for better process control, reduced handling, and reduced overall risk, compared to more laborious processes that require intensive manual manipulation and a significantly higher footprint to operate.

MB: It is ofwten thought that automation makes less sense for gene therapy applications that target smaller patient populations. I would argue that this is untrue. It

is true that automation helps in routine manufacturing, but when manufacturing is less routinely completed, such as for gene therapy applications, the chance for operator error is high. This is where automation can truly help.

Automation helps in the gathering, collecting, and controlling of data produced in gene therapy manufacturing. That is an area that does not scale with the size of the batch. In smaller batches, the effort to produce the batch reports required to release the drug substance remains the same, so having a proper digital infrastructure with automated systems to help collect data, still makes sense. This will enable you to move through the process of populating and releasing the batch reports quickly.

BIOGRAPHIES

DENIS KOLE serves as the Director of Process Development Services in the US, leading upstream, downstream, and bioanalytical assays teams working on developing and scaling up viral vector process to manufacturing scale. Denis has over 16 years of experience in industry and academia, holding various scientific and leadership roles. Prior to joining the Process Development Services group, Denis led the field applications team in the US, supporting the adoption and implementation of continuous bioprocessing and process intensification solutions.

JON PETRONE provides technical expertise, guidance and thought leadership for the process development (PD) and MSAT support for the purification of proteins and cell/gene therapy products. Previously, he led Pall Biotech's Process Development Services (PDS) team providing upstream, downstream, and analytics PD, scale-up, and tech transfer services to support emerging therapeutics such as gene/cell therapies. Mr. Petrone has over 35 years of experience supporting downstream purification applications from cell harvest through final bulk fill. Prior to joining Pall in 2005, he led a team responsible for PD and clinical/commercial support of the membrane-based separations and centrifugation steps at Wyeth BioPharma. He holds an MSc and BSc in chemical engineering from Worcester Polytechnic Institute.

MARC BISSCHOPS serves as Vice President, Manufacturing Sciences and Technologies at Pall Corporation and is responsible for all field support activities related to cell culture technologies, filtration technologies, single-use technologies and process intensification. In addition to this, he has been spearheading the development of the regulatory support initiative for process intensification and continuous bioprocessing within Pall Biotech. Prior to joining Pall, Marc was CSO at Tarpon Biosystems and held several scientific and management positions in the field of process development. Marc has been a pioneer in continuous bioprocessing and still is one of the leading scientists in this area.

NATHAN HAZI is responsible for managing the bioreactor applications team in the western hemisphere. He has worked in a technical support role of our bioreactors since joining us in

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2018. He has a BSc in Biochemistry and Molecular Biology from Penn State University and 10 years of experience in upstream process development and cGMP manufacturing.

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We hope you enjoyed reading this interview.
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INTERVIEW

Sharing best practices in AAV vector scale-up

David McCall, Commissioning Editor, BioInsights, talks to
Seth Levy, Director of Bioprocess Development,
Modalis Therapeutics

SETH LEVY, joined Modalis Therapeutics in 2021 as Director of Bioprocess Development to build and oversee internal process development and analytics efforts, as well as manage external manufacturing. Prior to Modalis, Seth lead teams in Manufacturing Science and Technology and Small-Scale Development for AAV and LV manufacturing at Viral Vectors Services. Seth worked in gene therapy R&D before his time at a CDMO and drove numerous AAV projects including basic biology research, capsid engineering, and translational gene therapy approaches at Sanofi Genzyme and academia.

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Q What are you working on right now?

SL: Our Bioprocess Development team supports research and development (R&D) efforts with viral vector lots for *in vitro* and *in vivo* work as well as oversees

external manufacturing and analytics. We have been putting the finishing touches to our adeno-associated virus (AAV) production and purification process to transfer to a contract development and manufacturing organization (CDMO), and we are also developing potency assays to transfer externally.

Q Can you give us some background on Modalis Therapeutics' specific platform/approach and R&D pipeline?

SL: Modalis' technology is what we refer to as CRISPR-GNDM, or guide nucleotide-directed modulation. We utilize an enzymatically-null Cas9 protein, so we do not actually cleave DNA, but rather we modulate gene expression. To upregulate gene expression, we attach transcriptional activators to the Cas9 protein to act as a transcription factor, or we simply park the enzymatically-null Cas9 on the DNA to prevent transcription and repress gene expression. This approach allows us to target many diseases that are not good candidates for traditional gene replacement therapy, such as those with large genes of interest, we can deliver our CRISPR-GNDM utilizing a single AAV vector either systematically or locally depending on the indication.

Q What are the associated considerations and challenges presented to you from a bioprocess development perspective?

SL: Since we are packaging our Cas9 and guide RNA in one AAV vector, we are approaching the limits of its carrying capacity so we are meticulous when looking at genome integrity and DNA-containing particles. In upstream, we optimize production parameters for a balance of total vector genomes (VGs) and the percentage of full capsids being produced, while in downstream, we have evaluated several anion-exchange chromatography (AEX) technologies to increase our full capsid enrichment. Analytically, we have explored several technologies to evaluate empty/full ratio and we have a multi-faceted approach to assessing genome integrity ranging from 'old-school' techniques such as alkaline gels, to newer technologies, such as multiplex droplet digital PCR (ddPCR) and nanopore sequencing.

Q What would you pick out as the key challenges relating to the scalability of rAAV vector processes at present?

SL: Specifically for Modalis, we have exciting data around biodistribution and gene expression utilizing novel AAV capsids. Unfortunately, these new serotypes do not

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always play nicely with the established techniques and available technologies. Some serotypes present challenges in terms of producing as many full particles in our process, while others have purification challenges. Whether changes are only a few point mutations apart from the wild-type capsids, or lengthy peptides have been inserted into the capsids, they can all have a wide-ranging impact on downstream purification efforts.

Q What do you see as the likely pathway towards resolving the uncertainties surrounding transient transfection processes at larger scales?

SL: The obvious resolution is scaling up to a certain point before scaling out. Is one 200 L batch enough for your clinical needs? Can your downstream process handle 2×200 L or 4×200 L worth of material? Are you willing to pay the money and spend the requisite amount of time to increase to a 1000 L or 2000 L process? Will baculovirus insect cell production gain more traction considering recent developments with BioMarin and CSL Behring products? Ultimately, from an R&D and safety perspective, we need better capsids so that the VG per patient needs are reduced, thereby reducing the strain on manufacturing capabilities.

Another area of innovation in the transient transfection space are the transfection materials themselves. Can we drive down the cost or reduce the lead-times of those reagents to AAV manufacturing? Will we see more alternatives to bacterially-produced plasmids such as doggybone DNA (dbDNA) come through? Will we see more transfection reagent options becoming available at a lower cost? Lastly, some groups are producing promising data around the use of producer cell lines at scale. This approach clearly has the potential to be a solution for many of the transfection process's woes.

Q Platform-based viral vector production processes are in vogue as a means of boosting scalability, among other things – which tools are driving progress in this regard at the moment, and equally, where is further innovation most needed in this area?

SL: Producer cell lines are a great start in the upstream process. In downstream, we need more affinity options. If we continue to engineer promising novel capsids that have increased potency or a desired biodistribution, will these remain difficult to purify? It remains to be seen if we can rapidly and cost-effectively produce new antibodies to employ in affinity chromatography for these new variants.

Analytical platforms are just as important. One example from here at Modalis, is to employ the same Cas9 target for our VG titer assay, which allows us to put production and purification differences in perspective when comparing different capsids, changing components

> "Do not underestimate the power of a strong analytics team. It can have immense repercussions if you wait too long through the process development lifecycle to implement analytical changes, such as changing from qPCR to ddPCR titering assays, from an ELISA to an HPLC method, or from one empty/full method to another."

of our gene of interest (GOI), or even across programs as we further develop our platforms. This approach can be employed elsewhere such as capsid-agnostic empty/full particle assays, and many process-related impurity assays can be used across programs. In general, many groups keep as many process parameters as similar as possible if the capsid is the same and they are only changing the GOI between programs. This is even easier if you target the same tissue type for multiple programs, as you can even employ the same promoter and poly(A).

Q Reflecting on your experience and your career to date, do you have any best practices that you can share that relate to successful scaling of viral vector processes?

SL: Do not underestimate the power of a strong analytics team. It can have immense repercussions if you wait too long through the process development lifecycle to implement analytical changes, such as changing from qPCR to ddPCR titering assays, from an ELISA to an HPLC method, or from one empty/full method to another. It is also never too early to start potency assay development. As a CRISPR-based company, we rely heavily on our potency assays. Guide RNAs may be different between species, so looking for equivalency in our GNDM expression and how GNDM expression relates to gene modulation in each species is key for us.

Another best practice is to think more collaboratively in terms of who can help you. Creating licensing agreements between several companies to develop a new platform or a new technology is great of course, but a quicker solution for many manufacturing and analytical problems can be to lean on manufacturers and contract testing facilities. Think of who can help you solve the problems instead of how you can solve the problems yourself or within your own team. Many vendors, whether they are CDMOs, transfection reagent suppliers, column providers, contract testing labs – all have vested interests in your program succeeding because they all want your returning business. They may have done targeted studies on something you may be struggling with or have strategic questions about, or have at least

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performed studies that are very similar to your issues and can provide some relevant insight. My advice is do not stay stuck working in a silo or only within your team.

Q What are some key goals and priorities, both for yourself in your role and for Modalis Therapeutics as a whole, over the foreseeable future?

SL: Modalis is planning to file two INDs in 2024, so significant effort is being spent supporting our two lead programs. Beyond that, the bioprocess development team is continuing to develop new production and purification processes of novel serotypes, and we are also growing our analytics team to help facilitate better vector characterization. Specifically for our development team, we plan to add more automation into our workflows to free up time for our operators so we can dive ever deeper into the interesting data that we continue to generate.

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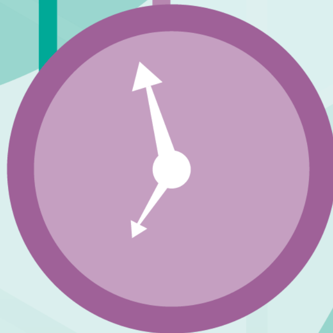
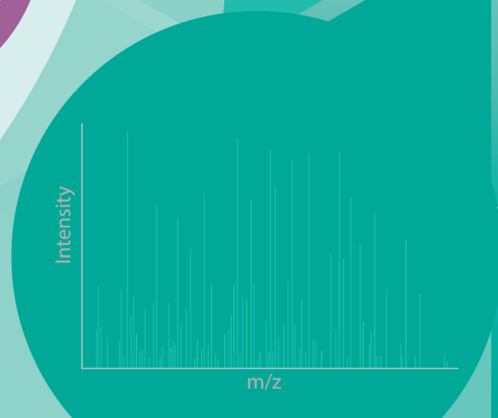
Analytics Channel





CELL & GENE THERAPY INSIGHTS

ANALYTIC CHANNEL:

February 2023
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INTERVIEW

Seizing the day: addressing the urgent need for standards development in regenerative medicine

Richard McFarland

INTERVIEW

Seizing the day: addressing the urgent need for standards development in regenerative medicine

David McCall, Editor, *Cell & Gene Therapy Insights,* speaks to **Richard McFarland,** President of the Standards Coordinating Body for Regenerative Medicine (SCB), Chief Regulatory Officer of the Advanced Regenerative Manufacturing Institute (ARMI), and Principal Consultant of BioFabConsulting

DR RICHARD MCFARLAND is the President and Chairman of the Board of the Standards Coordinating Body for Regenerative Medicine. He is also serves as Chief Regulatory Officer at the Advanced Regenerative Manufacturing Institute (ARMI) where he oversees regulatory affairs for ARMI and its BioFabUSA program. Prior to joining ARMI in 2017, Dr McFarland served at the FDA's Center for Biologics Evaluation and Research since 2000 in various positions. He was Associate Director for Policy of the Office of Tissues and Advanced Therapies (and its predecessor office) at the Food and Drug Administration's Center for Biologics Evaluation and Research (FDA/CBER) for eleven years after six years a reviewer. In addition, during his time at FDA he served on the several interagency standing committees including the Interagency Coordinating Committee for the Evaluation of Alternative Methods to animal use (ICCVAM) and, the Multi-agency Tissue Engineering Sciences group (MATES) for fifteen years, including five years as its Chair. MATES coordinated regenerative medicine efforts across the government.

Dr McFarland received his undergraduate, graduate, and medical school training at the University of North Carolina-Chapel and his post-graduate medical specialty training in anatomic/clinical pathology at University of Texas Southwestern Medical Center in Dallas.

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Q What are you working on right now?

RM: With respect to the Standards Coordinating Body for Regenerative Medicine (SCB), what I'm really excited about right now is the expansion of our educational offerings. The SCB was initially started to help coordinate and develop new standards, and to create a standards portal where people could see what standards are available that might relate to regenerative medicine. However, as we developed more standards, we came to appreciate that a standard without people using it is like a scientific paper that is never cited in the literature. So, with some funding from the US Food and Drug Administration (FDA) and Advanced Regenerative Manufacturing Institute (ARMI)'s BioFabUSA program, we are developing courses on implementing specific standards. These efforts should allow regenerative medicine firms to reduce development time and cost for commonly used unit operations and assays such as cell counting for example. This is something that we are going to be doing for all of the new standards that were are helping develop going forward, which is really exciting! We are starting to build that out and working with multiple channels to reach people all around the world, so that they can use those standards to help cut their costs, etc.

Turning to the ARMI, we are currently building out an early-stage manufacturing facility, in close proximity to our existing process development space. We currently have multiple process development projects ongoing in which we are closing and automating manual manufacturing processes for a diverse range of cell and tissue products for our members. ARMI incorporated standards whenever possible to increase process reproducibility and reduce cost of goods (COGs). ARMI, a member-based non-profit, has nearly 200 members across the United States representing a cross-section of the regenerative medicine ecosystem, from academic institutions and startups to multinational industrial firms that focus on providing the tools for manufacturing equipment. The new facility is going to allow us to really build toward our mission of advancing the nation's bioeconomy and creating a robust industry for manufacturing cells, tissues, and organs.

Q Let's dive deeper into your work on the standardization front – firstly, why is this such a critical time for the development of standards for the regenerative medicine field?

RM: Because we are sitting right at the beginning of the curve of potential transformation of the scientific field of regenerative medicine to the routine clinical use of regenerative medicine products. The window to set the expectation to use standards in such a potentially explosive field is limited.

If you go all the way back to the first cultured skin products, in the 1980s, there were just a few of them, and they were probably before their time in terms of how to deal with commercialization of these complex products. It was before we had induced pluripotent stem cells (iPSCs), before we had embryonic stem cells (eSCs), and before the potential for automation – manufacturing was just culturing adult cells in a lab using a biosafety cabinet (BSC) and an incubator. The way you scaled was adding another technician, another incubator, and another BSC. In reality, much of the manufacturing today for cell and gene therapies today is not too far beyond that highly manual system. (Viral vector-driven *in vivo* gene therapy is a little further ahead than cell therapy because gene therapy is more amenable to the processes that have been developed over the years in traditional biotech). But today, on the heels of the first few *in vivo* gene therapy and CAR-T cell therapy approvals, there is a surge in expectations for the field with a lot of money flooding as a consequence, even taking into consideration the current period of layoffs that we are going through right now. If this field is going to take off in a big way, right now is the time! However, it is also the time to realize that a lot of the unit processes for similar products in cell therapy and gene therapy really aren't necessarily all that different.

The regulators around the world have evaluated investigational products in terms of their individual manufacturing processes, and clinical performance of the final product in a given indication. They need to see convincing data demonstrating that the performance is going to be achieved reliably given the vagaries of the human body. As a result, without the development of standard ways to do things that everyone has to do – things like common measurements for counting cells, vector capsids, environmental factors (pH, oxygen, glucose, etc.), and standardization of common processes – every single company carries the burden of coming up with their own way to solve these problems. That's inefficient. Every hour, every dollar they spend doing that sort of work when they could be doing something standardized, they are taking that time and money away from understanding and demonstrating where their product's unique benefits and intellectual property (IP) resides.

I was at the FDA during what I would call the 'end of the beginning' of the monoclonal/recombinant antibody era. To my eye, it mirrors just about where we are now in the regenerative medicine field. Back then there were many protective measures being constructed around products, because everyone claimed all their techniques were so special and proprietary and everything was important IP. A few companies were saying 'our approach is so much better that even though we are making a therapeutic product, we are going to out license out some of these techniques, too.' That rarely happened, though. The issue is that the monoclonal/recombinant antibody field is still dealing with working out how to standardize things. One of the consequences of the lack of standardization in the antibody field is that it became a major factor in the increased COGs for those products. That's why it is critical for the regenerative medicine field to act now, and we only have a very short window in which to make a huge difference.

> "I think that the most fundamental consequence of not advancing the use of standards where appropriate in regenerative medicine today will be the field failing to fulfill its potential."

Q Can you expand on what the repercussions for the regenerative medicine field will be if it misses the current window of opportunity to advance standardization in the field, based on your previous experience?

RM: Given the fact that cell therapy COGs are currently considerably higher than those for protein therapeutics, and the manufacturing much more complex, I really don't think that anything other than the most rudimentary cell therapy modality can support a business with non-scalable, manual manufacturing. So, I think that the most fundamental consequence of not advancing the use of standards where appropriate in regenerative medicine today will be the field failing to fulfill its potential. The increased use of standardization must be one of the factors contributing to lowering the COGs for regenerative medicine products.

It is worth noting that with the first tissue engineered skin products that I mentioned earlier, one of the factors that caused the entire to field to shrink and almost vanish was a decision in 2014 about how the Centers for Medicare & Medicaid Services (CMS) was going to pay for skin substitute products for wound healing. The reimbursement decision was most problematic for the complex products with the highest COGs. We as a society do not have infinite dollars to pay for regenerative medicine products, no matter the number of years over which the payments may be spread. However, without adequate reimbursement, the field may cease to exist as an industry entirely, or could shrink back to highly novel products for a very small, niche indications where the question of treatment is as stark as imminent death from the disease, or living a full life free of the disease. Many of the early gene therapy products are certainly focused on those types of indications, but it is sobering to think: that may be all we ever get.

Q How are the organizations and activities of the SCB evolving and expanding to help drive progress in the regenerative medicine space?

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RM: The SCB is a relatively small organization, which I think at this point certainly boxes above its weight category – in part, because it manages to leverage a lot of expertise from subject matter experts. The SCB is designated in the United States as an IRS 501(c)(3) non-profit organization, which other regions would term a non-profit for the public good. We don't have any members; we don't lobby. The SCB really has no vested interest in anything other than moving standards and the regenerative medicine field forward. We have a small but growing group of talented PhD-level scientists, who for various reasons decided they didn't want to spend their whole life in the lab. So, they know the science, and they are able to communicate with subject matter experts in the field and draw all that information together. As the name of the organization suggests, these scientists don't develop their own standards, but rather coordinate standards development across the field. In doing so, they work with existing standards development organizations (SDOs) around the world – the likes of the International Organization for Standardization (ISO), ASTM International, US Pharmacopeia (USP), the Parenteral Drug Association (PDA), the list goes on, and it is increasing every day. I think the fact that the SCB has no vested interest other than coordinating these standards and getting them implemented gives both the regulators and subject matter experts from industry confidence that they can share information that they wouldn't necessarily share otherwise. In fact, the US FDA is involved frequently at the committee level in developing standards with SDOs. SCB also has some funding from the FDA for some specific projects – for instance, the SCB's Regenerative Medicine Standards Portal is an FDA-funded thing. We also work with the National Institute of Standards and Technology (NIST). Our headquarters are actually in the middle of the NIST campus, so we are right down the hall from some members of the NIST team who specialize in measuring cell and gene therapy products. We help to coordinate both their Rapid Microbial Testing Methods Consortium (RMTM) and their Flow Cytometry Standards Consortium. So, we are embedded in these organizations, and we also have good relations with the British Pharmacopoeia (BP) and other regulatory and standards agencies around the world. That's all we do at SCB: we wake up every morning wanting to help coordinate international efforts to standardize. That is, in my experience, fairly unique.

Q What is your perspective on recent innovation in the analytical tools area, particularly where it is helping to answer some of the key questions facing the regenerative medicine field? Do any specific recent advances stand out for you?

RM: I think that the progress in our ability to observe and measure compounds in the media, in the cells, or the culture vessel either online or near-line is very exciting, as are developments to measure multiple compounds with a single sensor. Those are exciting because traditionally, it has been a question of removing

> "In terms of analytical innovation, I think the first successful demonstration of using in-process analytes in a product, and even in an investigational new drug (IND) moving forward, will be a huge step."

samples from the process and performing sometimes multiple assays on them. The degree of complexity and time inherent in taking these types of measurements limited their use in real-time in-process control.

The ability to sense the health of a cell or virus in near-real-time enables the incorporation of Quality by Design (QbD) principles and process analytical technology into these complicated manufacturing systems. The capability to adapt your culture conditions and processing accordingly in near-real-time should decrease the number of lots one has to discard due to lot release test failures, thereby increasing the overall efficiency of manufacturing. As this approach continues to grow in acceptance, the range of 'off-the-shelf' sensors will grow, as will the need to standardize analytical processes and communication protocols to integrate them in a growing array of manufacturing and research applications. The need for standardization might conceptually extend to multiomic analytical approaches, enabling the incorporation of Quality by Design (QbD) principles into both these complicated manufacturing systems and the discovery of new analytes of interest. I am hopeful that creative use of these innovations in cell and gene therapy manufacturing systems will reduce the frequency of discovering that one's product performs differently in the clinic after scaling up and/or other major process change, despite the product's putative critical quality attributes (CQAs) remaining constant. I think that this is often due to the lack of a detailed understanding of the manufacturing system's critical process parameters (CPPs). Increased innovation and standardization of sensor technology and analytical techniques will reduce the barriers for firms to gain essential knowledge of their processes during product development.

Q Looking to the future, what will be some key areas for regulatory guidance development, for you?

RM: In terms of analytical innovation, I think the first successful demonstration of using in-process analytes in a product, and even in an investigational new drug (IND) moving forward, will be a huge step – that is analytes that are determined by looking at what the culture is, not *a priori*. We are not quite there yet but when it happens, I think it will be the start of a real avalanche.

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Six or seven years ago, nobody was talking about modular or automated or closed manufacturing for these systems. Today, everyone is talking about doing it. The next leap is moving toward QbD, and working towards quality assurance (QA) with that sort of approach. It would be helpful if the Agency provided some guidance on applying the principles outlined in ICH Q 8, 9, and 10 to cell and gene therapy manufacturing. The US FDA (across both CDER and CBER) and the Product Quality Research Institute (PQRI) hosted a workshop last Fall on distributed manufacturing, during which the possibility of distributed manufacturing for cell and gene therapy was discussed. I think regenerative medicine may have an opportunity to leapfrog other, more established areas, because it has the advantage of not having to adapt or replace huge manufacturing infrastructure with more forward-looking guidance from the agency and a community-wide effort to think innovatively with respect to manufacturing models.

To my mind, one of the teachings from the recent pandemic is that when they are presented with a real public health need, regulatory agencies can be inventive. The explosive growth of the CAR-T cell therapy field provides another example that is a little closer to home for cell and gene therapy. But firstly, sponsors must demonstrate they can solve that need. I think the agency will say 'yes' if they are given data to address the public health need.

Personally, I would love to see some more discreet guidance coming through on looking at manufacturing and GMP in a way that is more amenable to automated systems. I'd really like to see some discussion papers, like the one CDER produced recently on distributed manufacturing, which aim to bring people together in a way that is coordinated with agency involvement, so that everyone knows the key topics and focal points.

Q Can you sum up some key priorities that you have for your own work over the foreseeable future?

RM: I want to see a panel of standard education programs made available from SCB for newly developed standards. And I want to see standards implemented, because that will really cut down time to market. It will also help greatly in informing workforce development, because even with automation, we are going to need a larger trained workforce.

One of the encouraging things in terms of workforce is that we have received a lot of interest in non-four-year degree development. The bottom line is we can't afford to have PhDs running every single manufacturing process because frankly, they are expensive, and that is also not the best use of their training. (For one thing, PhDs don't tend to do things the same way every time, which is less than ideal in product manufacturing!) Increasing standards education and implementation will in turn create more opportunities to join the cell and gene therapy workforce for non-PhD/non-Masters employees. I would really like to see that happen in the near future.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES:

Optimizing vector production & purification to enhance scalable AAV manufacturing

Jonathan Zmuda, **Chantelle Gaskin**, **James Molinari**, and **Jenny England**

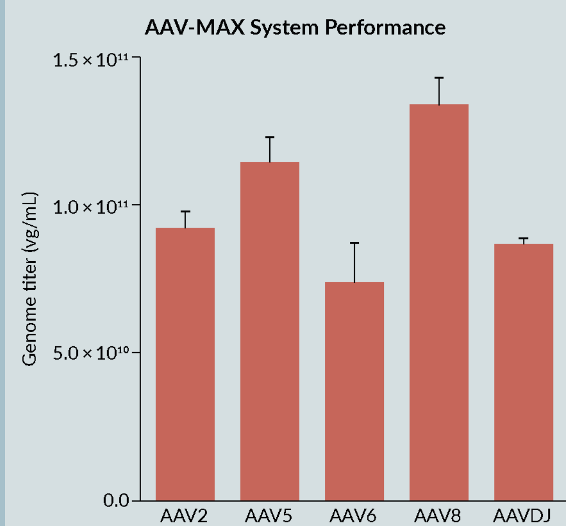
The rapid growth of the gene therapy field has driven demand for a reliable and scalable adeno-associated viral (AAV) vector manufacturing solution.

This poster describes how upstream production yields can be maximized using the Gibco™ AAV MAX helper-free AAV production system, and downstream recovery and purity can be optimized using the POROS™ CaptureSelect™ AAVX chromatography resin.

CTS AAV-MAX Production System

The Thermo Fisher Gibco Cell Therapy Systems (CTS) products are designed to enable clinical and commercial cell and gene therapy manufacturing. All CTS reagents are manufactured under cGMP, with cell and gene therapy-specific intended use statements, extensive safety testing, and proactive regulatory documentation provided. Within the CTS portfolio, the CTS AAV MAX production system is a fully integrated and optimized system for scalable, high-titer production of AAV (**Figure 1**).

Figure 1. Viral titers achieved with VPC 2.0 in different AAV serotypes.



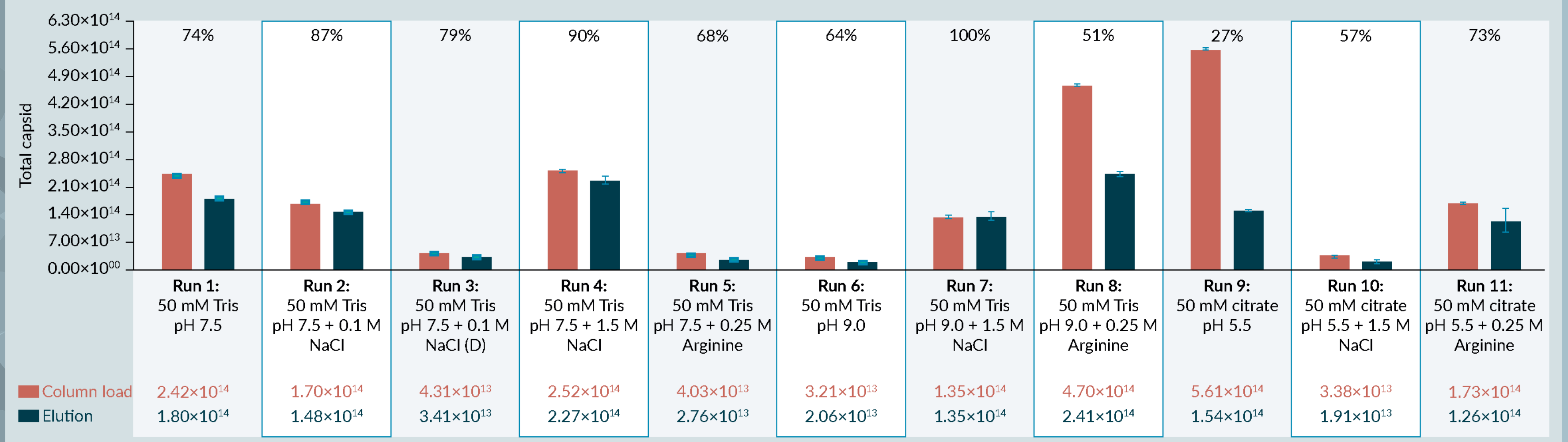
The heart of the AAV-MAX system is VPC 2.0—a clonal, 293F-derived suspension cell line for superior AAV production. In a typical shake flask, these cells will obtain 12 million cells per ml or greater and benefit from a non-clumping phenotype with robust scalability. The VPC 2.0 cell line will be available cGMP banked later in 2022.

POROS CaptureSelect AAVX wash optimization

Studies were carried out to determine optimum AAVX wash conditions. A total capsid ELISA was used to examine column load and elution and determine the total % recovery with different wash buffers **(Figure 2)**. Tris at pH 7.5 or 9.0 with 1.5 M NaCl resulted in the greatest recoveries. Citrate wash buffers resulted in the lowest recoveries, but recovery could be 25–50% improved with the addition of 0.250 M Arginine.

The wash conditions with the best % recoveries in the initial experiments were selected for additional combination wash purifications, which resulted in a smaller elution pool volume (1 CV versus 2–3 CVs) compared with a single wash. Overall, average log^{10} reduction values of 4.5 and 3.2 were achieved for residual host cell protein and host cell DNA removal.

Figure 2. Total capsid recovery with a range of wash buffers.



POROS CaptureSelect AAVX elution optimization

A further study explored the optimum elution conditions to maximize recovery of AAV6 capsids using POROS CaptureSelect AAVX. Various elution buffers were tested to determine the impact of pH (2.0, 2.5, and 3.0) and excipients (Arginine, $MgCl_2$, and Propylene Glycol) on AAV recovery.

Elution buffer conditions were tested using high-throughput screening, RoboColumn screening, and 1 mL column runs. In 1 mL column runs, the highest recoveries were obtained at pH 2.0 and 2.5, and the addition of Arginine allowed similar recoveries at pH 3.0.

Conclusion

Upstream production of AAV vectors requires a consistent and high-titer scalable production system, while downstream production must obtain a high-yield and high-purity product with a good safety and efficacy profile. The Gibco™ AAV-MAX Helper-Free AAV production system upstream plus POROS™ CaptureSelect™ AAVX chromatography resins downstream support a scalable, robust, and efficient AAV production workflow.

Wash optimization

Elution optimization

Watch webinar

CELL & GENE THERAPY INSIGHTS

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CELL & GENE THERAPY INSIGHTS

Implementing demand & operations planning in clinical cell & gene therapy

Peter Horton

The speed of breakthroughs in cell and gene therapy has resulted in explosive growth, presenting new challenges in the clinical trial supply chain. This rapid growth has exposed challenges that can disrupt the demand-supply cycle.

The emerging cell and gene therapy industry will require significant changes through the implementation of proven processes that have resulted in supply chain innovation. One of these key proven business processes is demand and operations planning (D&OP).

Companies that have implemented a D&OP process have seen drastic improvements in managing their demand–supply cycle. As successful as the D&OP process has been for many industries, it has traditionally been associated with large biopharma rather than small-medium sized CGT's clinical stage companies. This has created a slow adoption rate in CGT.

This article explains how D&OPs can be implemented in a clinical CGT environment and why it's important in implementing this process.

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For decades, the application of demand and operations planning (D&OP) has been used by large pharmaceutical and biotechnology companies with great success. D&OP is a key business management process that empowers leaders to focus on decision-making to drive smarter predictions related to the demand-supply cycles. The challenge has been making the case for adopting D&OP into the clinical stage cell and gene therapy companies.

Clinical cell and gene therapy (CGT) companies are generally smaller and without revenue from other products due to their

individual nature, smaller patient count, and limited clinical pipelines. According to 2021 Global Data, along with big biotech companies there are many small- and medium-sized startups that are developing the field in CGT. This includes over 300 European and 60 North American companies bringing custom therapies through a complex temperature- and time-sensitive supply chain [1,2]. Since most CGT companies have limited budgets they need to be more strategic on their investments and protect their program pipeline from risks associated with supply chain issues. The D&OP ensures that the demand-supply framework makes a perfect case to invest into as an efficient process that seamlessly coordinates chemistry manufacturing and control (CMC), clinical operations, and external functions to deliver an optimized patient-focused supply chain.

One of the biggest challenges facing many clinical stage CGT companies is their inability to manage demand-supply cycles effectively. Even with autologous treatment they are not immune against unplanned disruption in the demand-supply cycle. From the demand side, clinical designs and phases are increasingly more complex, as well as facing the realities of difficulties in recruitment and enrollment. This will drastically impact even the best clinical forecast for patient enrollment, attrition rate, site initiation, and dose escalation which have high variability and are therefore difficult to predict. On the supply side, there is high variability, especially with living organisms (molecules, cells, tissues, and organs), which are highly susceptible to mechanical stresses (heat, light, temperature) and donor pool availability. Further risk is added as many of the manufacturing materials required to produce cell and gene therapies are single sourced, with long lead times and short expiry. This critical area is often overlooked by CMCs in clinical study execution. The D&OP process can catch this oversight as part of the planning horizons and deliverables outlined in the charter and roles defined in the RACI Matrix [3,4]. The acronym RACI stands for responsible, accountable, consulted, and informed and is represented by a diagram that identifies the key roles and responsibilities of users for major tasks within a project. In addition, one of the vital parts of the D&OP process is the development of a supply continuity plan that is endorsed by senior management and implemented early in the D&OP process. An effective plan will minimize supply and supplier risks throughout the clinical supply chain process.

With the recent COVID global pandemic and force majeures in the chemical industry associated with winter storm Uri, the industry has experienced extensive lead times and shortages for critical polymer components, (e.g. filter adapters, filtration bags, and tubing sets) that are required for good manufacturing practice (GMP) CGT manufacturing. Without an integrated framework to balance demand-supply cycles, this would have created reactive and panic planning. Implementing a D&OP process would provide the necessary forum for on-going supplier surveillance and inventory strategy to proactively mitigate supply risk as part of short-range planning. Supplier surveillance and inventory strategy would be a key deliverable that would be outlined in the charter and roles defined in the RACI Matrix.

The impetus for change is evident for clinical CGT companies and the D&OP process will help deliver results through each clinical phase to commercialization. To do so, companies will need to have the right champion, sponsors, and framework in place [3,4].

D&OP CHAMPION

A D&OP champion will need to come forth and advocate for change by focusing on the benefits of establishing a D&OP framework [5]. To do this, the D&OP champion will need support from the head of CMC and clinical operations. These two primary functional heads will be critical to obtain commitment, as well as help advocate for change.

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The champion would find it beneficial to onboard the functional heads by outlining what makes clinical CGT supply chain demand-supply cycles different. The champion should draft an initial charter to include the scope, deliverables, and a RACI regarding team member roles in the process as outlined in Figure 1. Using a charter, the champion would present the sponsors with the big picture and ensure there are no gaps in their understanding. The champion is also responsible for escalating key issues to sponsors and/or a steering committee to support key decisions and mitigate any roadblocks in the process. A successful onboarding is critical as these functional heads will most likely be joint sponsors for the D&OP charter, as well as gain approval of the resources based on the RACI.

Once the sponsors have been onboarded and agreed to provide the resources required to support the D&OP process, the next step is to educate and train potential members. Using the draft charter and RACI the sponsors were onboarded with, the team members will need to know the following:

FIGURE 1

D&OP charter.

DEMAND AND OPERATIONS PLANNING (D&OP) CHARTER

Project Name:	**Champion**	**Project Manager:**
Demand and Operations Planning	Individual who will be responsible for project	Manager of the overall project and its deliverables

Project Priority –… .			**Project Complexity** – …		
High ☐	Medium ☐	Low ☐	High ☐	Medium ☐	Low ☐

Business Case and Objective
Document the business justification for initiating the project and the benefit realization to the company. Describe the current situation or problem that is driving the project.

Stakeholders	**Sponsor(s)**
Individuals or group that may be affected/impacted	Executive Management

In & Out of Scope
Scope describes the boundaries of the project. It should include what is included (In Scope) and excluded (Out of Scope) from the project.

Project Team Members	**Title**	**Function**
Those who will accomplish assigned project activities	Member's position	Member's role on the project team

Planning Horizons and Deliverables		
Deliverable	**Reviewed and Accepted By**	**Initial & Date**
List the individual deliverables that correlate to the top-level tasks that would be included in a work breakdown structure (WBS) on a project schedule. Deliverables should be outlined for short-intermediate-long range plans.		

Scenarios and Assumptions
Factors that, for planning purposes, will be true, real, or certain. Information that must be validated, or situations identified that needs to be modeled during the project to facilitate the planning process.
Constraints and Risk.
Factors that will limit the project team's option and known constraints: Time frame to completion, cost/budget, resources (internal/external), quality/validation steps, etc.

Sponsor(s) Approval			
Role	**Name**	**Signature**	**Date**

- Why do we need a D&OP?
- How do they fit within the D&OP process and expectations?
- What is the mission and what are the business challenges to address?
- How can D&OP be applied to the clinical phases, and what are the deliverables?
- The charter and RACI will help build consensus and ensure any potential team members' concerns are addressed immediately.

D&OP CHARTER

With the complexity of CGT clinical demand-supply cycles, the D&OP team will face many challenges and need to make informed decisions. By developing a D&OP charter, the team's scope, deliverables, and RACI roles are clearly stated [3]. As part of the charter the team should develop a business case, assumptions, constraints, and risk shown in **Figure 1**.

When developing the charter, it needs to be tailored to clinical CGT, tied into clinical phases, establish planning horizons, and provide ability to perform 'what if' scenario planning. The charter must be flexible to adjust to the different clinical phases. As such, the D&OP process should take a 'phase appropriate' approach to proactively address scale-up of the clinical phases shown in **Figure 2**. For example, where enrollment is less than 50 patients with a limited number of sites, a charter for Phase 1 would be different than for Phase 3, where patient enrollment could be in the 100's with study sites worldwide and manufacturing batch sizes increasing. As the clinical trials progress from phase to phase, the team's oversight to proactively manage deliverables would include formulating strategies that provide the most significant opportunities to mitigate risk and increase supply chain flexibility. To ensure that the D&OP process is proactively addressing scale-up issues, a specific planning horizon must be included in the charter.

Too often CMC and Clinical Operations are dealing with short range issues and tend to overlook next clinical phases. The D&OP charter should include three planning horizons that would reinforce the need to look ahead. The planning horizons would consist of:

1. Short-range (right now +3 months);
2. Intermediate range (up to 12 months), and;
3. Long-range (up to 24 months).

To appropriately identify risk and scale-up issues the D&OP charter needs to include performing 'what if' scenarios as part of the planning horizons. 'What if' scenarios should focus on those risks where the consequence is high, meaning that risk events with low consequences are not important to deal with from a planning perspective within the base case plan. Risk is distinguished by the initial risk assessment that looks at the structural part of the supply chain, probability of occurrence, their vulnerability and impact.

Scenario planning can help create different demand plans based on clinical phases and enrollment rates, or dosage proposals that would help exploit risk of shortages or costly material expiry.

Scenario should include:

- Base case: typically, what is in the company budget or clinical protocol;
- Low case: what are the potential demand downside scenarios that could include delay or slower enrollment rate, dosage, regulatory approval delays, etc;
- High case: what are the potential demand upside scenarios that could include accelerated enrollment rate or higher dosing levels, regulatory approval acceleration, etc.

The role for establishing different scenarios should be defined in the RACI. In most cases

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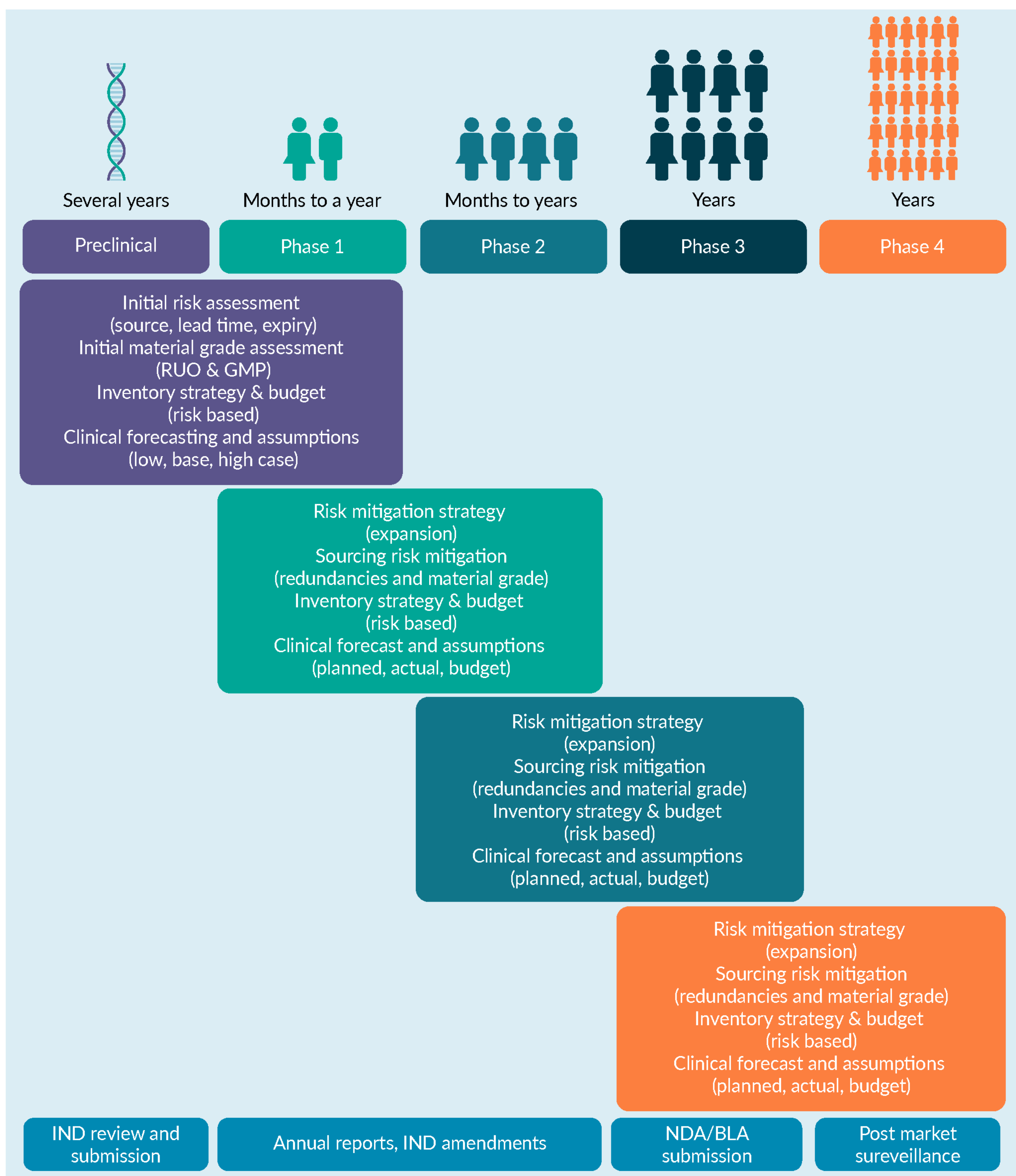
FIGURE 2

Clinical C&OP phase appropriate.



BLA: Biologics license application ; GMP: Good manufacturing practice ; IND: Investigation new drug ; NDA: Non-disclosure agreement; RUO: Research use only..

the head of clinical operations would be accountable for demand scenarios and the head of CMC would be accountable for supply scenarios. The clinical trial manager and supply chain manager would be responsible for executing the scenarios.

Once the charter has been developed and agreed upon by the team members it should be signed by both heads of clinical operations and CMC to indicate executive management support and commitment to the team. The sponsors should agree on the frequency of the D&OP meetings. Typically, D&OP meetings occur once a month, which is frequently enough to address any changes in the demand-supply cycles.

D&OP RACI MATRIX

D&OP requires high cross-functional engagement from key stakeholders for the process to be successful. When dealing with CGT start-ups the challenge for the champion is demonstrating the D&OP process is not too cumbersome to maintain with limited resources. To achieve this a clearly defined RACI Matrix will highlight each functional role as seen in Table 1 [4,5].

As part of the D&OP RACI Matrix, clinical operations will play a critical role. Clinical operation refers to the functions that support the clinical trial process from start-up to close-out. Within clinical operations, the primary contact for the clinical forecast is the clinical trial manager. They essentially perform the functional role of demand planning in terms of forecasting. The clinical trial manager would be responsible for clinical forecast and assumptions from the trial design regarding patient enrollment, study duration, dose escalation, site initiation, and many other forecast drivers. The clinical trial manager will also be responsible for reporting actual clinical trial performance and provide updates from the original assumptions. This ongoing forecast and assumption review will be one of the primary deliverables of the D&OP Charter.

The other critical role of the RACI Matrix is CMC. This function involves manufacturing practices and specifications that are followed to ensure product safety and consistency continues through all remaining stages of the drug development life cycle. Within CMC, the supply chain manager is responsible for the demand-supply cycles including drug product, drug substances, raw materials, and manufacturing consumables. As seen in Table 3, the supply chain manager can perform an initial risk assessment that would indicate potential issues and can proactively take appropriate steps to escalate into the D&OP process to mitigate supply risks that may lead to inventory shortages and trial delays. Their deliverables would be identified as part of the D&OP matrix as both accountable and responsible for the initial risk assessment as displayed in RACI matrix in Table 1.

One critical output of the initial risk assessment is the sourcing risk assessment (Table 2). As an example, single or sole source suppliers and research use only (RUO) grade materials are very commonplace in CGT. The supply chain manager could be Accountable to perform this assessment. The supply chain manager would be responsible to develop a detailed plan outlining key steps to mitigate risk based on specific planning horizons. For single source RUO suppliers, the short-term plan would be establishing an inventory buffer strategy and supplier surveillance meetings with critical suppliers to proactively assess risk and mitigate any uncertainty in supply. Since RUO's have a more scaled down change notification requirement than (GMP materials, an intermediate range plan could be establishing more robust change notification terms with the critical suppliers. Longer-range plans could include establishing incentive programs with key suppliers to qualify a GMP grade version of the material.

It is important to note a critical role is to include Regulatory especially in the areas where decisions could impact either regulatory strategies and/or timelines. As an example, a change in either materials, grade, or sources of supply could have regulatory impact.

CLINICAL D&OP PHASE APPROPRIATE

The D&OP process will evolve throughout the clinical phases. It will be important that

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TABLE 1

Clinical D&OP RACI.

Deliverable/role	Clinical ops	Trial manager	Program manager	CMC supply chain	Regulatory	Process development	Manufacturing manager internal and/or external
Clinical and regulatory milestones	R	R	A	I	C	I	I
Demand plan							
Clinical forecast (enrolment, site initiation and attribution rate)	A	R	I	I			
Clinical assumptions	A	R	I	I			
Supply plan							
Demand/supply scenarios	C	C	I	A/R			C
Drug substance/drug product supply	I	I	I	C			A/R
Risk assessment							
Initial risk assessment	C	C	C	A/R	C	C	C
Supplier surveillance	I	I	I	A/R	R		R
Capacity assessment	I	I	I	C		C	A/R
Sourcing risk assessment	I	I	I	A/R	C	C	C
Inventory strategy (buffer stock, expiry, spend)	I	I	I	A/R	R		C

A= Accountable, CMC: Chemistry, manufacturing, and controls; C= Consulted; I= Informed; RACI: Responsible, accountable, consulted, informed, R= responsible.

the D&OP team takes a phase-appropriate approach by focusing on current phases with an outlook up to 24 months (Figure 2).

As each clinical phase builds upon each other, so will the D&OP process with targeted deliverables for risk assessment and mitigation based on time horizons. This approach will allow the D&OP process to continuously adjust proactively to effectively manage future demand-supply cycles.

The best time to start the D&OP journey is during the pre-clinical stage, especially ahead of the investigational new drug (IND) application. One of the common CMC failure modes for cell and gene therapy during this stage is insufficient supply chain oversight. This lack of oversight will lead to poor risk management, resulting in inadequate budgeting into a sound supply chain and inventory strategy as an 'insurance policy; to buffer against unplanned disruptions in the demand-supply cycle. Early adoption of the D&OP process will turn panic into predictive planning and ensure a patient-focused supply chain.

Starting the D&OP process will immediately provide benefits, especially around risk management and mitigation [7]. Performing an initial risk assessment based on key variables is the feeder into the D&OP process for developing mitigation plans, as well as defining a sound buffer stock strategy as that 'insurance policy' to hedge against unexpected supply chain issues. Another outcome is understanding the current sourcing and material grade that will impact the IND application. During the pre-clinical stage, raw materials, consumables, and source materials are typically identified during research and development.

The bill of materials (BOM) is the best source to drive the initial risk assessment as it identifies the material type, the supplier,

TABLE 2

Sourcing risk assessment.

Sourcing assessment	Risk	Risk level	Short range	Intermediate range	Long range
Supplier 1	Sole sourced good manufacturing practice (GMP) grade	●	• Define and execute inventory buffer strategy • Initiate supplier surveillance meetings	• Execute master supply agreement • Strengthen supplier relationship management (SRM) • Identify alternative and regulatory impact	• Establish top to top senior leadership meeting with supplier • Evaluate equity stake with critical supplier
Supplier 2	Single source research use only (RUO) grade	●	• Define and execute inventory buffer strategy • Initiate supplier surveillance meetings	• Establish and deploy change notification terms for RUO grade material • Develop collaboration program with supplier to establish GMP grade version road map • Strengthen supplier relationship management (SRM)	• Establish top to top senior leadership meeting with supplier • Establish incentiveprograms for GMP grade version

cost, quantity, and other relevant data. By developing an initial risk assessment for each lead product candidate, it will provide the D&OP process with transparency on critical supply risk to help develop better planning models to avoid stockouts, costly expiry issues, or impact regulatory filing timelines.

The framework of the initial risk assessment will also afford the team a common purpose for their first tangible deliverable.

Using a risk assessment, teams can implement a framework that will provide:

- Balanced risk assessments, which are the perfect way to measure the balance between risks and controls;
- A common language to effectively understand and manage risk, and;
- A structured approach to assess risk and consistently apply the appropriate controls.

The risk assessment is one of many useful tools within the D&OP process that can drastically reduce risk and move from panic to predictive planning.

CONCLUSION

There is no question that the groundbreaking treatments being developed in CGT are fundamentally changing how patient care is managed around the world. As such, clinical CGT companies need to implement breakthroughs in supply chain management. The most important step companies can take to ensure their supply chains are fundamentally strong is adopting proven technologies. The D&OP process has become a commonplace over the past several decades and has transformed a wide range of industries by improving their demand-supply cycles.

For clinical CGT companies, the impetus to change clearly focuses on a singular point: to have the ability to execute the patient-focused supply chain flawlessly, bringing clinical cell and gene therapy products to patients safely and cost-effectively.

The D&OP process is a journey. The correct path starts with a champion who can scale up the organization's approach with a shared vision and knowledge to help stakeholders to adopt and do what it takes to build a sustainable, successful demand-supply process.

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TABLE 3

Initial risk assessment.

Assessment								Inventory strategy			
Supplier	Extended bill of materials ($)	Supplier surveillance program	Supplier 2022 performance	Sourcing Sole= red Single= orange Multiple= green	Qualification time High IND impact= red Low IND impact= orange No IND impact= green	Shelf life <3 months= red 4 to 12 months= orange >12 months= green	Overall risk level High= red Medium= orange Low= green	Buffer strategy months	Buffer stock	Cycle stock	Total stock
1	$$$$	Monthly	Green	Orange	Red	Red	Red	1.5	$$$$	$$$$	$$$$
2	$$$$	Monthly	Orange	Orange	Red	Orange	Red	4.5	$$$$	$$$$	$$$$
3	$$$$	Monthly	Green	Orange	Red	Orange	Red	4.5	$$$$	$$$$	$$$$
4	$$$$	Monthly	Green	Orange	Red	Orange	Red	4.0	$$$$	$$$$	$$$$
5	$$$	Monthly	Orange	Orange	Red	Red	Red	4.0	$$$	$$$	$$$
6	$$$	Monthly	Green	Orange	Red	Orange	Red	6.0	$$$	$$$	$$$
7	$$$	Monthly	Red	Orange	Red	Orange	Red	4.0	$$$	$$$	$$$
8	$$$	Monthly	Green	Orange	Red	Orange	Red	4.0	$$$	$$$	$$$
9	$$$	N/A	Green	Orange	Orange	Orange	Orange	4.5	$$$	$$$	$$$
10	$$	Monthly	Red	Orange	Orange	Orange	Orange	4.5	$$	$$	$$
11	$$	Monthly	Red	Orange	Orange	Orange	Orange	6.0	$$	$$	$$
12	$$	Monthly	Orange	Orange	Orange	Orange	Orange	6.0	$$	$$	$$
13	$$	N/A	Green	Orange	Orange	Green	Orange	9.0	$$	$$	$$
14	$$	Monthly	Green	Orange	Orange	Green	Orange	6.0	$$	$$	$$
15	$	N/A	Red	Green	Green	Green	Green	9.0	$	$	$
16	$	N/A	Green	Orange	Orange	Green	Orange	9.0	$	$	$
17	$	N/A	Green	Green	Green	Green	Green	12.00	$	$	$
18	$	N/A	Green	Orange	Orange	Green	Orange	9.00	$	$	$
19	$	N/A	Green	Green	Green	Orange	Orange	9.00	$	$	$
20	$	N/A	Green	Green	Green	Green	Green	12.00	$	$	$
21	$	N/A	Green	Green	Green	Green	Green	12.00	$	$	$
22	$	N/A	Green	Green	Green	Green	Green	12.00	$	$	$
23	$	N/A	Green	Green	Green	Green	Green	12.00	$	$	$
24	$	N/A	Green	Green	Green	Green	Green	12.00	$	$	$
25	$	N/A	Green	Green	Green	Green	Green	12.00	$	$	$
Grand total	$$$$								$$$$	$$$$	$$$$

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CELL & GENE THERAPY INSIGHTS

Advanced therapies, hospital exemptions & marketing authorizations: the UK's emerging regulatory framework for point-of-care manufacture

Edison Bicudo & Irina Brass

Hospital-centered manufacture, which consists in producing therapies close to the patient, within a hospital or in a nearby unit, is becoming increasingly viable and necessary. There are at least two modalities of this kind of manufacture: in what we name bedside manufacture, therapy production relies on hospital infrastructure and facilities, products can have all sorts of shelf life, and a small number of hospitals is involved; in the emerging modality called point-of-care manufacture, there is great reliance on portable manufacturing devices taken to the hospital, products have short or very short shelf life, and a large number of hospitals may be involved.

The UK's Medicines and Healthcare products Regulatory Agency (MHRA) has proposed a new regulatory framework dedicated to point-of-care manufacture. A large range of products can be manufactured this way, including some Advanced Therapy Medicinal Products (ATMPs), which are medicines based on cells, genes or tissues.

Bedside manufacture has been traditionally overseen via regulatory exemptions. In the European Union (EU), the manufacture of ATMPs in hospitals or for hospitals has been covered by the 'Hospital Exemption'. In the UK, another exemption, known as the Specials scheme, has been used. Both exemptions are grounded on the specificities of hospitals and clinical needs.

The MHRA's current regulatory proposal introduces a new rationale in which point-of-care manufacture will be subject to a flexible and proportionate framework while following the regulatory pathway now valid for commercial products, including the conduct of clinical trials and the issuance of marketing authorizations. This brings about a market route that will coexist with the clinical route of exemptions.

This article analyzes the implications and uncertainties of the UK's possible move from regulatory exemptions (bedside manufacture) to marketing authorizations (point-of-care manufacture) for hospital-produced ATMPs. It also sheds light on strategic issues triggered by the MHRA's proposal.

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INTRODUCTION

Therapy manufacturers, which have traditionally carried out their production in a few manufacturing units, are slowly proposing new approaches where much larger numbers of manufacturing units are mobilized [1,2]. Among these systems, there is hospital-centered manufacture, a model where therapies are produced in either hospitals or units adjacent to hospitals. This paper focuses on the UK's changing regulatory framework for this kind of production. We analyze this emerging model and ask: when new ways of manufacturing therapies are proposed, how quickly and effectively can regulators react to such changes, and what are the implications of those technical and regulatory shifts?

As we showed elsewhere [3–5], if there is little time available from completion of therapy manufacture to administration to the patient, hospital-centered production can be a viable option. Occasionally, it will be the only solution available, especially if it is not possible to freeze materials or products for transportation, as rapid manufacture and application at the hospital are required. This is likely to be the case for a range of products, including Advanced Therapy Medicinal Products (ATMPs), a group of cutting-edge therapies based on genes, tissues or cells [6]. (This definition of ATMPs has been proposed by the European regulator – the European Medicines Agency (EMA) – and continues to be valid in the UK even after the country's departure from the European Union [Brexit]). Further information about the European ATMP Regulation is provided below).

In addition to enabling expedient therapy delivery [7,8], hospital-centered production of ATMPs has been said to bring about benefits such as cost reductions [7,9–11], acceleration of bench-to-bedside innovation [7], and mitigation of risks generated by market shortages [10].

In order to make our analysis more precise, as well as account for the regulatory changes taking place in the UK, a distinction will be made between two kinds of hospital-centered manufacture, as summarized in Table 1.

In bedside manufacture, therapy production relies on manufacturing resources (facilities, devices, equipment) present in either the hospital or the unit of a company hired by the

TABLE 1

Two modalities of hospital-centered manufacture.

Characteristic	Hospital-centered manufacture: 1. Bedside manufacture	Hospital-centered manufacture: 2. Point-of-care manufacture
Product's shelf life	Long, medium, short or very short	Short or very short
Responsible organization	A hospital-centered team or a company hired by this team	In most cases, a company
Location of manufacture	The hospital or the company's manufacturing facility	The hospital
Infrastructure used	The hospital's infrastructure or the company's facility	Portable devices taken to the hospital
Number of hospitals involved	Small	Large
Kinds of therapies manufactured	Any kind of therapy	Therapies requiring rapid manufacture and immediate application

hospital. In point-of-care manufacture, the therapy is always produced in the hospital, by means of portable manufacturing devices taken to the hospital by a company. It is important to explain that, as our study has revealed, bedside manufacture has been practiced for decades whereas point-of-care manufacture is an emerging modality, with only a handful of companies having manufacturing systems with some of its features (Below we will focus on the example of Biotherapy Services, a company that is currently manufacturing, at some NHS settings, a product of very short shelf live). Furthermore, it is key to reiterate that point-of-care manufacture will be applied when products have short or very short shelf life, as freezing the product for transportation would compromise its stability or potency. This may be the case for different kinds of cells or tissues, as well as different disease areas. Ongoing and future research and development activities will therefore indicate what ATMPs will require this model of manufacture.

The difference between bedside manufacture and point-of-care manufacture, which we introduced above for analytical purposes, is not found in existing literature or regulatory texts. Even though many analyzts would consider these phrases as synonymous, the distinction is important here, as it will make it possible to analyze various regulatory aspects of hospital-centered manufacture, as well as the shifts now taking place in the UK. Furthermore, the distinction reflects a usage that is gaining recognition in the UK and may therefore not be in line with the terminology adopted in other countries.

So far the assumption made by regulators in different countries is that in clinical settings, it may be difficult or impossible to follow all the strict regulatory procedures typically adopted by companies producing ATMPs [11]. When therapy manufacture occurs in hospital, the manufacturing staff, equipment, and material infrastructure are present primordially for clinical reasons, not industrial reasons. Moreover, the production may occur under clinical pressure that is never faced by the industry. Manufacturing therapies within or close to the hospital would then justify the application of exemptions from some regulatory requirements such as conduct of some tests and specifications for final products.

For these reasons, exemptions have been used in different jurisdictions for the regulation of hospital-centered therapy production. Different exemption schemes may even coexist in the same jurisdiction, like in the UK's case. The country has had a regulatory exemption scheme known as the 'Specials scheme' [12–14]. In parallel, the UK recognizes the Hospital Exemption scheme, which was created in 2007 by the European Union and was transposed into UK law by means of the 2010 'Guidance on the UK's arrangements under the Hospital Exemption scheme' [12]. The EU's and UK's exemptions are similar but the British one is more flexible, as it enables the importation of unlicensed therapies, the prescription of therapies by dentists and supplementary prescribers, and the administration of products outside hospitals. Thus the UK constitutes an interesting case in which two exemption schemes that can be used for hospital-centered manufacture coexisted for over 10 years.

Technology advancements have heightened the viability of manufacturing ATMPs, including in hospital settings [7,8,15]. It is in this context that the UK has gained regulatory autonomy as a result of its departure from the EU (so-called Brexit), with the resulting Medicines and Medical Devices Act (MMDA 2021) [16], which provides the UK's Medicines and Healthcare products Regulatory Agency (MHRA) with authority to introduce or change regulations pertaining to human medicines and medical devices. The MHRA has been aware of the potential expansion in hospital-centered manufacture, including the emergence of point-of-care manufacture. As a result, the agency is proposing a new regulatory framework specifically designed for point-of-care

manufacture, which was submitted to public consultation from August to October 2021 [17]. If approved, the framework will apply to a broad range of therapies, which includes ATMPs but also products such as medical gases, blood-derived medicines, and 3D printed medical devices.

This proposal introduces, for point-of-care manufacture, a key change in relation to the ways in which bedside manufacture used to be regulated: point-of-care manufacture will take place, no longer in the framework of exemptions, but in the framework of marketing authorizations. In this way, it will be possible to manufacture therapies in hospitals while aiming for the conduct of clinical trials and, eventually, the issuance of a market authorization. This represents a considerable shift, as hospital-centered manufacture has traditionally warranted bespoke and special regulatory requirements, with products being delivered without the need for marketing authorizations, and therefore not provided as commodities on the market.

In this way, the MHRA's regulatory framework opens up a rich opportunity for the study of the move from exemptions (bedside manufacture) to authorizations (point-of-care manufacture). This article aims to identify the emerging issues, uncertainties, and potentialities of this passage. What are the challenges and promises of the UK's emerging point-of-care regulatory framework for ATMP development and production? What sorts of technical and political trends does it reflect or facilitate?

To address these questions, this article is organized as follows. Initially, we introduce the research methods on which our study has been based. We move on to analyze the UK's landscape for bedside manufacture and its dual exemption approach (with the Specials scheme and the Hospital Exemption). Subsequently, we describe the MHRA's point-of-care regulatory proposal, analyzing its rationales, potentialities, and challenges, with a focus on ATMP manufacture. The final section brings some closing considerations.

RESEARCH METHODS

This research project has been conducted at the Department of Science, Technology, Engineering and Public Policy of University College London (UCL) since 2017. Its main goal is to analyze the regulatory challenges in the manufacture of ATMPs. In addition to a literature review, the project mobilises the following three methods, which have been reviewed by and approved by UCL Research Ethics Committee.

First, in-depth qualitative, semi-structured interviews have been conducted with professionals involved in the development or manufacture of ATMPs, including entrepreneurs and academics working towards enabling hospital-centered manufacture. The range of the interviewee's expertise is summarized in Table 2.

In line with our research ethics approval, interviewees were given the option whether to associate their interview with their institutional affiliation. Therefore, in this article, not all interviewees have their institutional affiliation specified. All the interviews were recorded with informed consent from the interviewee.

The interviews explore the scientific, technical, institutional, and political challenges of ATMP development and manufacture, including the challenges entailed by hospital-centered manufacture. We also explored the interviewees' opinion about the UK's emerging regulatory framework for point-of-care manufacture.

For analysis, different parts of the interviews received codes based on the topics addressed by the interviewees (content analysis). In this way, it was possible to identify recurrent concerns, hopes, and uncertainties held by those who are somehow involved in, or aware of, ATMPs, their development and manufacture. The same codes were also used in the notes we have taken in our literature review, so we can relate what interviewees declared to other strands of our project. With this approach, relevant and recurrent themes

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TABLE 2

Qualitative interviews: interviewee's affiliation.*

Institution	Interviewees		
	In the UK	Other*	Total
Pharma/biotech company or contract manufacturing organization	6	4	10
Hospital department	8	2	10
University research laboratory	6	3	9
Regulatory agency, regulatory consultancy firm or government agency	11	6	17
GMP manufacturing facility†	5	0	5
Total	36	15	51

* Ireland, Germany, Spain, Switzerland, Belgium, Israel, and United States.
† Good manufacturing practice facilities are therapy production units funded by public bodies; contract manufacturing organizations are companies selling therapy manufacturing services to other companies.

emerge from our research data, in such a way that we are then able to connect themes in a coherent interpretation.

Second, a quantitative analysis of the UK Specials scheme was conducted. This was done with data published by the MHRA on its website [18]. Some charts, tables, and maps were thus produced, providing an overview of this regulatory scheme. For data processing and visualization, the R programming environment [19] was used (more specifically the following libraries: dplyr, readr, stringr, PostcodesioR, pdftools, sp, rgdal, and ggplot2).

Finally, we hosted an online workshop that addressed the challenges of ATMP point-of-care manufacture, as well as the MHRA's regulatory proposal. The workshop took place in June 2021 and was joined by 32 specialists in the field of ATMPs, pharmaceuticals, therapy manufacture, and regulation. Prior to the event, all participants were informed that the workshop would also involve information collection for our research project. The discussions of the event were the object of a separate publication [5].

Based on these research methods, we present here an analysis of the rationales and trends introduced by the MHRA's point-of-care regulatory proposal. Initially, the following section outlines the ways in which regulatory exemptions have traditionally been used for hospital-centered therapy production.

BEDSIDE MANUFACTURE: THE IMPORTANCE OF REGULATORY EXEMPTIONS

The international landscape

Regulatory exemptions have long been used, in many economic sectors, as they enable flexible and fine-tuned regulations [20]. They may receive different names: exceptions, waivers, variances or adjustments [21]. They are put in place whenever the regulator frees some people or entities from certain obligations or requirements. In other words, we are dealing with "[…] those exemptions granted by agencies exercising their inherent authority to make exceptions to general regulations" [21]. In spite of its frequent occurrence, this mechanism is not always studied in detail [21–24] and there is still much to be analyzed about "the little-known nature of regulatory exemptions" [21].

Exemptions can be considered as necessary when certain activities are carried out in unusual locations. This is what happens

when therapies are produced in hospitals instead of specialized manufacturing units. In the case of ATMPs, regulators have indeed been aware of the specificities of hospital-centered manufacture. In 2001, for example, the USA Food and Drugs Administration (FDA) created the 'Same surgical procedure exception' [25], allowing the manufacture of human cells, tissues or cellular-centered products with no need for the mandatory registration, as long as the product is autologous (derived from biological samples of the patient to whom the therapy is destined) and collected, produced and implanted in a single surgical procedure [26].

Another key example has been the 'Hospital Exemption' created in the European Union (EU) [12,27–32]. The scheme was introduced in article 28 (paragraphs 2 and 3) of the Regulation 1394/2007, the so-called ATMP Regulation [6], which came into force in 2008. This Regulation created the Hospital Exemption by supplementing the provisions for marketing authorizations under Directive 2001/83/EC. The supplementation also introduced the definition of ATMPs, products based on cells, genes, or tissues. When a medical device or implantable medical device is also present, this constitutes a combined ATMP. In this way, the European regulation states that these therapies should be considered as medicinal products (or 'advanced' medicinal products) and should therefore be developed in accordance with the requirements valid for such products, in terms of manufacturing, quality controls, and pharmacovigilance. However, exceptions are granted when ATMPs are manufactured in hospitals.

As is often the case with regulatory exemptions, the EU's Hospital Exemption has been adopted because of specificities and special needs. In other words: "The exemption was included in the Regulation in recognition of the small scale and developmental nature of activity carried out in some hospitals, which argued for a degree of flexibility over the nature of regulatory requirements" [13]. Furthermore, as the industry may eschew the development of some therapies of risky development processes and unclear intellectual property prospects, hospitals may have to fill the gap by taking on the manufacturing responsibility [29]. Finally, in some rare disease areas, patient populations may be too small for the conduct of classical clinical trials, which can be solved by delivering the therapy under a Hospital Exemption [30], thus speeding up access to therapies that might take too long to be approved via traditional routes [33]. For these reasons, the European regulation allows the use of an exemption, so that hospitals manufacture and deliver some therapies as unlicensed products, that is, products that do not have a marketing authorization.

It has been claimed, especially by industrial players, that regulatory exemptions may create potentially harmful loopholes. The Hospital Exemption is sometimes said to "constitute a disincentive to develop ATMPs to current regulatory and manufacturing standards" [27]. To be sure, the EU's exemption allows manufacturers to follow flexible pathways in terms of therapy risk-centered assessment [29] and efficacy criteria [32]. However, those who are exempt still need to keep high standards for patients [27], as well as comply with traceability, quality, and pharmacovigilance standards for ATMPs [31,32]. Indeed, regulatory exemptions never amount to full regulatory freedom and could better be described as a special zone in the regulated field, as illustrated in **Figure 1A**.

Activities in the regulated area follow all the regulatory requirements whereas in the exempted area, they are not unregulated but are 'subject to less regulatory intensity' [23] for some specific reasons. This does not allow us to hastily conclude that unlicensed medicines are riskier that the ones with a marketing authorization. Such conclusion would need to be supported, for example, by the conduct of a systematic study comparing the occurrence of severe adverse reactions in unlicensed and marketed medicines, with all the methodological complications that this kind

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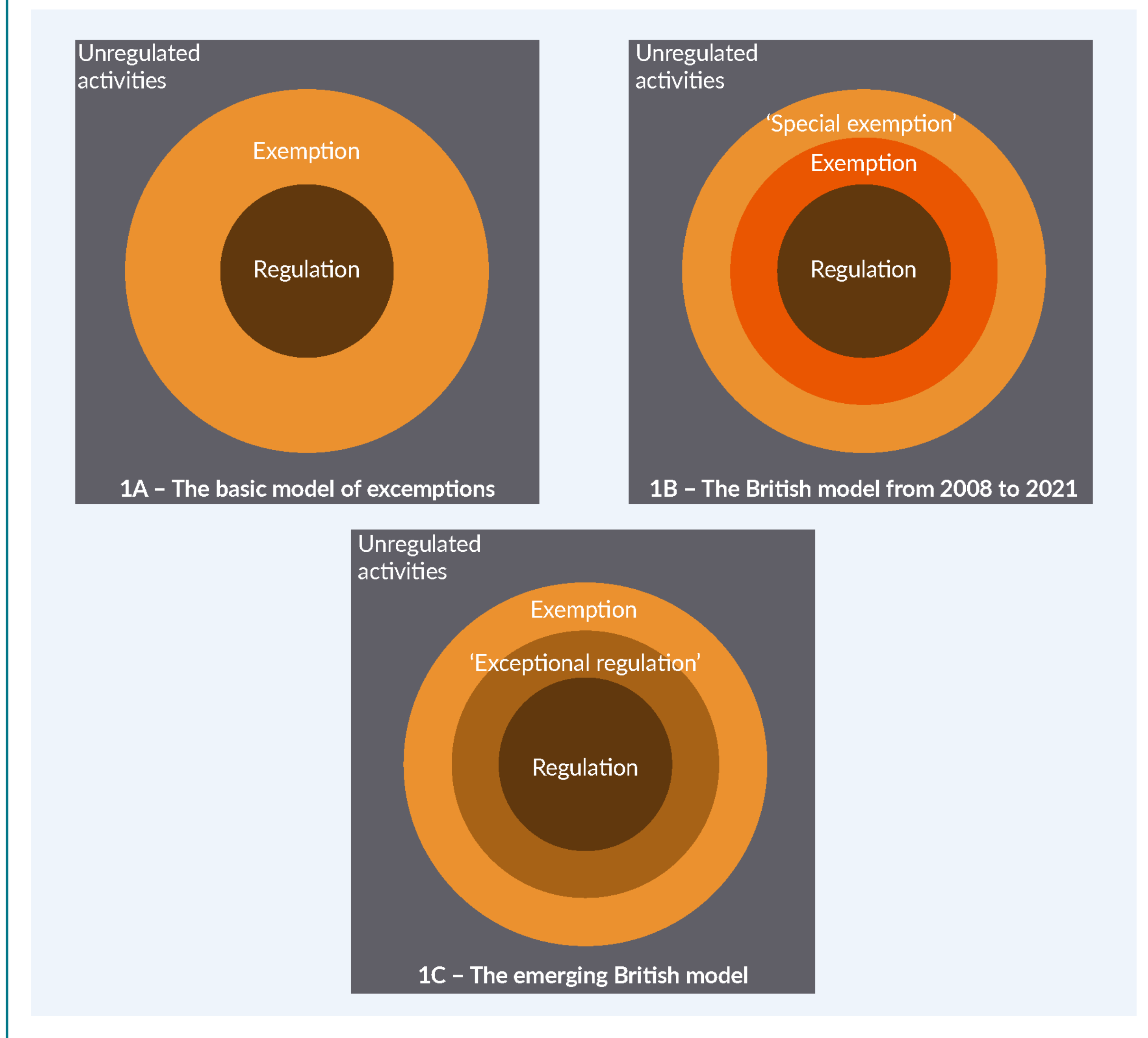
FIGURE 1

The role played by exemptions in regulatory frameworks.



of study would entail. Both classes of medicines are approved and overseen by regulatory agencies, which, in both cases, strive to make sure that patients can access therapies with as few risks as possible. If regulators decide to provide some manufacturers with some exemptions, this is due to the very specific characteristics of some products, which may, for example, target too small patient populations or require variable and tailored manufacturing processes.

Therefore, the EU's Hospital Exemption is grounded, on the one hand, on clinical needs, and this is why the exempted therapy should be manufactured "[…] under the exclusive professional responsibility of a medical practitioner, in order to comply with an individual medical prescription for a custom-made product for an individual patient" [6]. On the other hand, the unlicensed therapy should be produced in exceptional circumstances, being recognized that it will be "prepared on a non-routine basis" [6].

However, considerations other than clinical needs can be taken into account when regulations are designed or revised. In the UK,

a new rationale is emerging for a standalone regulation of point-of-care manufacture, as explained below.

The UK landscape

The UK has had, since 2010, two exemption schemes for bedside manufacture: its domestic Specials scheme and the EU's Hospital Exemption. The former has been much more frequently used than the latter. According to Interviewee 28, based in the MHRA, so far a Hospital Exemption has been obtained by only one British site, namely the Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Foundation Trust. However, because the EU law brings no precise regulatory definition for non-routine manufacture, there are doubts about the volume of therapies that can be produced under this scheme. In a written communication with the Cell-Therapies facility of the Trust mentioned above, we found out that this uncertainty was the reason why its Hospital Exemption was subsequently converted into a license under the Specials scheme, with less doubts in terms of quantities manufactured.

In EU Member States, Hospital Exemptions have not been numerous but have been used more frequently than in the UK. In their 2020 study, Coppens and colleagues [29] identified, for example, eleven exemptions given in France, eleven in Netherlands, and seven in Germany.

In the UK's case, there are two reasons for the underutilization of the Hospital Exemption. First, the Specials scheme is older than the Hospital Exemption, being therefore more familiar to British organizations. Indeed, companies and not-for-profit organizations attached to NHS Trusts have been created for manufacturing, exclusively, unlicensed medicines under the Specials scheme. There is even an Association of Pharmaceuticals Specials Manufacturers, now with twelve companies, including some non-British players [34]. Interviewee 2, a representative of this Association, explained that in order for companies to become members, "[…] the bulk of their commercial activity should involve the manufacture of unlicensed medicines." Second, the Specials scheme, compared to the Hospital Exemption, contains some additional authorizations [13,14]. The MHRA's guidance [13] spells out these authorizations, which are present in the scheme and absent in the EU's exemption: license holders can import therapies that are also unlicensed in other countries; the therapy may be commissioned by dentists and supplementary prescribers; and the therapy can be administered outside hospitals, at locations not specified by the regulation.

In this way, the UK's regulatory landscape has taken the form depicted in Figure 1B; there is, on the one hand, the EU's scheme (Exemption) and, on the other hand, a further withdrawal of regulatory requirements (a sort of 'Special exemption') promoted by the British scheme.

Like the EU's exemption, the UK scheme is based on clinical demands, as therapies must be manufactured 'to meet the special needs of individual patients' [13]. For this reason, the prescription of such therapies is subject to guidance from the General Medical Council, an independent body which sets clinical standards [35]. Furthermore, the prescription may be made only if there is no licensed therapy on the market for the particular disease [35].

With this format, the Specials scheme has attracted a variety of license holders. In January 2021, 56 institutions held these manufacturing licenses, most of which were obtained at an early period (from 2007–2010). Most of these license holders are NHS hospitals or Trusts, as shown in Table 3.

The high proportion of Trusts and hospitals licensed under the Specials scheme suggests that private companies are leaving manufacturing gaps to be filled by clinical institutions. For instance, Interviewee 38, based in an NHS Trust, explained why this institution decided to manufacture unlicensed medicines in a unit located in a

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hospital: "Because they tend to be products that are not used enough to make it worthwhile investing to get a product license." This holds not only for relatively simple products but for ATMPs as well. For example, the Newcastle upon Tyne Hospitals NHS Foundation Trust has used its Specials license for manufacturing a sight-saving product for an eye condition called limbal stem cell deficiency, as there is no licensed product on the market [36]. Furthermore, some ATMP areas tend to be underexplored. For example, Dimitropoulos and colleagues [30] claimed that companies are unlikely to focus on cell therapies for severe burns, which are not profitable enough for the industry.

Institutions with a Specials license must declare where their manufacturing activities will happen. In January 2021, there were 71 manufacturing sites registered, as shown in Figure 2.

For the production of Figure 2, we considered only licenses given from 2007, the year when the EU's ATMP Regulation was approved. In addition, we are considering only the MHRA's categories numbered from 1.3.1.1 through 1.3.1.7, encompassing: blood, immunology, cell therapy, gene therapy, biotechnology, human or animal extracted, and tissue engineering. These are, roughly, the categories also covered by the EU's ATMP legislation. (The limitation of our map is that institutions may be licensed but have no actual manufacturing activity. However, given the administrative work and economic investment required for obtaining a license, it is fair to consider that these players are at least planning to perform some manufacture, unless they are using their licenses only to import unlicensed products from other countries.)

Of the 71 sites seen on Figure 2 (represented by dots), 40 are Trusts or hospitals, constituting hospital-centered manufacture. An interesting case is provided by Biotherapy Services [37], a company offering a medicinal blood product (not considered as ATMP) for treating complex and chronic wounds that has been manufactured under the Specials scheme. Even though this company is not offering an ATMP, its manufacturing system has many characteristics of what the MHRA sees as point-of-care manufacture, especially the very short shelf life of its products, which amounts to less than twenty seconds. According to MHRA's data, the company had, in January 2021, six hospitals registered as manufacturing sites on its license, but this list is constantly updated. As explained by Interviewee 6, a Biotherapy Services employee: "To retain a site, we would need to hold equipment there, and routinely audit or attend the site to keep it ready to go. If we know we'll be very infrequently at a site, the upkeep becomes too much of a resource drain." Thus the Specials scheme, although useful, has displayed some limitations, especially for companies that need to constantly update their list of manufacturing sites.

Another of its weak points is the fragmentation it creates. As the Specials license is strictly bound to particular sites, it is frequently difficult to diffuse promising products and projects, a typical feature of regulatory exemptions. Speaking of the EU's Hospital Exemption, Interviewee 1, a European regulator, made this point: "[…] those things very, very rarely move out of that particular hospital, and if the Professor who does it retires, it disappears." Therefore, regulatory exemptions may lead to regulatory fragmentation, as it is frequently difficult for exempted players to engage in collaborations and mutual support.

This difficulty has been noted before. Beck [22], for example, claimed that in federalist

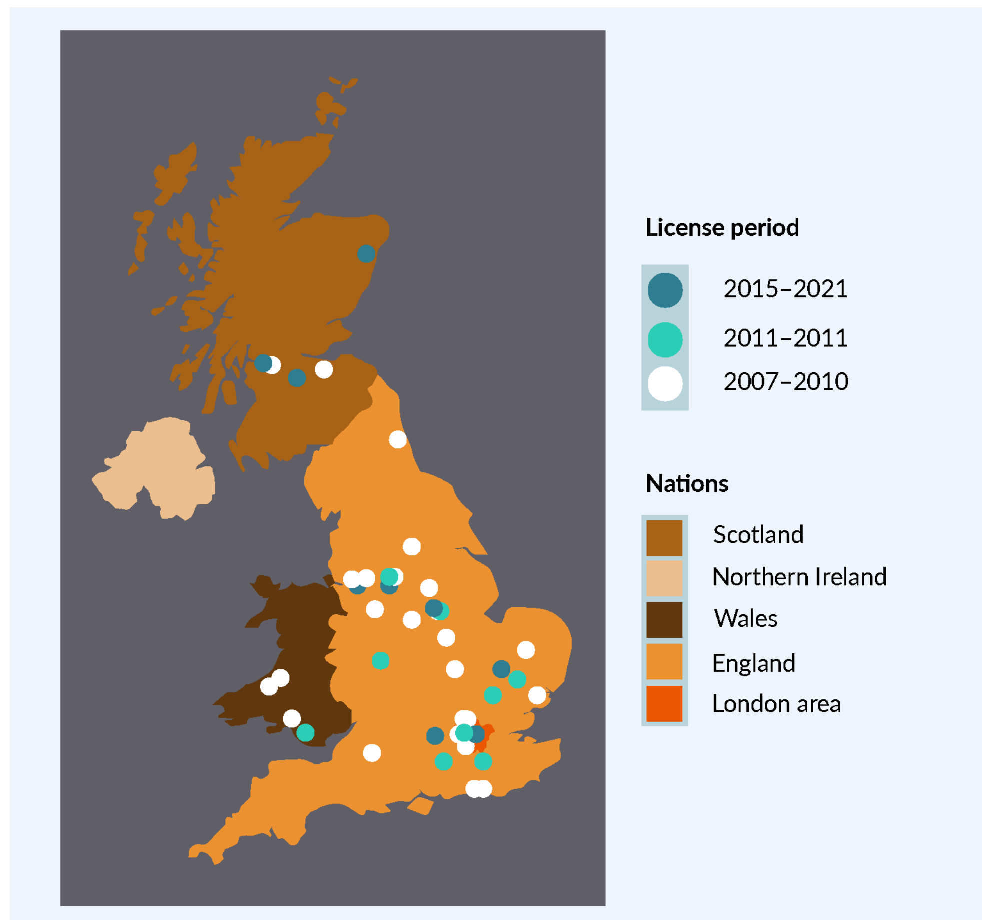
TABLE 3

Specials license holders, by nature of institution: January 2021.

License holders	Number
NHS trusts	26
Private companies	24
NHS hospitals	3
Universities	3
Total	56

FIGURE 2

Sites where unlicenced medicines (Specials scheme) may be manufactured: January 2021.



countries, regulatory exemptions subject organizations to state or local rules that foster local solutions. Even though the EU is not a classical federalist union, it has features of federalist political organization, as noted by some authors [38–40]. In this sense, the Hospital Exemption would promote regulatory disintegration by transferring key decisions to Member States. If, on the one hand, this creates a flexible regulatory scheme, the resulting landscape can, on the other hand, prove piecemeal. For the EU's Hospital Exemption, it has been stressed that Member States have transposed Article 28 into national regulation in disparate ways [12,29,31]. Thus, regulatory exemptions might be inappropriate when one expects, and wishes to promote, a controlled diffusion of technologies and products. This is one of the reasons why the UK's MHRA has proposed a new regulatory framework where a particular modality of hospital-centered manufacture is no longer managed by means of exemptions, as we see in the sequence.

POINT-OF-CARE MANUFACTURE: THE MHRA'S REGULATORY FRAMEWORK PROPOSAL

In Table 1, it was seen that one of the main characteristics of point-of-care manufacture is the use of portable manufacturing devices. Interviewee 11, based in Cancer Research UK, gave the following description:

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"[…] you are literally manufacturing the pharmaceutical in the hospital environment or in an outpatient care center, with the patient right there. So, if you think about dialysis. Dialysis uses a medical device which attaches to a patient […] So, blood is drawn from a patient, it is changed in the process, and then it is reintroduced into the patient. In many respects we could consider a point-of-care manufacture […] in the same way. You could be producing the biological drug or a small molecule even if you're, like, within a small device or a set of devices which mimic your manufacturing plant or your process."

As explained by Interviewee 17, based in University College London, this kind of highly automated manufacture has been attempted, at clinical trial level, in some hospitals of different countries. It is an emerging approach that "makes the patient part of the supply chain" [41].

In 2020, the MHRA organized two consultation meetings with specialists in the field, aiming to identify the challenges of point-of-care manufacture, as defined in Table 1. At the beginning of 2021, an additional online workshop was held to introduce the main lines of its regulatory proposal (Members of our research team participated in all those meetings). In mid-2021, a public consultation was carried out. Elsewhere [5], we described this process and outlined the regulatory framework, which is also briefly presented below.

One of the guiding considerations of the proposal is that for some products, including some ATMPs, the shelf life (that is, the length of time for which the product remains viable and safe after its production has been completed) will be short (some days), or extremely short (hours, minutes or seconds). This is so because manufacturers may need to handle cells and tissues whose therapeutic potential is minimized or destroyed if the product is frozen for transportation. Other products have short life spans, being incapable of surviving long periods outside the human body, as is the case of pancreatic islets [42]. In this way, manufacture must happen near the patient, being performed close to the hospital or even within it, sometimes very quickly. As explained by Interviewee 28, an MHRA regulator: "[…] you now have to go from your [manufacturing] room, up the corridor, to the operating theatre, you've got […] seconds to do that […]."

The MHRA also considers that for some products, manufacturing capacity and activities may be spread across a large number of hospitals. Some days prior to the 2021 workshop, the agency circulated a regulatory proposal document. One of the points made there is: "An application currently at clinical trial stage is projected to involve approximately 200 […] sites in the UK, which would manufacture a total of about 12000 products per year" [40]. With such large number of sites, the MHRA's inspection capability would be put under much strain.

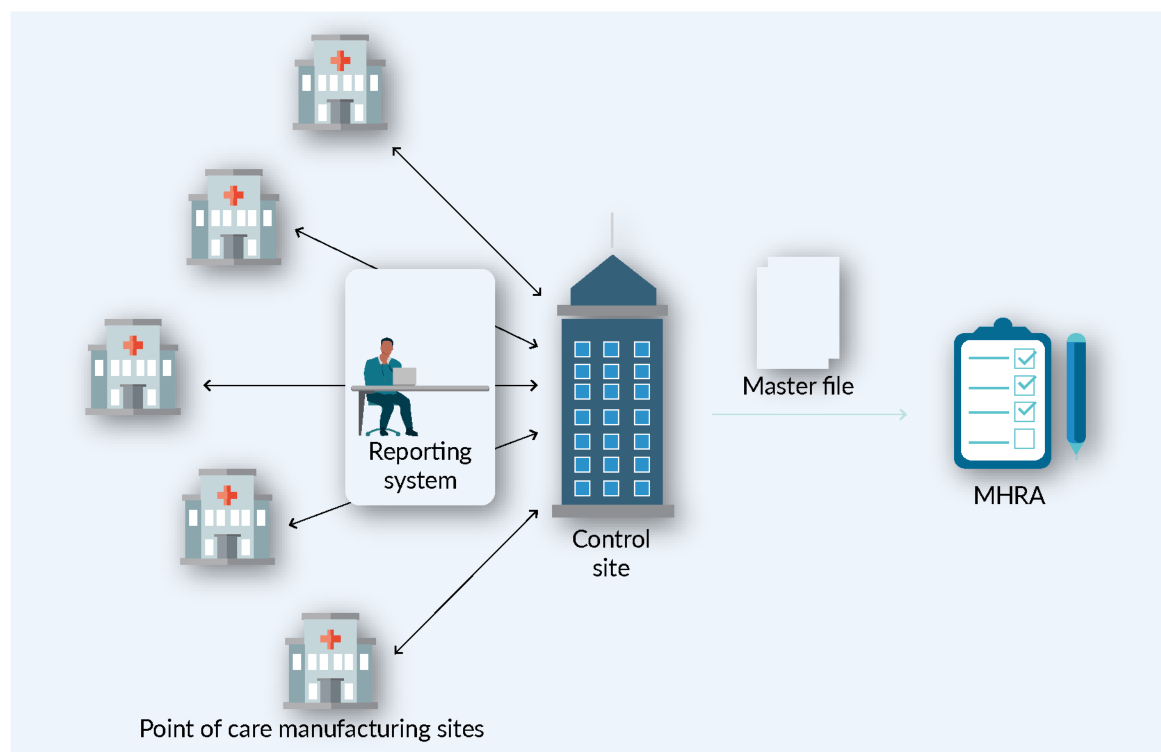
To face these challenges, the agency has proposed the new regulatory framework for point-of-care manufacture, as illustrated in Figure 3.

In this model, the key player is the so-called Control Site, the institution (probably, a private biotech or pharma company) responsible for various tasks: procurement of starting materials, manufacture, quality control, inclusion and exclusion of Manufacturing Sites, inspection of sites, traceability, and so on. Here we are no longer dealing with a regulatory exemption; thus the Control Site is expected to obtain a clinical trial authorization, run the trial, and eventually get a marketing authorization for the product, which will be sold as a commercial medicine [17,44].

Communication between the Control Site and Manufacturing Sites will take place through a reporting system whose sophistication will depend on the product's characteristics. When high risks are present, the system may involve real-time communication so adverse events can be reported with no delay. The Control Site will keep the MHRA informed about all the relevant

FIGURE 3

The MHRA's regulatory proposal.



Source [44].

aspects by means of a Point-of-Care Master File. This document, whose contents are yet to be fully specified, will contain information such as product properties, Manufacturing Sites details, adverse events, and GMP inspections [17,44]. The MHRA also wishes to create a framework where site management is dynamic and simple, so the Control Site can '[…] add new manufacturing sites in order to increase manufacturing capacity […], without the lengthy and expensive regulatory processes of repeatedly updating clinical trial or marketing authorizations and manufacturing authorizations' [45].

The MHRA is willing to create a framework that can be functional for a very broad range of products, from relatively simple medicines to ATMPs. For this reason, the agency points out its openness to adjust various aspects of the system, according to more precise guidelines to be published once the framework has been passed into law (probably in the course of 2023). For example, in the public consultation, the MHRA [17] declared: "Data requirements for finished product testing, batch analyzes, stability testing and labelling will be dependent on the nature of the product and the shelf life; these could differ significantly from conventional pharmaceuticals and may need to be agreed on a case-by-case basis." Other elements that may be subject to adjustments include the frequency of site inspections, the contents of the Master File, and the format of risk management plans.

In this way, the MHRA is proposing a proportionate approach for its framework regulation, with features of so-called 'adaptive regulation' [46,47] whereby requirements are adjusted when new knowledge becomes available. The approach also has some aspects resembling 'enforced self-regulation' [48,49],

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also known as 'management-centered regulation' [50], whereby regulated entities help establish the parameters for risk management and quality control.

This flexibility announced by the emerging regulatory framework seems to be well-regarded by both the industry and academic players in the field. In the workshop we held in June 2021, participants were polled on what they considered as the strongest aspect of the MHRA's proposal. We received feedback from 17 participants, as summarized in Figure 4.

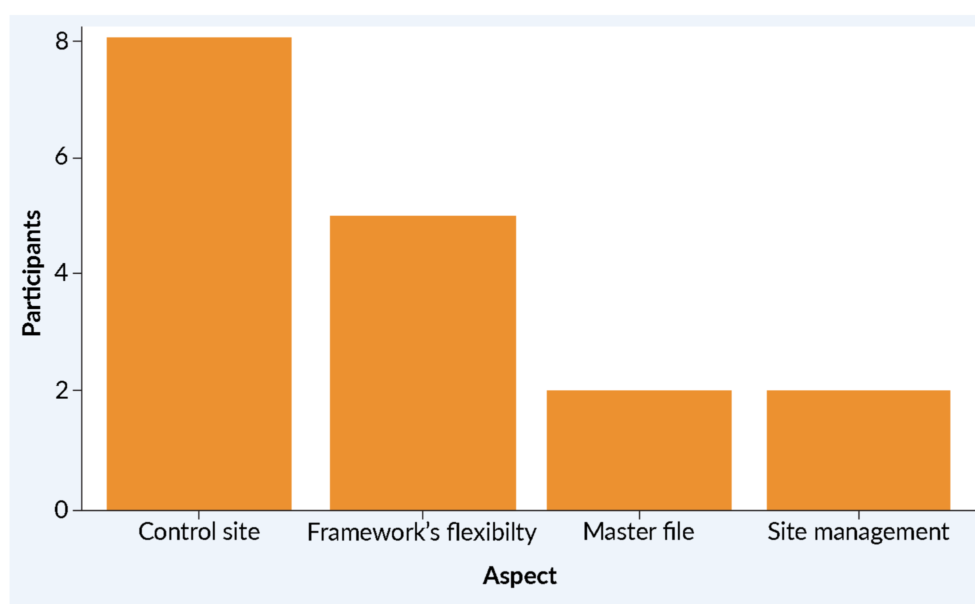
After the concept of Control Site, the aspect most appreciated was precisely the proposal's flexibility. It can then be argued that the MHRA is designing a regulatory landscape (as illustrated in Figure 1C) where the regulated area is split into two zones, one with strict requirements leading to marketing authorizations and post-market surveillance (for conventional centralized manufacture) and another one (for point-of-care manufacture) where the agency can adjust requirements to make them fit a variety of situations, products, and manufacturing systems. Stringent requirements are then combined with a regulatory flexibility generally obtained via exemptions, and the outcome is a sort of 'Exceptional regulation' zone (Figure 1C). At the same time, the exemption area (for bedside manufacture) will be kept, because as explained by Interviewee 28 (MHRA), the Specials scheme will not be extinguished. "[…] we can't force people and say: 'Right, you've got to stop now this unlicensed [production]; you've got to apply for a marketing authorization.' They'll just walk away."

Once again, the MHRA, by maintaining the Specials scheme, is not subjecting some patients to unnecessary risks. There will be risks associated with any category of medicines, whether they are unlicensed or sold commercially, especially when it comes to ATMPs. With the continuation of the Specials scheme, the MHRA is guaranteeing that some medicines not yet explored by the industry can be produced, with as few risks as possible, bearing in mind that clinicians may need to produce medicines requiring very bespoke and variable manufacturing procedures.

The MHRA's framework proposal summarized above is inspired by concerns that

FIGURE 4

Strongest aspect of the regulatory proposal, according to 17 workshop participants: June 2021.



had been absent in regulatory exemptions for bedside manufacture. The rationale of the Specials exemption is based on medical needs "[…] and does not include reasons of cost, convenience or operational needs" [35]. In its turn, the emerging point-of-care authorization is decisively informed by technical and operational considerations, namely the short shelf life of products and the diffusion of manufacture across many sites. Therefore, this regulatory shift, with a marketing authorization being made to coexist with exemption schemes, implies the creation of a new rationale, in addition to generating concerns and debates which are briefly highlighted in the next section.

REGULATORY RATIONALES & STRATEGIC DECISIONS

As explained above, the MHRA has proposed a regulatory framework highly informed by technical and operational considerations. The latter are surely key but there is much more to point-of-care manufacture. In the literature, as well as the interviews we have conducted, various other issues have been highlighted and some grey areas of the MHRA's proposal have been identified, especially with regard to its on-the-ground implementation and enforcement. The remaining parts of this section provide a brief description of some of these issues.

Infrastructure & investments

When discussing the MHRA's proposal, some interviewees highlighted that, for the most part, the role of Control Site will be played by middle-sized and large companies. This expectation is sometimes coupled with the view that researchers based in university hospitals are frequently not prepared to develop and manufacture products in the most robust and effective ways. As claimed by Interviewee 1, a European regulator:

"[…] when they arrive to us, with an academic dossier, you don't know where to start. You know, you don't have a single patient that you can compare with the other, because you have tried different things in all of them […] The companies do it the other way around. "Don't change anything. We'll do a full batch, compare the batch, we need to know all the parameters of solubility, viability, the reagents, the number of hours, the conditions of the incubator, we can scale it up, we can have, you know, the potency assays…" The academics just don't think that way."

Nevertheless, there have been some successful projects conducted by clinicians-academics. In Spain, at the Hospital Clinic of Barcelona, for example, a CAR-T cell product (which derives from gene-editing technology and fights drug resistant cancers) has been manufactured and delivered to patients since 2017. Nowadays, around forty patients receive the product every year. According to Interviewee 5, a member of this clinical team, the project's main advantage is medical autonomy, as the team has full control over the process, from collection of starting patient samples to administration of the final product. For this product, the Spanish team is using a Hospital Exemption, capitalizing on its less stringent requirements in terms of data collection and efficacy parameters.

In its turn, the UK's emerging framework brings the rationale of marketing authorizations to hospital-centered manufacture. If many companies end up acting as Control Sites and routinely visiting hospitals with their portable systems to perform manufacturing activities, hospitals will need to implement changes in terms of available technologies and staff [3], enhancing their preparedness or 'institutional readiness' [51]. In addition, it will be necessary to establish workflows and standard operating procedures for handling materials, liaising with manufacturers and couriers, scheduling patients, and so on. These demands can become particularly pressing in the largest hospitals, which may be mobilized, as Manufacturing Sites, for a range of products on a daily basis. Elsewhere [52], we showed how the rules and standards

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of clinical trials bring to hospitals rigid mandates in terms of skills management, contract clauses, and physical space. Equally, the UK's emerging framework can potentially introduce new demands and pressure into the premises of NHS settings turned into manufacturing units of commercial products.

In the field of ATMPs, regulatory requirements have caused drastic redefinitions of public entities' scope of actions. It has been claimed, for example, that the EU's ATMP Regulation has expanded market opportunities but restricted the range of actions available to public hospitals and research institutions [53]. While the UK's emerging framework raises questions about the role to be played by companies (as a potential key group of Control Sites), the adaptations required from hospitals seem to be even more drastic, as some of the manufacturing activities taking place in their premises are to be covered by requirements to which only market players are fully accustomed. Possibly, they will be witness to the installation of new standards, data management systems, auditing procedures, and others practices whose impact on routine clinical activities is yet to become clear.

The strategic value of hospital-centered manufacture

Academic or clinical teams manufacturing ATMPs in a hospital explain that one of the advantages of such endeavor is that in the medium or long term, the hospital is able to save costs [7,54]. According to Interviewee 5, a clinician-researcher based in the Clinic Hospital of Barcelona, where a CAR-T product has been manufactured, the therapy's price is around one-quarter of the average price of the CAR-T product which the health system would have to otherwise purchase from the industry.

However, the economic effects of ATMP hospital-centered manufacture are controversial. For Interviewee 17, a researcher based in University College London, hospital-centered manufacture of cell and gene therapies is likely to be more economically sustainable when hospitals partner with companies or when adjacent manufacturing facilities, run by technology companies, are built up. This interviewee concluded: "I think there's a need for clinical trial manufacture […], which I can see some benefit and argument for. But for routine manufacture, I don't think a hospital can sustain that activity, and it's a very, very different skillset that's required."

This view, according to which hospital-centered manufacture of ATMPs should be market-driven, seems to prevail in the UK. Administrators of hospital manufacturing facilities, including the largest and most experienced institutions such as NHS Blood Transplant (NHSBT), prefer to shun ATMP production when it does not target clinical trials. The NHSBT, for example, runs GMP manufacturing facilities in six cities, including one in a hospital in Oxford. Interviewee 31, a NHSBT employee, explained that the institution would be reluctant to be involved in ATMP manufacture not related to preclinical or clinical trials, because in addition to the costs involved, it would be necessary to engage in an unknown regulatory field, including building capacity related to risk management and liability issues.

The MHRA's emerging point-of-care manufacture framework can strengthen or solidify such views and expectations, as it introduces the need for running costly clinical trials and obtaining marketing authorizations. Eventually, hospital administrators, policymakers, and entrepreneurs operating in the UK might eventually be convinced that investments in the hospital-centered manufacture of licensed ATMPs should be always carried out by those players already used to develop commercial products. Moreover, it can be claimed that the MHRA's proposal is creating a market opportunity, as it introduces or enhances the distinction between bedside and point-of-care manufacture, providing the latter with a clear marketing authorization route, in a move appreciated by the industry.

In the 1980s and 90s, NHS Trusts decided to create some manufacturing organizations, such as Torbay Pharmaceuticals [55] and Newcastle Specials [56], which are now providing routine, non-ATMP medicines to several hospitals across the UK, frequently filling market gaps. This has been possible thanks to the regulatory exemption constituted by the UK Specials scheme, as well as the decisions made, at that time, to provide NHS Trusts with manufacturing capacities, technical skills, and regulatory compliance expertise that seemed strategic. Nowadays, a new phase is emerging in which ATMPs can, and in some cases must, be subjected to hospital-centered manufacture. Once again, the resulting landscape will depend on how regulations are designed and investment decisions made. As for investments, the UK has witnessed a decade of declining public spending in its health system [57]. In this context, one might hope that the lack of government investment could be offset by the investments made by Control Sites willing to visit Manufacturing Sites and mobilize miniaturized manufacturing systems, in a new model of mobile commercial therapy provision. In terms of regulations, the nascent framework proposes to subject point-of-care manufacture to requirements and licensing pathways that are more familiar to companies than hospital researchers. In this way, the MHRA's regulatory proposal is made in a context where the technical, operational, and commercial challenges of hospital-centered manufacture (in its two modalities) are clearly identified, but its strategic value, from the viewpoint of public healthcare policies and technology governance strategies, tends to be neglected.

CONCLUSION

Exemptions have been used by regulators to account for the diversity of players subjected to the law. In this way, they can be considered as instruments which promote regulatory precision [21,24] and social welfare [20], showing that "exemptions are not just random loopholes" [22].

Precisely because of the flexibility they provide, exemptions have been adopted in the regulation of ATMP manufacture, especially when such manufacture happens in hospitals or other clinical settings. Key examples have been the EU's Hospital Exemption (part of the ATMP Regulation) and the UK's Specials scheme.

Expecting to observe a rise in hospital-centered manufacture in the years to come, and wishing to regulate such activities in a more precise and dynamic way, the UK's MHRA is now proposing a new regulatory framework for point-of-care manufacture. The proposal brings the concept of Control Site, the institution that will be responsible for the overall management of the manufacturing system, taking the product from the development phase, through clinical trials, to the stage of licensed medicine. In this way, a regulatory domain is being created where point-of-care manufacture ceases to follow the rationales of exemptions to follow those of marketing authorizations.

In this article we have analyzed this process aimed to generate a new regulatory framework. From a theoretical point of view, it is interesting to see how the MHRA makes efforts to take a branch of hospital-centered manufacture (point-of-care manufacture) towards the logic of marketing authorizations while keeping the regulatory flexibility that will be needed to oversee manufacturing systems populated with diverse companies, hospitals, and technical solutions. In doing so, the MHRA proposes a proportionate and adaptive framework (or a zone of "Exceptional regulation" as we was called it in Figure 1C) where expectations and requirements will be highly dependent on the product's characteristics and associated risks.

From a practical point of view, most interested players, such as experts and practitioners based in companies, academic departments, and even NHS pharmacies,

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welcome the regulatory change, as pointed out by the MHRA [45]. At the same time, however, some issues of concern have been detected in our study, such as the differences between hospitals and the readiness of different institutions for the emerging framework. In this way, when the regulatory proposal goes through the parliamentary process and is subjected to political assessment, some of its key concepts and proposals may change either slightly or substantially. Even if the proposal passes as is, both companies and NHS Trusts may prefer to continue to use the Specials scheme and produce unlicensed medicines. Thus the upcoming regulation may turn out to be of little impact in spite of its innovative nature. As explained by Fuller [58], laws can "fail," and they do so when they posit requirements that cannot be followed by those supposed to follow them.

The rationale of exemptions, which prevails in the Specials scheme, can generate regulatory fragmentation, in the sense that different procedures, quality standards, and data management systems are put in place in different hospitals. Thus, it may be too difficult, or even impossible, to disseminate solutions that are working well in particular settings. In this respect, the MHRA's proposal can be very helpful to promote higher levels of standardization and technical efficiency, fostering the dissemination of promising ATMPs.

At the same time, however, it is important to strike a good balance between operational and technical requirements, on the one hand, and the medical reasons that have been the main motivators of both the EU's Hospital Exemption and the UK's Specials scheme, on the other. As the proposal goes through the parliamentary process, the interests of key stakeholders are likely to sharpen, impacting political choices that will define the models with which ATMPs will be developed, distributed, and (hopefully) accessed in the years to come.

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INTERVIEW

Next steps for MSC innovation

David McCall, Commissioning Editor, BioInsights, talks to **Jacques Galipeau,** Associate Dean for Therapeutics Development at University of Wisconsin-Madison, and President of the International Society for Cell & Gene Therapy (ISCT)

JACQUES GALIPEAU, MD FRCP(C) is the Don and Marilyn Anderson Professor of Oncology within the Department of Medicine and UW Carbone Comprehensive Cancer Center at the University of Wisconsin in Madison, and is Associate Dean for Therapeutics Development at the University of Wisconsin School of Medicine & Public Health. He is the director of the University of Wisconsin Advanced Cell Therapy Program whose mission is to develop personalized cell therapies for immune and malignant disorders and to promote and deploy first-in-human clinical trials of UW cell therapy innovations to improve outcomes for children and adults. Dr Galipeau leads a research program in the study and use of mesenchymal stromal cells as an immunotherapy of catastrophic illnesses including cancer and immune disease. He has also developed the field of fusion engineered cytokines known as fusokines, as a novel pharmaceutical means of treating immune disorders and cancer. He is an internationally recognized expert in translational development of cellular pharmaceuticals and the sponsor of a series of FDA-sanctioned clinical trials examining the use of personalized cell therapies.

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Q What are you working on right now?

JG: At the University of Wisconsin-Madison School of Medicine, my remit is to develop technologies within an academic center of excellence that are pharmaceutical in intent, particularly of the cell therapy flavor, and to move them along the de-risking food chain. I am a university-based scholar with a research lab with students and grants, but I am also a card-carrying hematologist and practicing physician, so I still have my toe in the clinical pond. The reality of clinical medicine is useful to keep you grounded when you are interested in translational research.

I define 'translational' as moving from preclinical to first-in-human. ('Translational' means different things to different people: cell biologists think that translational is going from a cell to a mouse, whereas biochemists might think it is going from a protein to a cell). My day job is operationalizing first-in-human clinical trials and investigational therapies to meet unmet clinical needs, and dealing with regulators. It is the operational realities of making that happen in a not-for-profit, academic healthcare center of excellence context.

Q What have been some of the key advances and major barriers to success you have experienced in your time in the MSC field?

JG: The main vexation is that there have been dozens of rigorous, scientific, peer-reviewed papers published demonstrating unequivocally that mesenchymal stem cells (MSCs) can have a substantial clinical impact on murine models of human disease, with many mechanisms of action, especially in the autoimmune, inflammatory, and regenerative medicine spaces. By these criteria, this should work in people, because it works so well in mice and other species. However, pivoting to the many clinical trials that have been conducted in people since 1995, when the first-in-human MSC clinical trial was done, very few have received full marketing approval. So, there is a disconnect between the unambiguous effectiveness of MSCs as a cell pharmaceutical in preclinical models, and the inability to meet the primary endpoints of efficacy in people.

However, it looks like we are now turning a corner - for example, with the approval of TiGenix's Alofisel and predicated on that success, there are now second-generation MSC platforms coming through. Although there have been many ups and downs, there are exciting emerging therapies using MSCs. These often work best using a tissue engineering approach. (Indeed, the TiGenix/Takeda Alofisel platform is a tissue engineering approach, because they are injecting the cells at the physical site at which they are to exert the therapeutic effect).

The Alofisel platform uses allogeneic, adipose-derived, culture-adapted MSCs that were culture rescued, meaning they were frozen but then put back in culture for a few days to recuperate. These cells are injected locally into the fistulas of individuals with Crohn's-related

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> "Although there have been many ups and downs, there are exciting emerging therapies using MSCs. These often work best using a tissue engineering approach. (Indeed, the TiGenix/Takeda Alofisel platform is a tissue engineering approach, because they are injecting the cells at the physical site at which they are to exert the therapeutic effect)."

perianal fistulas. That approach worked and the therapy received European Medicines Agency (EMA) approval. There are other exciting developments using the tissue engineering approach, too. Recently, there has been an early-phase clinical trial from Jan Nolta of UC Davis, where they are using *in utero* MSCs to correct spina bifida in neonates. Those MSCs are put on a patch to treat the fetus before birth.

So, I think we are going to see an upswing for the MSC field. However, people do still have to get over the hangover of those major clinical failures, especially those led by industrial concerns. Even in the recent past, there were some expectations that MSCs could move the needle in COVID-related acute respiratory distress syndrome (ARDS), but this did not eventuate.

Q Where specifically do you predict that the future innovations in the MSC field will occur?

JG: Up until very recently, the platforms used were virtually unchanged from those used in the mid-90s. What will make a big difference moving forward is not so much the tissue source, as the three prevalent tissue sources for MSCs remain the same: bone marrow, umbilical cord, and adipose tissue. Rather, it is going to be about the augmentation or alteration of the cell attributes of MSCs. In particular, this will be the case for tissue engineering approaches that utilize gene-enhanced MSCs, whether they are engineered using synthetic mRNAs, gene editing, viral vectors, or another platform.

MSCs can be grown in large numbers *in vitro* and are fairly easy to gene engineer. It is true that when you begin using gene engineered cells and tissues, the safety requirements that regulators impose requiring investigational studies become more burdensome. However, I believe those who move forward with these platforms will eventually unlock the full potential of MSCs. I also think that all the money that is currently chasing CAR-T is eventually going to have to find another home. Once investors see that cell therapeutics are commercially viable, margin-friendly, industrializable products, they will need to find other opportunities in the field. I think that is going to be the next big opportunity for MSCs.

Q What are the most significant advances in enabling technology for the cell and gene therapy space that you see coming through, and why?

> "Gene engineering of cells in tissue, especially those approaches that will render allogeneic off-the-shelf products useful and margin-friendly to effect human outcomes, is going to be the next big thing for human clinical trials."

JG: Whomever can improvise the use of off-the-shelf immune-evasive cell platform therapeutics will be a game changer. Allogeneic and immune-evasive are two different things.

The industry prefers allogeneic because you can manufacture at scale, it is off-the-shelf, and you do not have to deal with the quality issues in manufacturing cells from an individual patient. We have learnt from the Japanese study where induced pluripotent stem cells (iPSC) were used to generate retinal pigment epithelium (RPE), as it cost a million dollars per patient to manufacture the cells. This resulted in a pivot to allogeneic, but with this move, you must deal with mother nature and immune rejection. The issue that must be addressed, then, is how to have an allogeneic off-the-shelf but also immune-evasive therapeutic, which allows for substantial long-term engraftment - that is key for the CAR-T space in particular right now. I believe to achieve this, inevitably, you are going to have to use a cell product that was gene engineered. Gene engineering of cells in tissue, especially those approaches that will render allogeneic off-the-shelf products useful and margin-friendly to effect human outcomes, is going to be the next big thing for human clinical trials.

Q You recently became President of ISCT. What attracted you to put yourself forward for the role?

JG: I like the International Society for Cell & Gene Therapy (ISCT) for many reasons. It is the society if you have preclinical or translational research interest in MSCs and cell therapeutics. If you are interested in the pivot point of first-in-human trials, but also issues relating to manufacturing, commercialization, and regulatory, it is the only society that bundles everything together. It is a society with knowledge transfer, where you can see today what will be out there in two years through the abstracts and talks at the various meetings we stage.

Coming back to the translational side of things, I ardently believe that academic hospitals can serve an important role in deploying some of those advanced cell therapies that are meritorious with regard to their impacts on human outcomes, but are ill-fitted to the

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traditional industrial, margin-friendly, commercialization at scale model. A good example is bone marrow transplant – if bone marrow transplant were a commercial product, it would never have been developed. There are a lot of cell therapies out there that fall into that sort of gray zone. It's not a criticism of my industrial colleagues, I totally get it. They can't go back to their board or investors and make the case to develop such products – they are not charities, after all.

But we as care providers, I think we can deploy these products as services. And today, I believe academic health centers might be able to become masters of their domain and be able to move technologies all the way through the equivalent of conditional approval, as they are doing in Japan. They can deploy those technologies in parallel to the traditional pharmaceutical industrial development efforts.

Q What are your key goals for ISCT and its activities over the course of your tenure, and why are they important for the future of cell and gene therapy?

JG: An important developing theme right now is that our society can play a meaningful role in workforce development. Many of our members and member organizations are involved in hands-on cell product development and manufacturing, which is where the most pressing workforce requirements are. The skillsets of the people that do the work of preclinical R&D, product development, and manufacturing, are shared and applicable typically across the whole spectrum of cell therapeutics. Whether working with CAR-T cells, natural killer cells, iPSCs, or MSCs, the learnings of cell culture, good manufacturing practice standards, and best practices are relevant throughout. ISCT is uniquely well suited to playing a meaningful role in that kind of workforce development through, for example, hybrid webinars and hands-on workshops at regional meetings. There are, however, limits to what that can achieve, as it is difficult to use a hybrid substitute for in-person communication and education.

Lastly, I am keen to continue the legacy of my predecessors with regard to one of our major efforts, which is in international regulatory ethics of cell therapy. There are still a lot of scoundrels in this space and you can never let your guard down. We have to continue to call them out and keep issue this high in the awareness of all our membership, whether they be from industry, academia, or regulatory bodies.

Of course, the regulatory side is a moving target. Many people have the impression that the US Food and Drug Administration or the EMA or Australian Therapeutic Goods Administration– all these regulators are sitting in an ivory tower deciding everything on their own. In fact, many of them look towards us for guidance (typically, through our position papers and the like). This helps them to frame how to develop the regulatory landscape – to relax it where it needs to be relaxed, to tighten it where needs to be tightened, but always with the end goal of what I would call distributive justice, which is maximizing the potential for access to all those in need of these therapies.

Q Finally, can you distill one or two critical learnings relating to successfully fostering innovation that you have picked up over the course of your career?

JG: In academia, everybody is an independent entrepreneur. Many people remain in academia because they can be masters of their own domain and pursue their own ideas, as long as they can convince someone to fund them. University-based research and its intellectual ferment matters a lot because in industry, you have to be more task-focused. You need to have bold, creative ideas, but the problem is, often those bold, creative ideas are not recognized for what they are for a very long time. For example, the mRNA that Moderna used – for the longest time, the development of those versions of mRNA could not get funded, because nobody recognized their importance.

There are no bad results – the only bad results are uninterpretable results. So, my encouragement to students, grad students, and post-docs is that if you have a great idea, if you can convince yourself it is something good that moves forward, then go for it. And if you can convince yourself that something clearly, unambiguously does not work, then that's important as well, and at least you pursued a creative idea.

This is critical because solely banking on safe bets is not going to make the difference, especially in this space. Here, we are throwing mother nature's textbook in the dustbin with things like genetically engineered cells. We're rewriting the books.

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INTERVIEW

Vaccines & beyond: a decade in mRNA delivery

David McCall, Commissioning Editor, BioInsights, speaks to **Russell Johnson,** former Vice President, Formulation Research, RVAC Medicines

RUSSELL JOHNSON was until recently the Vice President of Formulation Research at RVAC Medicines, a clinical stage mRNA-focused healthcare company with a strategic focus on global health. He brings more than 10 years of industrial R&D experience in the delivery of RNA in preclinical and clinical programs. Prior to RVAC, Russell was the local-head of drug product discovery at GSK's US-Vaccine Research center and worked across GSK's vaccine technologies and platforms. He has deep experience in lipid, polymeric, and inorganic nanoparticles used for a variety of mRNA delivery, with more than 40 peer-reviewed publications and presentations.

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Q How did you first get involved in the RNA field?

RJ: I entered the space during a senior fellowship at the University of Washington. I worked in Suzie Pun's lab, which had a key focus on the non-viral delivery of genes.

As part of that, we investigated RNAi, and saw some direct potential for the delivery of genetic materials.

From there, I moved to a couple of startup companies, where I received a great sense of innovation in the delivery space for nucleic acids, and how the vehicle had to provide and support the payload. Those early experiences in startup environments were transformative. I had the chance to work broadly across the small biotech company I was in, doing chemistry, formulation, and bioanalytical analysis for testing *in vitro* and *in vivo* systems. I worked to generate key datasets to provide proof of concept data for the treatment of potential target diseases.

There was a significant pivot in my first company from 2012–2013, from an initial focus on RNAi and oligo delivery to a focus on mRNA delivery. At that point, my whole career pivoted towards the delivery of mRNA.

I then moved into a big pharma environment at GSK. There, I led a team focused on early discovery programs, pivoting towards development and clinical studies. Doing that within GSK was amazing and I learned an incredible amount. Being able to work across disciplines within a big pharma company gave me a lot of license and opportunity to deeply investigate key questions in mRNA delivery. This included the general ways that mRNA was pivoting towards use in vaccinations, which my role was specifically geared towards.

Since then, I have worked at RVAC Medicines, which is a small but growing biotech company.

Q Obviously, the COVID-19 mRNA vaccine platform development gave mRNA technology areas an enormous boost – as someone who had worked in the space previously, what for you were the most significant advances and learnings that the pandemic-related activities delivered for the field as a whole?

RJ: Generally, there are many early-stage technologies and innovations that have the capacity to contribute to healthcare. However, the reality is, even in a pandemic situation, that real critical platform knowledge is required to enable innovative technologies to make an impact on healthcare.

In terms of key learnings, the biggest one was the basic understanding of the technology's performance in the clinic. This type of knowledge has an enormous dividend – even if those initial clinical experiences were not directly promising, they still provided pivotal information on a new technology's capacity, and revealed liabilities that must be resolved before direct application to a successful product.

mRNA and lipid nanoparticle (LNP) platforms were present before the COVID pandemic. There were quite a few mRNA studies in clinics before 2019. From those experiences, we saw that potency was not on target, and there were some key safety concerns that needed to be resolved before the true potential for an mRNA-based vaccine product could be realized.

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The timing for these learnings was impeccable because shortly after the clinical knowledge for mRNA vaccines was demonstrated, the pandemic occurred. Those companies that had done the legwork in the clinic drove forward mRNA vaccines in response to the pandemic.

In response to COVID, what mRNA specifically could do in comparison with other vaccine platform technologies was move quickly from concept to discovery, and then into a manufacturing environment. This was transformative for the pandemic response to COVID, and is unique to the mRNA platform, or to the family of technologies that belong to mRNA medicines.

From an innovation perspective, there were two other key advances that existed prior to the pandemic, and that enabled an mRNA vaccine response to COVID. One is the synthesis of mRNA *in vitro* and the ability to purify mRNA to become a viable drug substance. The second was to ensure that mRNA delivery was potent and safe enough for clinical success. Those two things built together allowed us to catapult into creating an amazing, miraculous medicine.

Q Can you introduce us to RVAC Medicines and the company's R&D platform and pipeline?

RJ: RVAC Medicines is a young company - our direct founding was just 18 months ago. Nonetheless, in that time, we have been able to do some remarkable things. A big part of that was to raise funds, which is the case for any startup in this environment. On top of that, RVAC has brought forward a vaccine that is now in a Phase 1 clinical study. We also have a good manufacturing practice (GMP) manufacturing capability, which will be a part of our strategic interests as the company gains momentum and builds potential.

On the business side, we are a Singapore-based company that has a key strategic focus in Southeast Asia. That being said, our research and development (R&D) centers are in both the US and China. I am presently at an R&D center in the Boston area.

RVAC is unique in the sense that most startup companies focus on a proof of concept of their technology or therapeutic target. At RVAC, while there are many discovery efforts, we have tried to build out towards end-to-end capabilities where our preclinical programs, after demonstrating proof of concept data, can pivot quickly towards Phase 1 activities in clinic.

Q What differentiates RVAC Medicine's approach in what is an increasingly competitive space?

RJ: It is a very competitive space, and we see that. There are many people who have seen the potential of RNA medicines and are moving towards them. The RVAC approach centers on bringing forward the best possible product and using innovation in a way that is unique to a biotech environment to enable us to move quickly. We also aim to target key areas of performance that we believe are important for a successful program.

I am a delivery and formulation leader and scientist. My job is to use the capacities and capabilities of mRNA, plus the ability and flexibility of non-viral delivery vehicles, to home in on key tissues of interest. Across both prophylactic vaccines and therapeutics approaches, we look for ways to match delivery strategy and formulation with a top-flight mRNA, so that our products can perform to their maximum potential and have a potentially tremendous impact on global healthcare.

RVAC also has a direct focus on global health, which for me is a long-running passion. We have the technology and innovation present now to move forward, and see those medicines and programs return a benefit for human society as a whole.

Q What are the key considerations and challenges in the bioprocessing and formulation of next-generation RNA therapeutics?

RJ: With mRNA medicines, one of the biggest challenges in manufacturing and producing materials is that they are metastable. RNA is intrinsically unstable, so you need to heavily modify it or do something else that can provide it with a stable form. On top of that, LNPs and other delivery vehicles are meant to have a staged disruption, so one challenge is to provide a process to give a well-characterized and uniform product that can perform in expected ways. The process is somewhat challenging in the manufacturing environment. Understanding that makes you push for efficiency so that your final drug product is what it is guaranteed to be.

From a formulation standpoint, there are two sides to future improvement: manufacturing and innovation. On the manufacturing side, there is a push to have components that are well characterized, have a great safety profile, and give you strong confidence in the outcome. On the innovation side, we need to use the dynamic nature of our delivery systems to build more functionality for that delivery vehicle. There is great work being done in both of those areas. RVAC is a part of the effort to continue innovating.

Right now, there is a tremendous push towards bioprocessing and manufacturing in the mRNA industry. If you look at the landscape of where the synthetic vehicles are moving,

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there is strong potential to continue to unlock the potential of RNA medicines as a broader set of vaccines and therapies.

Q What are the issues and opportunities relating to thermostable RNA formulations?

RJ: Obviously, something requiring cold storage at -80°C does not have a good chance of impacting healthcare in certain regions of Africa or Asia, for example.

From a scientific standpoint, we must bear in mind the intrinsic nature of RNA. That is not to say it cannot be improved, though. In fact, there are many forms of RNA that are very stable, even in liquid environments. Understanding the RNA molecule in its micro- and nanoscale environments, including molecular dynamics, will help to prove the potential of making mRNA medicines stable enough to have a real shot at providing durable care, even in distant reaches of the globe.

That is where the field is heading. There has been some great information publicly disclosed already, indicating that the stability of RNA medicines will be addressed and become much less of a limiting concern. We are on the cusp of seeing more stable and potent mRNA medicines advance. If you are passionate about global health, that should be great news. When you couple both improvements in stability and the potential for modular manufacture, it is amazing to imagine what mRNA medicines could become.

Q What role can the manufacturing side play in improving the safety profile of mRNA vaccines and therapies to support applications beyond COVID-19?

RJ: First of all, mRNA vaccines are safe. Personally, I would like fewer aches and pains related to mRNA vaccines, but that is also true for most flu vaccines as well.

In response to the question, there are probably several answers. It is impressive how mRNA technologies have performed during the COVID pandemic given how novel they are, especially when they are compared to any other platform that is available in vaccines. When you consider how new the field is, the impact it has had, and its capability to be properly dosed, that provides a lot of hope.

This being said, mRNA vaccines can get better. They will become more efficacious and better tolerated, with improved safety profiles. This can only be done through learning and clinical investigation. Given that most of the other platforms have existed for a long time, their safety profiles have been provided through many years of trials and observation. mRNA is getting there. Because of the pandemic, much of the data have become available. Those data will strengthen mRNA vaccines and medicines that move forward in future programs and products.

As we move forward, mRNA must develop better biomarkers for safety to help us to understand most adverse events in the clinic related to local and systemic reactogenicity. On a molecular level, there is still a lot being learned and a lot of progress being made, and there will be a great return on that knowledge. The other part that is important is the bioprocess and formulation perspective, and the ability to modulate those vaccines.

I am confident that with a little more time, and the understanding that we are getting right now from mRNA vaccines and medicines, future mRNA-based products are going to be better. That includes efficacy, safety, and tolerability profiles. mRNA has unique potential in this regard, which I am excited about.

Q What supporting enabling technology is proving key in moving this area forward? And what are the chief areas of need for future innovation in this regard?

RJ: As I mentioned earlier, there is the innovation side and the manufacturing side, and key to enabling both is the great potential to scale. That is unique to mRNA vaccines because they are synthetically devised. Being able to make all the materials from synthetic origins means that scalability, characterization, and purification all follow in a close sequence. Alongside the fact that it is such a varied platform, these are the things that have differentiated mRNA from any other technology that exists.

In terms of innovation, the process is somewhat complex and involves a lot of effort. We have a manufacturing process that is parsed into several steps that can only be carried out in batch processes, which limits the amount of material you can make. On top of that, the complex process and extension over several steps means that the in-process controls are a bit more challenging. My wishlist in that regard would be to have a continuous process, in-process controls, and characterization, that can be coupled with a digital twin and learning capability that gives you exquisite control over manufacture. This would allow you to make tightly defined and controlled products with high performance.

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In terms of working in a global environment, once you have that continuous process with integrated in-process analytics, that then moves you towards modular systems. There have been press releases from other companies that have shown this, but there is still some evolution needed to take it forward and maximize the potential. There is a significant possibility that this field of medicine could become individualized. This would globalize the potential to make high-quality and high-grade medicines all over the world.

> "For mRNA technologies, what is needed is the next product, and the demonstration that the next product is viable in a typical vaccine or therapeutic target compared with other technologies."

Due to the fact we have a composition-driven vehicle, there is the potential to control the physical and chemical attributes to the point where you can drive towards diseases and take advantage of pharmacokinetics and dynamics that support the programs being targeted.

Innovations in all these areas will be significant. The future is very bright.

Q What would you identify as some key likely future trends for ongoing platform development in the mRNA field, and what does the industry need to do now to prepare?

RJ: For mRNA technologies, what is needed is the next product, and the demonstration that the next product is viable in a typical vaccine or therapeutic target compared with other technologies.

In the long term, there are a couple of things. One is the presentation of product and enabling the product to fit the needs of the medicine. That means vaccines that are portable and can be deployed in a way that provides access to the global population. It also supports healthcare providers' needs for dosing.

There is huge market potential in other diseases with a focus on cancer or immunology and driving potential for the fields and indications that have a critical unmet need. The therapeutic and gene editing applications require targeted and robust mRNA delivery in precise locations, and in a way that gives durable treatment. These areas need some advancements in delivery and specialization of the payload.

Q Finally, can you sum up some goals and priorities, both for yourself in your own role and for RVAC Medicines as a whole, over the coming 12–24 months?

RJ: For RVAC, our goal is to continue to provide medicines that reach the clinic and finalize our GMP capabilities and capacity. We want to connect that process end-to-end from discovery programs all the way through to clinical investigation, and potentially, even through commercialization.

Personally, my goal is to enable the best products and facilitate the growth that RVAC is trying to capitalize upon. I am focused on growing my organization to a point where we reach our full potential and capability. I want to implement innovation for mRNA delivery and drug product to strengthen the future of mRNA medicines. To do this, I will focus on the areas we have covered and ensure that the delivery and formulation innovations move toward clinical testing. This is where my focus is specifically directed, and I believe there is tremendous potential here. We have good investors supporting us, so the potential for reaching those goals both for RVAC and myself personally is very high.

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Current trends in advanced therapy development & commercialization for rare diseases

Alan Boyd
CEO & Founder, Boyds

"Since those early days, financing has increased in the gene therapy space, with the commercial advances that have followed resulting in multiple product approvals, mainly in rare diseases, both in North America and Europe."

VIEWPOINT

On January 26th 2023, David McCall, Commissioning Editor, *Cell & Gene Therapy Insights*, spoke to Alan Boyd, CEO & Founder of Boyds, about gene therapy trends and innovation in the rare and ultra-rare diseases space. This viewpoint has been written based on that interview.

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Cell and gene therapy has focused strongly on rare diseases since the field's earliest days. The first clinical trials were conducted in the early 1990s, and I joined one of the first gene therapy biotechs, Ark Therapeutics, a few years later.

At that time, big pharma was actively involved – Novartis, Pfizer, and Roche all

had gene therapy programs, for example. However, funding for cell and gene therapy biotechs was scarce. Rare disease clinical trials were small due to the low patient numbers, and this also meant that the studies could be completed faster. This became a strategy that Ark and other smaller companies began to follow, as running very large Phase 3 studies in many hundreds of patients at that time was not commercially viable. By pursuing rare disease indications, companies like Ark could conduct preclinical testing with small batches of product, perform tox studies, and advance their product candidates into and through the clinic. Indeed, Ark managed to raise £150 million in the space of 5 years, which was enough to allow one product, Cerepro®, a treatment for malignant glioma, to be the first gene therapy to be submitted to a regulatory agency for approval. Unfortunately it was not approved, but what had been demonstrated by Ark was that it was possible to take a gene therapy product from research up to a regulatory submission. This work with Cerepro® then paved the way for all the other gene therapy products that were to follow.

Since those early days, financing has increased in the gene therapy space, with the commercial advances that have followed resulting in multiple product approvals, mainly in rare diseases, both in North America and Europe. Also, it should be noted that there has recently been the first gene therapy approval for a major cancer (bladder) with the product Adstiladrin® by the US Food and Drug Administration (FDA). The FDA has also recently stated its expectation that there will be six gene therapies approved annually over the next couple of years.

COMPETITIVE LANDSCAPE FOR RARE DISEASE GENE THERAPIES

Although many advanced therapy biotech companies have developed and are developing treatments for rare disease indications, in reality, comparatively few companies are working on the same indications, and even though the same vector delivery systems are being used, this is not really being considered as a competitive situation. This is because the range of actual viral vectors we can use is limited, and it is in the interest of all gene therapy developers to get their products into cells so they can have a therapeutic effect to bring benefits to patients, leaving the specific transgene as the unique component.

Therefore, what is competitive is the transgene that is being used and its individual construction. There was an example of an indication where more than one gene therapy was being developed to treat it. At one time, no fewer than four products were in development that all targeted the RPE65 gene, which due to a mutation in the gene leads to blindness in the individual carrying it. Spark Therapeutics won that particular race with the approval of Luxturna®, and since then, the other candidates seem to have fallen by the wayside. While developing gene therapies for the condition of hemophilia is considered to be a highly competitive area, there are in fact a number of different transgenes being developed that are targeted at a different mutated gene in the clotting pathway, which is the hallmark of the disease.

I do not see a lot of competition in rare disease clinical trials either. The main issue here is not one of patient supply, but rather one that lies with the hospitals that deal with the gene therapies. For one thing, there are still only relatively few centers that will conduct an early-stage gene therapy trial. Later in development, the amount of money required to support the clinical sites and hospital pharmacies during a Phase 3 trial can become a key limiting factor. Fortunately, clinical trials are not necessarily the key bottleneck for the gene therapy field at large, including those developers targeting rare diseases. That continues to reside with the manufacturing of the product.

Since the commercial development of gene therapies began, manufacturing capabilities and capacity have been and

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remain a major limiting factor. For example, the first adeno-associated viral (AAV) vector-driven gene therapy to be approved by the European Medicines Agency, Glybera®, ran into production problems due to its administration and dosage requirements. Originally, Glybera® was produced in HEK293 cells and administration involved up to 70 injections into each leg per patient. During its development, it was found that the HEK293 cells and the roller bottles used in manufacturing process could not produce enough product to satisfy demand for the clinical trials. Consequently, the company developing Glybera - uniQure – had to switch to the baculovirus expression vector system (BEVS) platform. This necessitated the company to restart the whole development process from the beginning.

GENE THERAPY MANUFACTURING PLATFORMS

Taysha Gene Therapies is one biotech in the field that is being built around the concept of a standard gene therapy manufacturing platform. They are working to generate an AAV platform by standardizing release criteria, for example. There is a lot to recommend this approach, however, developing a gene therapy platform technology is not always straightforward given that different transgenes need to be introduced into the viral vector according to the target indication, and this can then cause issues with the activities required for the manufacturing process.

As an example, when Ark Therapeutics' cardiovascular program was established, it began with using an adenoviral vector and the vascular endothelial growth factor A (VEGFA) gene. It was then discovered that vascular endothelial growth factor D (VEGFD) was a better option for the indication being investigated, so a switch was made with the new gene of interest while using the same adenoviral vector. When it came to manufacturing the virus, the cytopathic effect (where the culture cells burst and release the virus) typically takes around 4 days with VEGFA, but once we changed to VEGFD, we just could not get the cells to reach this cytopathic effect. Then, one bank holiday weekend, a technician didn't clear up the petri dishes properly on the Friday night. They came back into the lab after the 3 day break and found that all the cells had burst. Through serendipity, we discovered that we had just not left the new transgene on the cells for long enough.

This was a clear, early example for the gene therapy field that the transgene makes a big difference to the manufacturing process and although a company is developing a platform technology, there may well be differences that develop with the product's characteristics, meaning greater flexibility is required.

IMPACT OF ACCELERATED CLINICAL TRIAL TIMEFRAMES ON CMC

I spent the first half of my career developing new chemical entities (NCEs), the manufacturing for which had usually been signed, sealed, and delivered by the time we got to the Phase 2 clinical development activities. As the Head of Clinical Research at Zeneca Pharmaceuticals, I was always the department which was under the time pressure to deliver the results of the Phase 2 and 3 clinical studies, as the manufacturing of the product was not usually a rate-limiting step post the initiation of the Phase 2 studies. However, with biologicals in general, and with cell and gene therapies in particular, the polar opposite is true. Having now worked on several advanced therapy development programs up to approval, the chemistry, manufacturing and control (CMC) and manufacturing activities have typically been the final piece of the jigsaw in every case.

Today, when I start working with a new client, I tell them honestly that they will have trouble with their manufacturing process and analytical procedures. It does not matter if it is a relatively straightforward

and well-established manufacturing process; they will have trouble defining and validating the potency assay; they will also likely have trouble with things like host cell DNA removal and host cell protein contamination, etc. Therefore, anticipating these issues early is vital to the future success of the product.

One often hears it said that regulators' CMC requirements for gene therapies have grown more stringent over the years. However, I do not know if stringent is the right word – although I do believe the requirements have become more focused and specific, because we have learned more about the quality and stability of the product and what is important in the long run. For every advanced therapy product type that is in common development, there is now a standard list of what regulators expect in terms of what needs to be measured for each batch, which basically falls into three areas: potency, purity, and identity. With potency, regulators will let you go initially into the early translational activities with an *in vivo* bioassay, but they want an *in vitro* assay by the time you get to Phase 3. That is a challenge, but something that it is necessary to work on. With purity, it's largely about limiting host cell contaminants and most importantly, addressing full-empty capsid ratio. There are now standard lists for AAVs, adenovirus, lentivirus vectors, etc. – the difficult part is getting the results into the range expected.

CHALLENGES IN CLINICAL TRIAL DESIGN FOR RARE DISEASES

The first clinical study for Glybera was conducted in The Netherlands. At that time, there were only around 6000 people in the world who had the target indication – lipoprotein lipase deficiency – and some 5000 of them were in the Saguenay region of Quebec, where ancestors with this gene mutation settled from Europe in the 18th century. By the end of that study, Amsterdam Molecular Therapeutics (which would later become uniQure) had already treated all 12 Dutch patients available there. When patients are so scarce, it is necessary to adapt a clinical trial to meet the limitation in numbers.

Another problem with developing treatments for rare diseases is which clinical endpoints to use. The majority of rare diseases have never had therapies developed and approved to treat them and so it is unknown what should be measured as an endpoint to demonstrate efficacy. Consequently, over the last decade in particular, people have started running natural history studies as part of their development program, in order to learn more about the disease and what may be measured as possible endpoints. At the same time, there has been a strong focus on increasing patient involvement in study design, to learn what is most important to and for the patient.

Interacting with those affected by rare diseases is very often an eye opener. I was involved in developing a treatment for Niemann Pick disease, which is a rare central nervous system disorder in children where cholesterol is deposited in the brain. Having read the scientific papers and talked to one or two clinical experts, I thought I knew what was probably important and what we could measure, namely cholesterol clearance. However, I subsequently spent a weekend with the Niemann Pick Society in the United Kingdom with parents and families of children with the disease, where they get together and talk about problems, issues, and potential treatments. Talking to the parents, I was struck that the key thing to them is the fact that as the disease progresses, their children cannot communicate with them because of the effects on the brain, with them losing their speech and hearing abilities. That was what was important to them, as well as things like walking and talking. I went home after that weekend, tore up the protocol and started again, this time focusing specifically on the items that the parents of the children considered to be important and were clinically meaningful – not just conducting a laboratory assessment.

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SECURING A BRIGHT FUTURE FOR GENE THERAPIES FOR RARE DISEASES

While gene therapy as a sector continues to expand and look to new indications and more prevalent diseases, the rare diseases research and development (R&D) pipeline remains healthy. Importantly, big pharma companies have now re-engaged, with companies like Novartis, Roche and Pfizer now involved again. Pfizer recently took a step back from direct involvement in rare diseases R&D, but are still active through their investments in smaller companies in the space. Roche are doing a similar sort of thing with Spark Therapeutics, whom they acquired – allowing the biotech to continue in its work without undue outside interference, while providing financial stability and resources. I feel this is a sensible approach for big pharma to take, and I think it will continue. There will certainly be more gene therapies for larger patient population indications coming through in the wake of Adstiladrin®, but, at the moment, gene therapy as a field remains very strongly associated with rare and orphan diseases.

Now that we have technology that has been established over the past 25 years, with many more people and companies involved in the development of gene therapy products, I am sure that the product pipeline will remain very full, and many more therapies will be approved. This can only bring enormous benefits to the people who need them, and long may it continue.

BIOGRAPHY

PROFESSOR ALAN BOYD is the President and CEO of Boyds. He founded the company in 2005 to support the translation of ideas into medicinal products and treatments for patient benefit. He has since grown the company globally with offices in the UK, Ireland, and the US.

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INNOVATOR INSIGHT

Enhancing non-viral gene editing, processing & expansion of T & NK cells

Sung Lee & Deepak Kumar

The cell therapy manufacturing process is extremely labor-intensive with a high degree of complexity, regardless of the cell type in use. One key focus area in the field includes developing closed, automated manufacturing processes to help reduce costs and increase the speed of getting treatments to patients. Cell and gene therapy workflows involve cell collection, isolation, activation, and engineering of cells followed by expansion and concentration, and then either cryopreservation or infusion. To better serve the cell therapy industry, Thermo Fisher Scientific has created flexible, modular systems that can be easily adapted into existing workflows. This article will highlight two recently introduced Thermo Fisher instruments: the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System and the Gibco™ CTS™ Xenon™ Electroporation System.

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INTRODUCING THE GIBCO CTS ROTEA SYSTEM

The Gibco CTS Rotea Counterflow Centrifugation System applies a proven counterflow centrifugation method for a broad range of cell processing applications such as chimeric antigen receptor T cell (CAR-T) therapy, stem cell therapy, and peripheral blood mononuclear cell (PBMC) isolation. This system offers exceptional flexibility in cell therapy development and manufacturing systems. The Rotea system is designed to handle a wide range of input volumes from 50 mL to 20 L and output volumes as low as 5 mL. The system is powerful but gentle on cells, compared to other separation methods. It enables over 95% cell recovery

while maintaining cell viability and achieving high throughput. The single-use kit interface enables an easy transition to commercial manufacturing and helps enable good manufacturing practice (GMP) compliance with industry standards.

Whilst viral delivery has been used for some time, viral methods have limitations such as safety concerns, immunogenicity, mutagenesis, increasing test burden, payload limitations, and cost. Viral engineering of cells can lead to poor and inconsistent regulation of CAR expression. Non-viral engineering approaches are attractive because they allow more specific control of engineering. Electroporation (EP) is an interesting alternative to viral delivery due to its simplicity of use and ease of large-scale production.

The Gibco CTS Xenon Electroporation System is a closed and scalable EP system for GMP-compliant cell therapy manufacturing. The system can transfect up to 2.5×10^9 T cells/25 mL in less than 25 min. It shows up to 95% gene knockout with CTS TrueCut™ Cas9 protein and 80% cell viability. The user program enables the creation and optimization of EP protocols for various cell types and payloads, from process development through to commercial manufacturing. It can be used to deliver DNA, RNA, and protein payloads. The Xenon MultiShot Electroporation Cartridge helps enable sterile welding to PVC or C-Flex® tubing. The system can be integrated with other Thermo Fisher Scientific instruments and consumables into a complete closed-cell therapy manufacturing workflow.

Thermo Fisher Scientific also offers reagents, buffers, and consumables for the CAR-T workflow. The SingleShot chamber designed for processing development can transfect 2.2×10^9 cells in one batch. The 5–25 mL MultiShot chamber can transfect 0.1–2.5 billion cells in a continuous process, with an intuitive rapid user interface. The Gibco CTS Xenon editing buffer is designed to improve performance with gene editing-specific payloads, such as CRISPR/Cas9, for knockout or knock-in applications in a variety of human primary cells. Bottles (100 mL) or bags (100 mL) are available. The non-viral workflow is shown in **Figure 1**.

EP SYSTEM TESTING FOR CAR-T CELLS

The Invitrogen™ Neon™ Transfection and Xenon Electroporation systems were compared in an investigation. Flow cytometry was used to assess the gene editing efficiency and phenotype. The V5 antibody was used to detect part of a CAR antigen on T cells to quantify how many cells expressed the CAR on their membrane.

Superior efficiency was observed with the CTS Xenon system (22–44% knock-in efficiency) compared to the Neon system (15–23% knock-in efficiency), which suggests that the CTS Xenon system can be used to easily scale and optimize the transfection process in a closed system **(Figure 2)**.

T cell phenotype was assessed on the Invitrogen™ Attune™ NxT Flow Cytometer. Compared to no EP controls, there is minimal or no phenotypic change across the EP volumes tested.

CAR-T cells generated by the Xenon EP from donors A and B were tested for functionality in a cytotoxic assay. Effector CAR-T cells or control cells were seeded into a 96-well plate containing GFP and nalm-6 target cells. Effector:target ratio ranged from 10:1–0:1. The effector and target cell mixtures were incubated for 6 h and then analyzed for present cytotoxicity using the Invitrogen™ EVOS™ M5000 Imaging System and flow cytometer. The results showed that 48.2–60.8% of CAR-T cells demonstrated the ability to efficiently kill the GFP neighbors and nalm-6 target cells in a dose-dependent manner compared to the control cells *in vitro*.

OPTIMIZING CAR-T WORKFLOWS WITH THE CTS ROTEA SYSTEM

Optimization of the cell and gene therapy workflows can be complex due to the number

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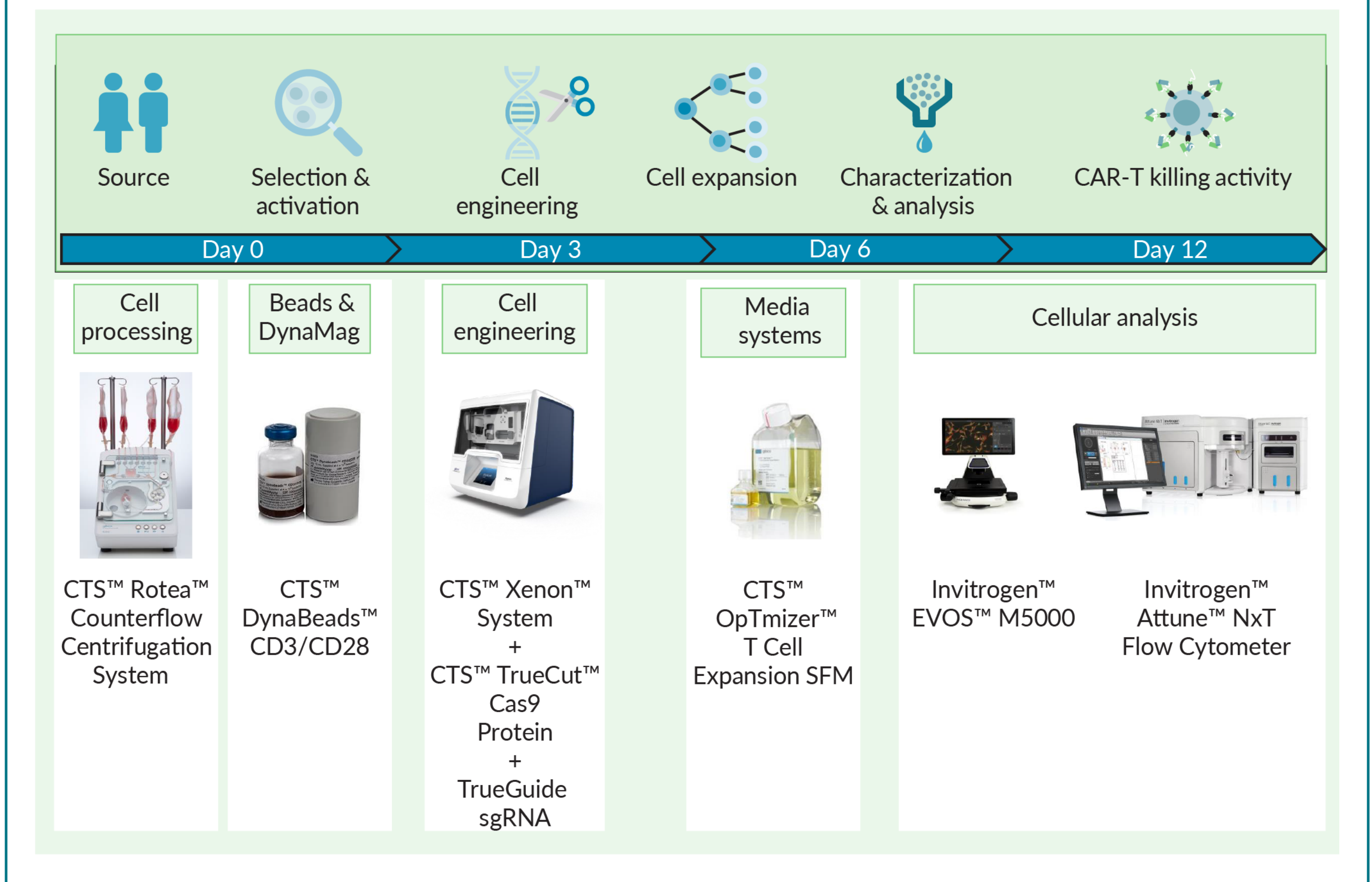
FIGURE 1

The non-viral CAR-T cell workflow.



of process steps and variables included. Here, variable conditions were tested with the modification of the workflow. First, buffer exchange was performed by Rotea system prior to EP. Second, to test the impact of activation time on editing efficiency, T cell activation was performed over 2–3 days with CTS DynaBeads™ CD3 and CD28. Three different donors were used for the EP steps. The comparisons are the closed and semi-automated process on the Rotea system versus the open and manual process for buffer exchange. Additionally, the Xenon system was compared to the Neon system for EP, and time to T cell activation was assessed.

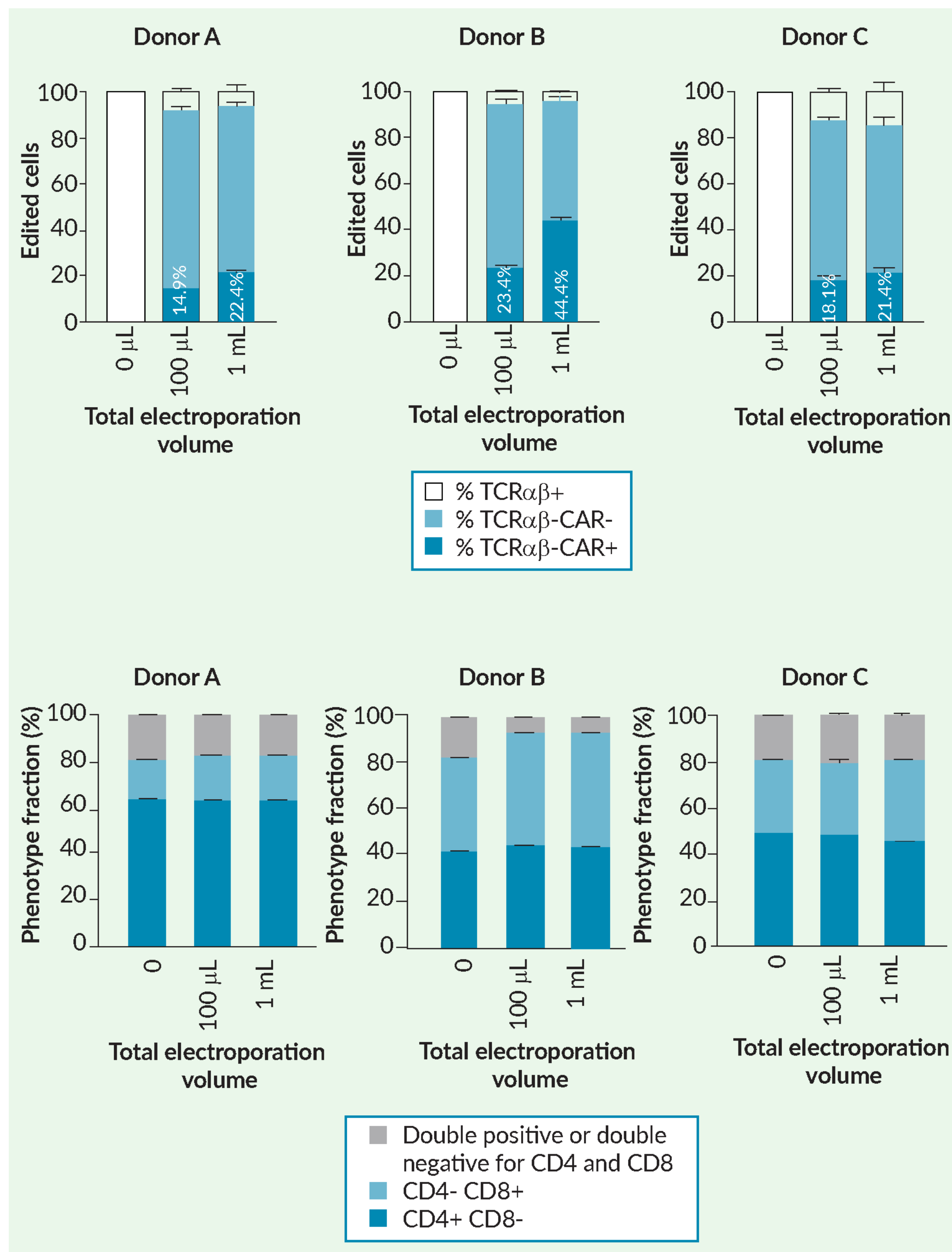
The CTS Rotea system can be programmed to perform effective washout of media and buffer components. See the application note for additional information: Residual washout on the CTS Rotea Counterflow Centrifugation System [1]. Wash buffer can be washed through the fluidized cell bed, enabling over 95% removal of original medium components with minimal cell loss and maintenance of cell viability.

The Gibco CTS Rotea single-use kit was primed, then cells were washed, concentrated, and harvested. The viability and recovery percentage of T cells were measured on days 2 and 3. The cells were then debeaded before being washed and concentrated either manually, or using a Rotea system. The viability of the cells recovered by both methods was over 89%. On day two, the Rotea system and manual methods showed similar recovery, although on day three, the Rotea system outperformed the manual method in terms of recovery rate. With the Rotea system, the results were well over 85% for all conditions tested.

EP efficiency knock-in versus knockout was assessed 3 days post-EP (Figure 3).

FIGURE 2

Transfection efficiency (top) and CD4/CD8 phenotype (bottom).



Manual versus the Rotea system processes were tested for buffer exchange, and the Neon system versus the Xenon system were tested for EP.

As expected, there was donor-to-donor variation with a knock-in efficiency of up to 36.8%. Knock-in efficiency of the Xenon system was consistently higher than the Neon system for all three donors across all conditions.

The result was analyzed by evaluating the total number of adhesive cells for donor three. Activations for 2 days showed higher EP efficiency compared to 3 day activations across all donors and conditions. For the 2 day activation, the protocol efficiency was

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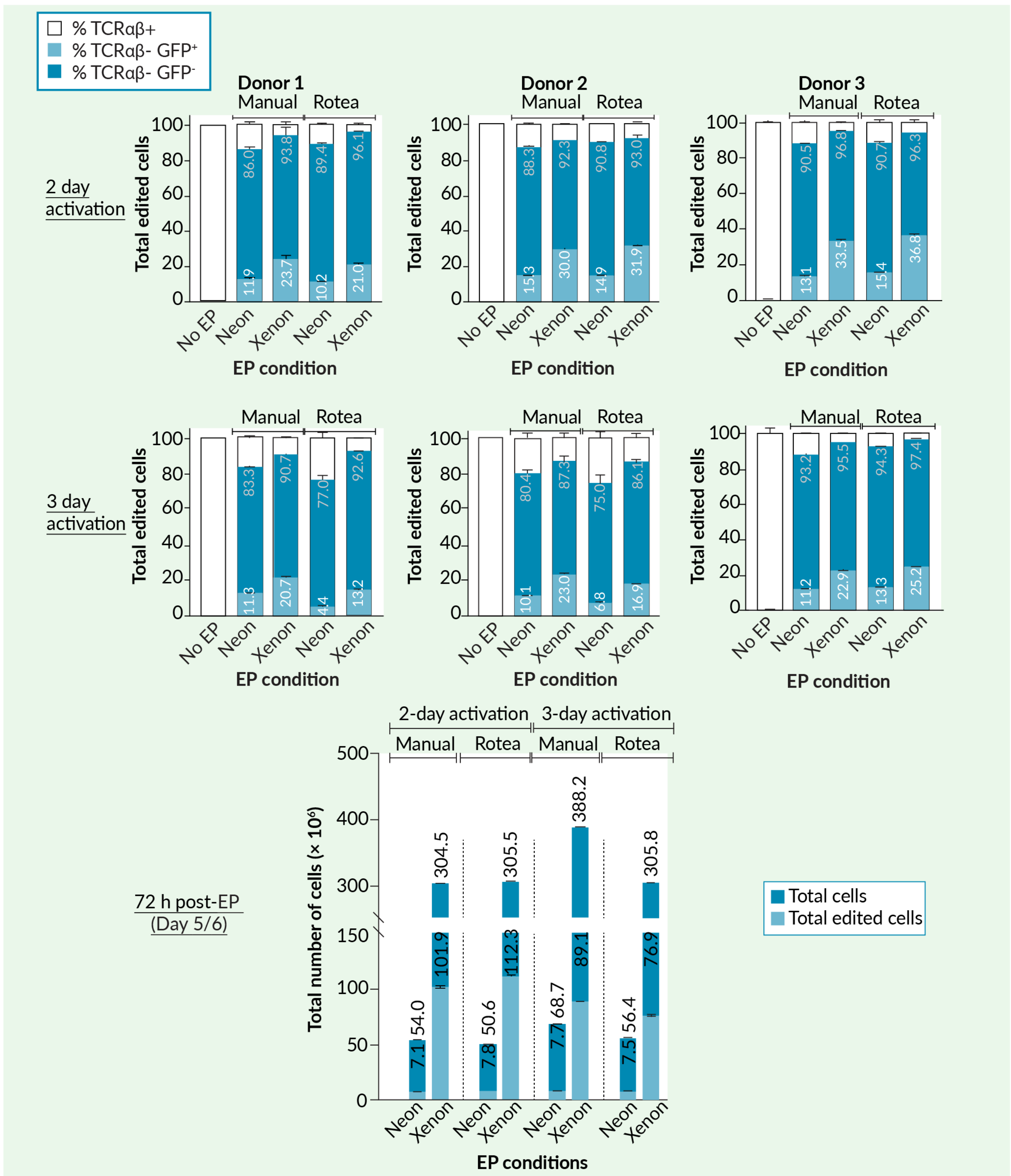
FIGURE 3

Electroporation efficiency 72 h post-electroporation.



EP: Electroporation.

similar using manual versus the Rotea system process for buffer exchange. The 3 day activation resulted in lower efficiency for donors one and two on Rotea system with similar efficiency for donor three.

Phenotypic characterization of CD4/CD8 ratio and CD69 and CD25 activation markers was also assessed. No significant difference in the phenotypic analysis was seen between day two and day three activation. Testing on either day two or three resulted in sufficiently activated CD4/CD8 T cells. We observed no significant difference in activation markers between the days.

Cells were evaluated for viability and growth after EP on the Xenon system. Good viability of more than 80% was observed for all conditions compared to no EP controls. Cells from the 2 day activation protocol showed a slightly improved growth over those from the 3 day activation protocol, but overall, growth scores showed a similar trend in both groups.

In conclusion, the 2 day activation protocol showed higher knock-in efficiency, the CTS Rotea system outperformed the manual buffer exchange, and the CTS Xenon system outperformed the Neon system. The CTS Rotea system and the CTS Xenon system are powerful modular tools in the quest towards creating a closed cell therapy manufacturing process by providing exceptional performance and helping to reduce contamination in a cell therapy manufacturing workflow.

GENOME EDITING OF NATURAL KILLER CELLS USING THE CTS XENON ELECTROPORATION SYSTEM

Natural killer (NK) cells are innate immune effector cells that can rapidly identify and kill abnormal, virally infected, and tumor cells. They can be genetically modified to obtain capable effector cells for adoptive cellular treatment of cancer patients. CAR-NK cells may represent a valuable complementary tool to the use of CAR-T cells in the treatment of adoptive immunotherapy of leukemia and solid tumors. However, gene transfer or gene editing of human NK cells is a challenging task.

NK cells for cell therapy applications can originate from multiple sources including peripheral blood, cord blood, induced pluripotent stem cells (iPSCs), and NK cancer cell lines. To improve the immune cell function against cancer or other diseases, cells must be engineered.

Engineering of NK cells is challenging using conventional methods because plasmid transfection has limited efficiency to express the transgene, and retroviral transduction requires a high viral titer and poses concerns around insertional mutagenesis and oncogenesis. Furthermore, lentiviral transduction is inconsistent for NK cells, even at a high multiplicity of infection (MOI). A robust and precise toolkit is urgently needed for NK cell engineering and expansion.

CTS NK-Xpander™ Medium is designed to meet the needs of transitional- and clinical-stage cell therapy developers by expanding human NK cells without the need for feeder cells. With this medium, cells have been proven to expand and maintain CD56 and CD16 expression as well as having robust cytotoxic capability. The NK cell process workflow is shown in **Figure 4.**

In this experimental design, on day six of post-isolation, NK cells were counted and suspended in genome editing (GE) buffer. The CTS TrueCut guide cas9 was used with the target of B2M knockout. Re-suspended NK cells were electroporated using either a Neon (10 µL) or a Xenon (1 mL) EP system to assess scalability. The same EP parameters were used for both Neon and Xenon systems. After 3 days of EP, editing efficiency was analyzed using flow cytometry.

First, PBMC were isolated using the CTS Rotea system and then characterized for CD56, CD16, and CD3 populations **(Figure 5)**.

The NK cells were isolated from three different PBMC donors and enriched. On day

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FIGURE 4

NK cell workflow.

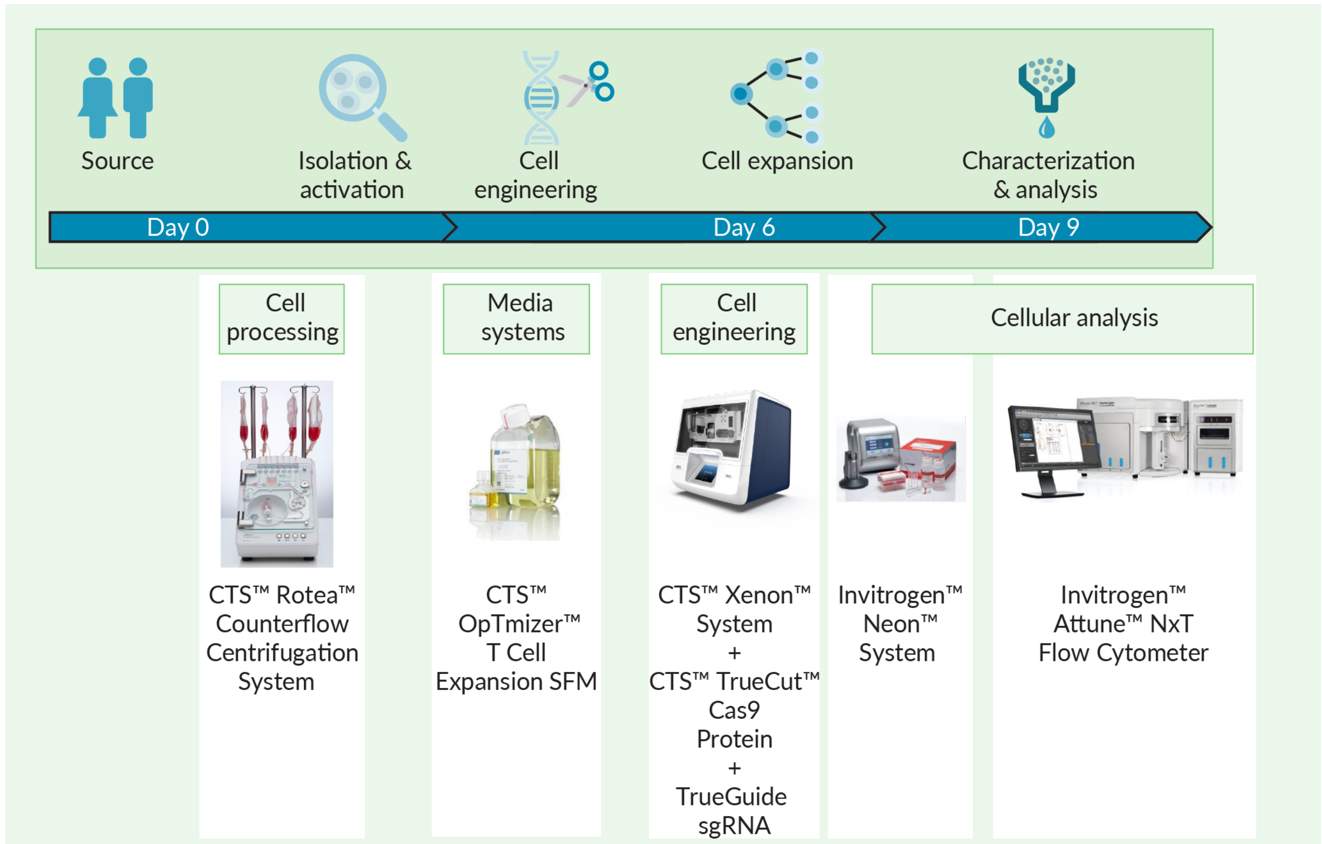
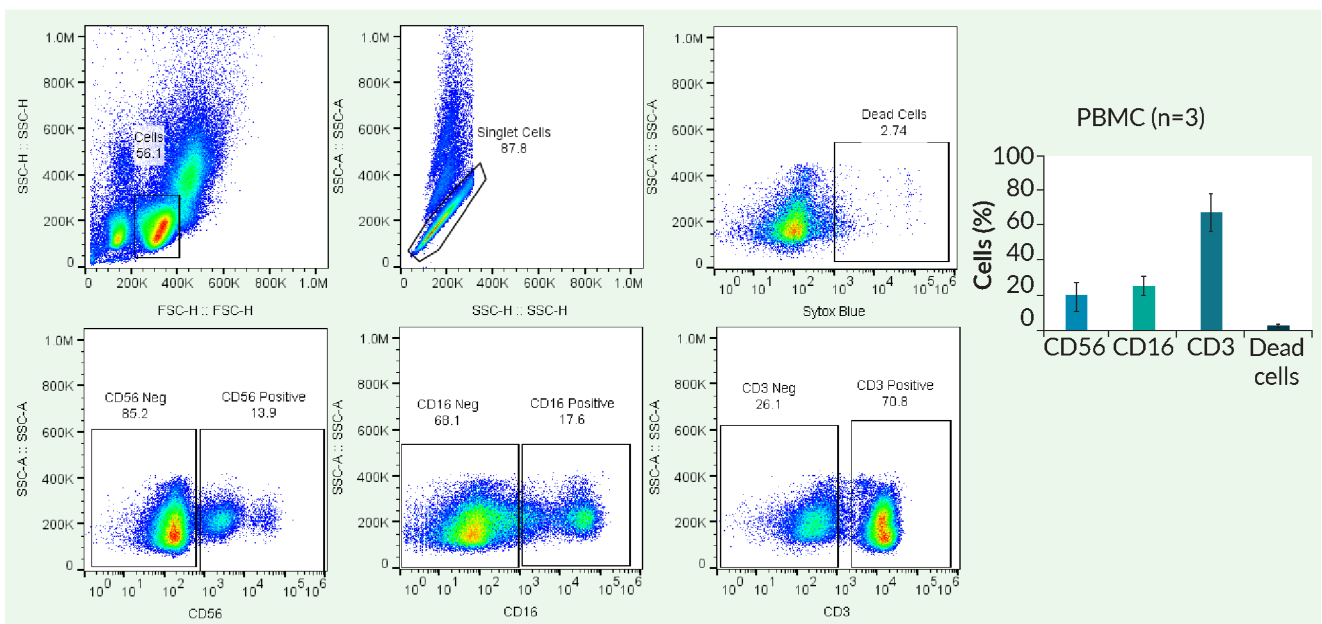


FIGURE 5

Phenotypic analysis by flow cytometry of PBMCs isolated using a CTS Rotea Counterflow Centrifugation System.



zero, the CD56, CD16, and CD3 NK cells were expanded. After the NK cells were isolated, NK expander media was added, supplemented with 5% human serum, and 500 units per mL IL-2. The cells were fed every 2–3 days. At the beginning of day five, the total fold expansion viability and phenotypes were analyzed (Figure 6). Across the three donors, CTS NK-Xpander expanded the cells by an average of 70-fold in 2 weeks.

For NK cells to be successful in allogeneic therapy, they must maintain their functionality post-expansion. All three donors maintained CD56⁺ at more than 80–90% and maintained 70–80% CD16⁺. This means they all maintained their functionality.

Additionally, a different Neon program was used to identify optimal conditions for NK editing efficiency. Consequently, on day six of the NK cell culture, cells were electroporated using Neon programs 1–24.

After 72 h of EP, a genomic cleavage detection (GCD) assay was performed to measure the knockout efficiency. Data suggested that program five showed the best editing efficiency. The NK editing efficiency between GE buffer and EP buffer was also compared. It was observed that GE buffer has better editing efficiency than EP buffer.

For NK cell therapy requiring a large number of NK cells, high efficiency of NK cell editing on a larger scale is required. For this, the same CTS Xenon Electroporation system can help to edit various cell types on a larger scale, including NK cells. Data for NK cells are shown in Figure 7. The NK cells were isolated at day six and expanded on both Neon and Xenon systems. On average, the Xenon System showed greater knockout efficiency than the Neon system.

To summarize, PBMCs were isolated using a Rotea system. Pre-isolation, the CD56 NK cell population was 13.9%. NK cells were isolated from the PBMCs of three different donors, and 88.3% of CD56 cells were purified. NK cells were expanded using NK-Xpander medium method to achieve 65-fold expansion. NK cells were edited using non-viral

FIGURE 6

NK cell expansion and characterization.

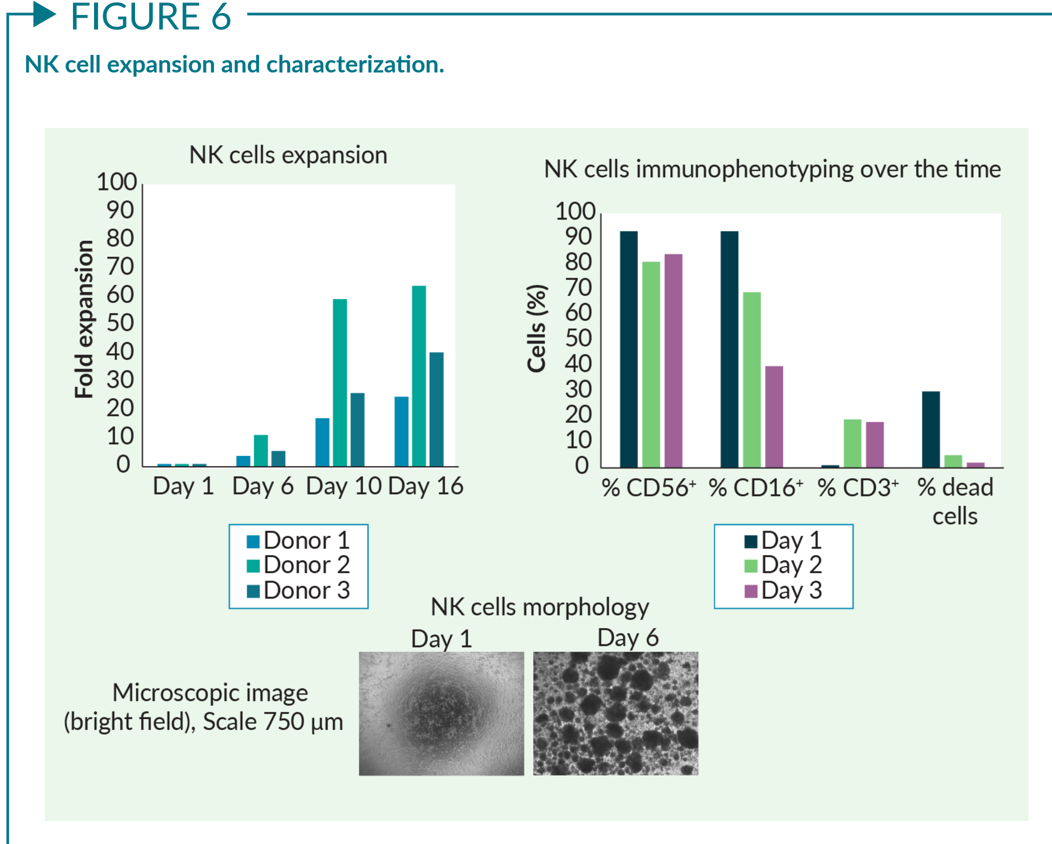


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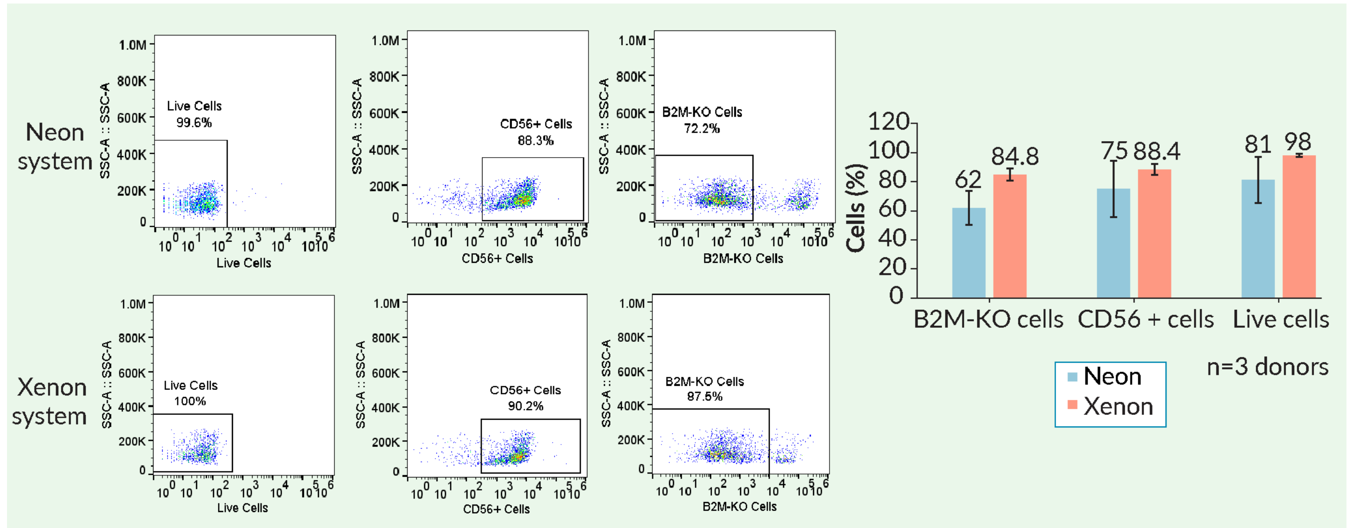
FIGURE 7

Comparative analysis of gene editing efficiency in expanded NK cells using different electroporation systems.



methods, with B2M as a knockout target. When compared, the Xenon system demonstrated greater knockout efficiency than the Neon system, with the Xenon system achieving approximately 85% knockout efficiency on average across three donors.

For more information about the Xenon system, visit thermofisher.com/xenon, and for additional information about the Rotea system, please visit thermofisher.com/rotea.

REFERENCE

1. Application note: residual washout on the CTS Rotea Counterflow Centrifugation System.

Q&A

David McCall, Editor, BioInsights speaks to (pictured left to right) **Sung Lee, Scientist, Thermo Fisher Scientific** and **Deepak Kumar, Scientist,Thermo Fisher Scientific**

Q Can you comment further on the donor-to-donor variability observed with CAR-T workflows?

SL: The donor-to-donor variability is due to the difference in cell types. The cell cycle dynamic usually significantly affects cas9 efficiency. In our case, primary T cell editing efficiency could be affected by T cell donor-to-donor factors, such as genetic factors, recent infection, T cell activation stages, and/or the changing of gene locus due to chromatin stages. Characterizing these variables and further optimizing genomic engineering efficacy can increase the therapeutic editing of the T cells using cas9 protein.

Q Can the CTS Xenon system edit NK cells at a large scale?

DK: In cell manufacturing for cell therapy, a large scale is often required. We observe that the Xenon system is a powerful tool and can perform larger-scale NK editing. The Xenon system can be used to edit up to 50–100 million NK cells. We can also edit different cell types, such as T cell samples.

Q What is the recovery time post-EP?

SL: Recovery time is the time that the cells rest post-EP in our EP buffer prior to adding the media. Typically, a shorter recovery time is better. After 0–60 min, we try to put the cells back into the media after EP.

Q Can CTS NK-Xpander Medium be used to expand pluripotent stem cell-derived NK cells in a feeder-free system?

DK: Our team has found that for an induced pluripotent stem cell (iPSC)-derived natural killer (NK) cell expansion, we can use our NK cell Xpander medium with good results.

Q Do NK cells maintain their phenotype after editing using a Xenon Electroporation System?

DK: Yes, we observed that after EP, the NK cells maintained their phenotype. After EP, we measured the percentage of CD56 and CD16 and observed that they maintained their cell surface markers. After using the Xenon Electroporation System or editing the NK cell by using the Xenon system, we observed more than 90% CD56 was maintained.

Q Why does the Xenon system show higher editing efficiency than the Neon system?

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DK: The Xenon system is a closed system while the Neon is an open system. This could explain the higher editing efficiency of the Xenon system.

Q What are the payload considerations for EP?

SL: You must consider whether the payload is toxic to the cells, as well as payload purity and quality. You also must optimize your concentration of the payload and consider the size of the donor DNA and what kind of buffer you need to use.

Q What is the maximum number of cells that can be washed and concentrated using a CTS Rotea System?

SL: From our study, we used up to 1.9 billion cells and then concentrated the cells in 50 million per mL for the output. We achieved a 76–80% recovery rate. Internally, the Rotea team used a single-use kit and showed the capability of 5–500 billion cells per mL for the maximum output.

Q How do the expansion rates compare between primary NK and iSPC-derived NK cells in NK Xpander media?

DK: When we isolated the NK cells and compared these, we monitored the expansion rate every 3 days. We found that both iPSC-derived NK cell and primary NK cells have the same expansion rate.

Q What is the viability of cells post-EP and what impact does dead cellular material have on the final product from a safety perspective?

SL: The shorter the processing time, the better. We are trying to optimize conditions to better serve the patient. EP is harsh, but we see that 3 days post-EP, we have high viability of about 80–90%. Depending on how you optimize the condition, you can achieve higher viability of cells. Post-EP, we have observed some cell debris, but we try to remove as much as possible and we are currently working on that further.

Q For routine assays in GMP labs, is the Xenon electroporation device reliable for generating reproducible transfection efficiency?

DK: In our group currently, we are not currently performing any good manufacturing practice (GMP) runs. However, this EP system can also be used for GMP.

Q What were the target cells used in the T cell cytotoxicity assay?

SL: The target cells used were NALM6, which are GFP-positive cells that also express CD19. We are using the CD19 CAR-T as our effector cell and then the target cell is GFP-expressing NALM6.

Q What is the expansion rate with NK-Xpander plus modified feeder cells?

DK: I have not used any feeder cells in these experiments as our NK cell expander media is a feeder-free media.

Q Is your process a closed system from start to finish?

SL: From beginning to end, this is not yet a fully closed system, but we are working to try to connect each instrument to transfer to each step in a closed way. Recently, we also launched the Gibco™ CTS™ DynaCellect™ Magnetic Separation System. We are working on minimizing any open steps.

Q Have you used any human serum in your media and what types of cytokines did you use for cell survival?

SL: We are not using any human serum – instead, we use Gibco™ CTS™ Immune Cell Serum Replacement which is a xeno-free formulation. We use animal-free components in the system. The cytokines in this experiment used are interleukin (IL)-2, although we are also testing IL-7 and IL-15 in different studies. You can expect to learn more about that in the future. With IL-7 and IL-15, it is important to maintain the stemness of the cells.

Q What optimal voltage strength can enhance EP without killing NK cells?

DK: Here, we use 1700 volts. After trying different voltages, we found that this was the best way to not kill the cells and still have the best editing efficiency for EP systems.

Q Do you check for viability of cells after EP?

DK: Yes. Checking for viability is important. We waited for 30 min, as there is some repair of the cell membrane after you perform EP. The cells electroporated with the Neon or Xenon systems both showed more than 90% cell viability.

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BIOGRAPHIES

SUNG LEE, PhD, is a Scientist at Thermo Fisher Scientific where he focuses on non-viral delivery for cell and gene therapy applications. Dr Lee received his PhD in Medical Immunology from University of California, Irvine and performed his postdoctoral work at City of Hope, Brigham and Women's Hospital (Harvard Medical School) and UCSF Medical Center. He also worked in two start-ups (Pro-Drug/Immuno-Oncology related) prior to joining Thermo Fisher.

DEEPAK KUMAR, PhD, is a Scientist at Thermo Fisher Scientific where he focuses on NK Cells gene editing using viral and non-viral approach to support cell and gene therapy workflow applications. Dr. Kumar received his PhD in Molecular Biology and Genetic Engineering from the Institute of Life Sciences (Department of Biotechnology, Government of India), India. After his PhD, he was a Postdoctoral Fellow at the Moores Cancer Center, University of California, San Diego and then worked as a Project Scientist at the Department of Medicine, University of California, San Diego.

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AUTHORSHIP & CONFLICT OF INTEREST

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FASTFACTS

Technology innovation to address challenges in gene therapy manufacture

Tania Pereira Chilima, Chief Technology Officer, Univercells Technologies

The gene therapy industry is striving for highly efficient viral vector manufacturing processes, and working towards increased capacity in upstream development. Technology selection will have a significant impact on a multitude of critical process parameters including product titer, quality and the level of impurity. Process intensification achieved through biomass immobilization in a homogeneous low shear environment will promote cell health leading to high capacity and product quality while reducing footprint and materials usage.

DUAL-LAYER TECHNOLOGY

The scale-X™ bioreactor is a dual-layered structured fixed-bed bioreactor, which can be used with adherent as well as suspension adapted cells. The bioreactor is designed to reduce cell culture volumes and to increase productivity and product quality in the upstream process. Cell retention in the fixed-bed matrix also positively impacts downstream processing, by facilitating continuous processing and reducing process impurities during harvest.

The fixed-bed comprises a cell immobilization layer and a flow distribution layer, rolled together to provide a structured, homogeneous environment (Figure 1), maintained throughout the bioreactor as well as throught different batches and scales.

The two layers combined enable high cell density with cells transfected at ~35M cells/mL without aggregation. The homogeneity has been demonstrated across the scale-X range achieving mixing times of ~23s and even radial and axial cell distribution of +-16% at commercial scale (600m²).

Figure 1. Key features of the scale-X bioreactor.

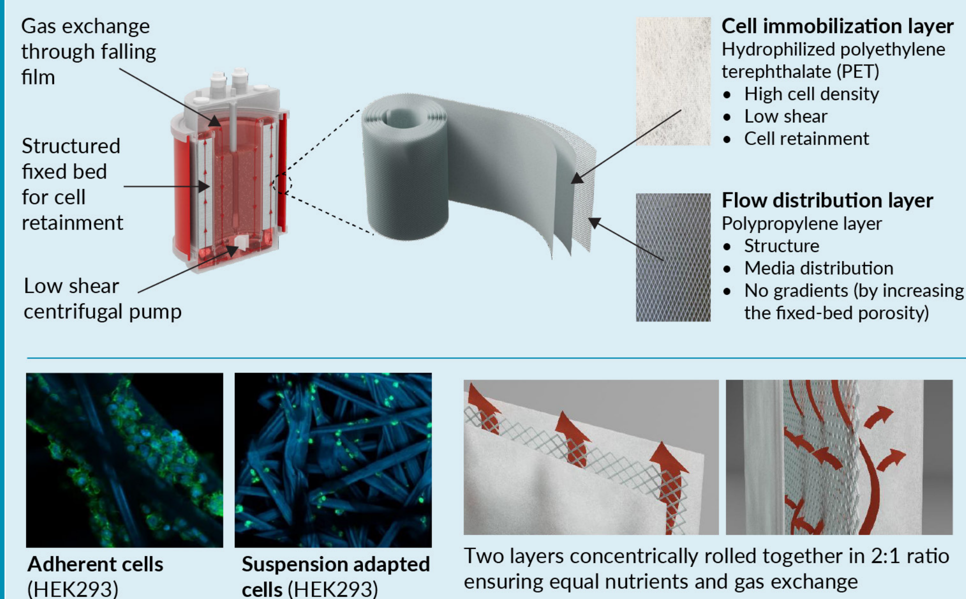
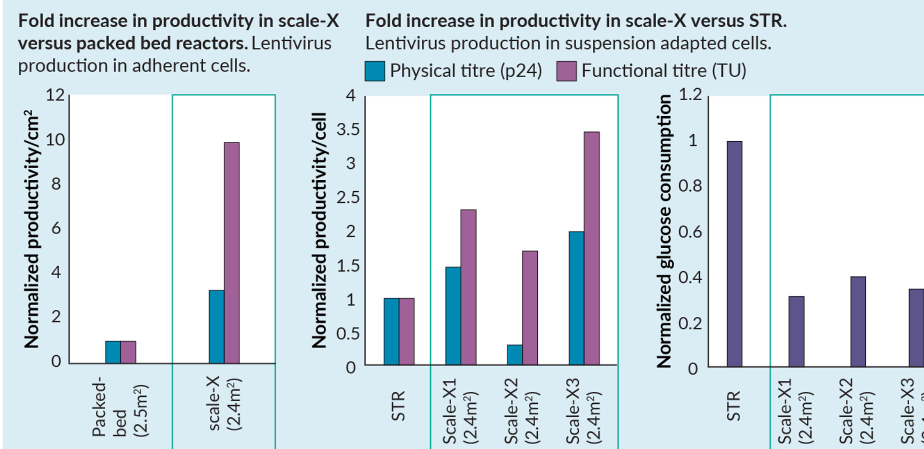


Figure 2. LV production in adherent HEK293 cells using a packed bed versus the scale-X bioreactor (left) and a comparison using suspension adapted HEK293 cells in a stirred-tank bioreactor versus the scale-X bioreactor (right).



The conditions in the bioreactors in the middle graph differed (different transfection reagents, feeding strategies and inoculation densities).

LENTIVIRUS & ADENO-ASSOCIATED VIRUS PRODUCTION

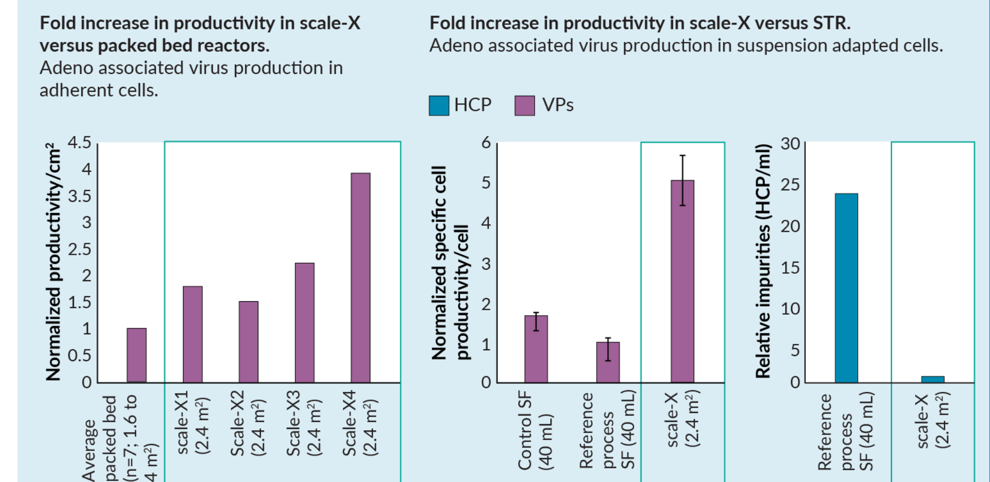
To demonstrate that a homogeneous and low shear environment promotes higher productivity and product quality, comparison experiments were conducted using various culture systems to produce lentivirus (LV) and adeno-associated virus (AAV). Tests were performed using adherent as well as suspension adapted HEK293 cells. Physical titers were determined to show productivity. Functional titers were measured to confirm product quality. The results for LV production are presented in Figure 2. Both the adherent cell cultures and the suspension adapted cell cultures show elevated physical titers compared to the alternative culture systems used. Next to higher physical titers, an even larger increase in functional titer was observed, indicating product quality is greatly improved. Another noteworthy fact is the difference in glucose consumption of the cells grown in the scale-X bioreactor, which was significantly lower compared to other culture systems (seen in suspension only).

A similar productivity experiment was performed producing AAV in adherent and suspension adapted HEK293 cells, comparing various culture systems to the scale-X bioreactor. Results are shown in Figure 3. The AAV culture experiments exhibit comparable results to those seen in the LV production experiments. The scale-X bioreactor shows elevated productivity compared to the other culture systems used. Furthermore, the system enables a high reduction of host cell proteins resulting in an improved impurity profile when moving into the downstream process.

CONCLUSION

The results of the experiments performed demonstrate that selection of the appropriate technology can increase capacity and product quality while reducing manufacturing footprint as well as facilitate downstream operations through lower volumes, lower impurities and integrated continuous processing.

Figure 3. AAV production in adherent HEK293 cells using a packed bed versus the scale-X bioreactor (left) and AAV production in suspension adapted HEK 293 cells using a shake flask versus the scale-X bioreactor (right).



The conditions in the bioreactors in the left graph differed.

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Simplifying lentiviral downstream processing with a novel affinity resin & robust analytical tools

Chantelle Gaskin & Suzy Brown

Recombinant lentivirus has become a vector of choice for many gene-modified cell therapies, including several US Food and Drug Administration-approved cell therapies, due to its broad tropism and long-term, stable gene expression in non-dividing cells. The safety and efficacy of lentiviral-based therapies depend greatly on optimized and controlled lentiviral production. Downstream purification of lentiviral particles presents unique challenges, and robust analytics are critical to verify both the recovery and infectivity of the purified product. In this article, an overview of challenges and newly developed solutions for robust lentiviral purification and rapid analytical methods for titer determination and impurity quantification will be presented. Details of a new affinity chromatography resin to purify vesicular stomatitis virus glycoprotein pseudotyped lentivirus, as well as two qPCR-based genomic and proviral infectious titer assays, will be discussed.

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As of the first half of 2021, there had been an estimated 288 cell and gene therapy programs in clinical trials using lentiviral vectors. Of US Food and Drug Administration (FDA)-approved cell and gene therapies, there are four CAR-T cell therapies and one *ex vivo* gene therapy that use lentiviral vectors. Characteristics of the recombinant lentiviral system, including long-term transgene expression, high packaging capacity, and the ability to transduce both actively dividing and non-dividing cells are advances used in *ex vivo* gene-modified cell therapies. Lentiviral vectors have also found use in therapeutic gene editing and genetic vaccine platforms. As a result, the demand for high-quality lentiviral

vectors for therapeutic applications continues to be strong.

To meet the growing demand for lentiviral vectors, advances have been made to improve yield and turnaround in large-scale production, with robust analytics to ensure vector quality and safety. From a manufacturing perspective, lentivirus is produced using cell-based bioproduction systems of adherent or suspension HEK293, or 293T cell lines, co-transfected with multiple plasmids carrying transgene packaging and enveloped elements to assemble into the recombinant viruses. Stable producer cell lines can also be used. Viral vectors are then purified, concentrated, and formulated. Although the manufacturing process is similar to other viral vectors, the characteristics of lentiviral vectors are different, which needs to be accounted for in downstream purification and analytics.

PROCESS-RELATED SOLUTIONS FOR LENTIVIRAL PRODUCTION

Thermo Fisher Scientific offers a complete solution for the production and purification of lentiviral vectors. The Invitrogen GeneArt™ brand services provide flexible, reliable custom gene synthesis, with short turnaround times. For vector production, the Lipofectamine™ 3000 transfection reagent for adherent cell systems produces efficient transfection and high titers with lower reagent requirements. The LV-MAX™ system is a next-gen lentivirus production system and can be used alongside the newly launched CaptureSelect™ lentivirus affinity resin for vector purification.

The LV-MAX™ lentivirus production system includes high-density HEK293 suspension cells, chemically defined media, production supplements, transfection reagent, optimized lentiviral packaging plasmid, and a novel enhancer reagent. The system was designed to be scalable with no animal-derived components and includes regulatory support files for GMP manufacturing. Customers have been able to increase their titers 10-fold using the LV-MAX system.

MYCOPLASMA TESTING

At lentivirus vector harvest, manufacturers need to test for *Mycoplasma* to ensure product quality and patient safety. While there are several available *Mycoplasma* testing solutions, few meet the sensitivity, specificity, and robustness required for regulatory agencies. One available solution that meets these criteria is the MycoSEQ™ assay.

The MycoSEQ™ Mycoplasma detection system has been designed to fulfill the regulatory guidance in European Pharmacopeia section 2.6.7 on *Mycoplasma* testing with nucleic acid-based methods. The qPCR-based system provides clear, objective, multiparameter data interpretation using three acceptance criteria for the identification of a positive result. The assay is proven to detect over 90 different *Mycoplasma* species, with no cross-reactivity to off-target bacterial organisms. It is also highly sensitive and enables validation of less than/equal to ten genome copies per mL in test samples. This off-the-shelf kit also contains a patented discriminatory positive control that can eliminate the concern of any potential cross-contamination, as it is easily differentiated from a true positive result.

A global support network of experienced Field Application Scientists (FAS) can help deliver full workflow training, from sample preparation to results interpretation. Additionally, a drug master file is held with the FDA for this solution, and Thermo Fisher Scientific offers in-house experience in validation design and regulatory support. Instrument installation qualification/operational qualification (IQ/OQ) services and computer system validations are provided for the integrated data analysis AccuSEQ™ software, which features 21 CFR part 11 compliance features.

Following validation, regulatory filing, and review, customers have received regulatory acceptance to use the MycoSEQ assay for lot release testing applications across multiple therapeutic modalities. This includes cell culture manufacture, cell therapy, and tissue therapy.

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Over 40 customers have already received regulatory approval for a drug filing that uses the MycoSEQ assay for *Mycoplasma* testing.

PURIFICATION CHALLENGES

The most cited purification challenges in the field stem from the fact that lentivirus is an enveloped virus. It requires processing within narrow ranges of pH, temperature, conductivity, and shear. Many current purification processes either have low feasibility for scale-up or require longer processing times to produce the required levels of purification, such as centrifugation and tangential flow filtration. Longer processing times also translate to lower recovery. There are also several anion exchange processes that use different chromatographic support technologies – monolith or membrane absorbers – to reduce process time. However, anion exchange typically requires exposure to high salt concentrations, which might impact the infectious titer. Current purification methods are also unable to distinguish between infectious product and product-related contaminants.

One of the most requested solutions has been an affinity chromatography method that could leverage gentle buffer conditions and reduce processing times while retaining infectious titers. In general, current methods do not typically yield more than 30% recovery. In many cases, process development scientists are seeing even lower recoveries than that.

To understand why we see low recovery, we must look at the lentiviral particle structure. The genetic payload is encapsidated by capsid proteins such as P24 and enveloped proteins such as vesicular stomatitis virus glycoprotein (VSV-G). Lentiviral tropism is determined by the ability of the enveloped proteins to interact with the receptors on the cell surface. VSV-G is one of the most used pseudotypes in cell therapy due to the broad tropism across different species and cell types.

During production in human cell lines like HEK293, a variety of particle species are generated in addition to the infectious viral particles. These are product-related contaminants: virus-like particles (VLPs), non-infectious particles, and exosomes, with and without envelope proteins. The envelope proteins found on the infectious particle are fragile and sensitive to the conditions that are commonly used in the purification of hardier molecules like monoclonal antibodies (MABs). This leads to low recovery during processing.

ANALYTICAL CHALLENGES

Analytical methods for lentiviral quantitation include the P24 ELISA for capsid-based titers, reverse transcriptase (RT)-qPCR or digital PCR for genome-based titers, and particle counting systems for all physical particles in a sample. A combination of these methods is often used. Each method has its own advantages and disadvantages but regardless of the method used, manufacturers face challenges including poor reproducibility, high variation, difficulty optimizing assays in complex matrix conditions, and inefficient recoveries. These challenges, coupled with the lack of a lentivirus reference standard, make it difficult to accurately quantify yields.

Once cells are transduced with lentiviral vectors, they must be tested to ensure the safety, quality, and potency of the gene-modified cell therapy product. There are several different ways to assess integration and lentiviral infectivity, including flow cytometry or fluorescent-activated cell sorting, which look at transgene expression, whereas qPCR and digital PCR measure copy number of the provirus in the genome itself. Cell-based assays can be used to calculate the infectious titers of the lentiviral vectors. As high integration may be a safety risk, these assays must be sensitive and accurate. Vector copy number (VCN) is tested for each lot of a transduced cell product, so the assay must be amenable to use in a quality control (QC) environment with rapid turnaround and minimum manual intervention preferable.

Cell lines and healthy donor cells used for infectious titers may have different transduction efficiencies to patient cells, which poses additional challenges. Frequently, transgene-specific assays have been used, which measure multiple attributes of a final product. However, this limits the ability of an assay to be used across a program as a platform approach and will add to assay development and program timelines.

SOLUTIONS FOR LENTIVIRAL PURIFICATION & ANALYTICAL TESTING

The newly launched CaptureSelect™ Lenti VSV-G Affinity resin was designed for specificity to VSV-G pseudotyped lentiviral vectors. The resin provides high-level purification in a single step with gentle elution conditions at neutral pH to maximize infectious particle recovery.

To determine the dynamic binding capacity, experiments were performed using a 1 mL, 3 cm bed-height column, equilibrated in 50 mM HEPES, 150 mM salt pH 7.5 **(Figure 1)**. The titers of the load material were determined by p24 ELISA to be 4×10^{9} particles per mL. Flowthrough fractions were collected in 5 mL increments and sampled for titer determination. As can be seen from the breakthrough curve, there was a 10% breakthrough corresponding to 1×10^{11} particles per mL total capacity.

On a 10 ml CaptureSelect Lenti VSV-G column (1.6×5 cm) equilibrated in 50 mM HEPES, 150 mM NaCl pH 7.5, 250 ml clarified suspension harvest with a titer of 1.1×10^{10} total particles/ml was loaded at a flow rate of 5 ml/min (150 cm/h, 2 min residence time). The load was washed out with equilibration buffer and the column was eluted with 50 mM HEPES, 150 mM NaCl, 0.8 M Arginine pH 7.5 **(Figure 2)**.

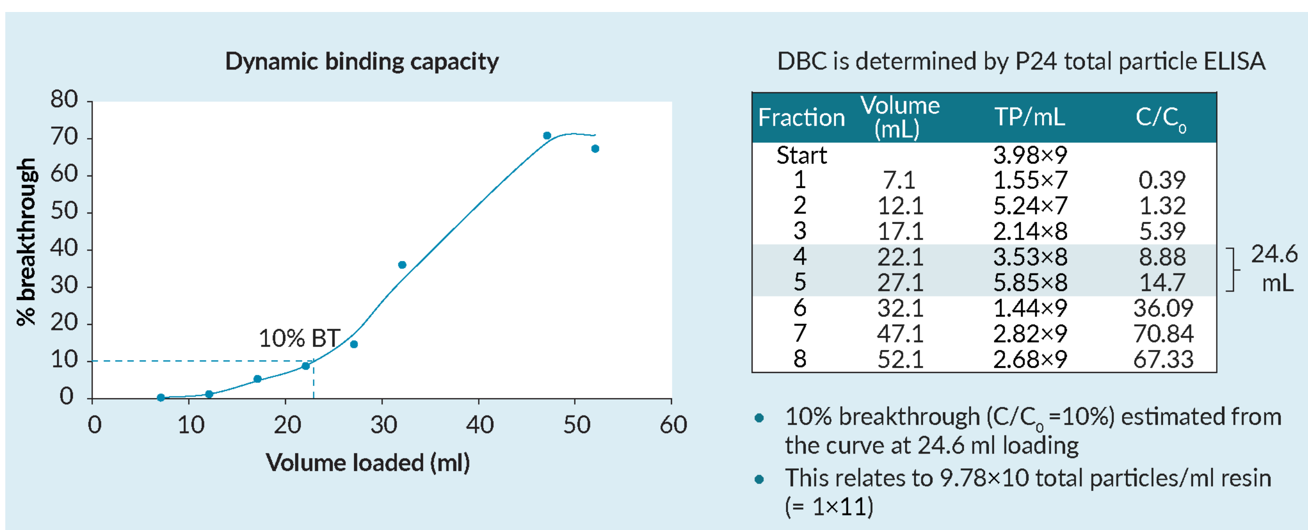
The elution was efficient and showed good compatibility with the enveloped virus particles, resulting in high concentrations of infectious particles in the elution fraction. Depending on the feed and application, optimization of the elution buffer might be needed with adjustments of the arginine concentration, pH, or combinations thereof.

A summary of the recovery results by total and infectious particles, as determined by p24 ELISA and a cell-based infectivity assay, is shown in **Table 1**.

This data demonstrates that the infectious particle titer increases after purification. As the process progresses, the total to infectious particle ratio decreases. This results in a more

FIGURE 1

Dynamic binding capacity determination on a 1 mL column.



Fraction	Volume (mL)	TP/mL	C/C_0
Start		3.98×9	
1	7.1	1.55×7	0.39
2	12.1	5.24×7	1.32
3	17.1	2.14×8	5.39
4	22.1	3.53×8	8.88
5	27.1	5.85×8	14.7
6	32.1	1.44×9	36.09
7	47.1	2.82×9	70.84
8	52.1	2.68×9	67.33

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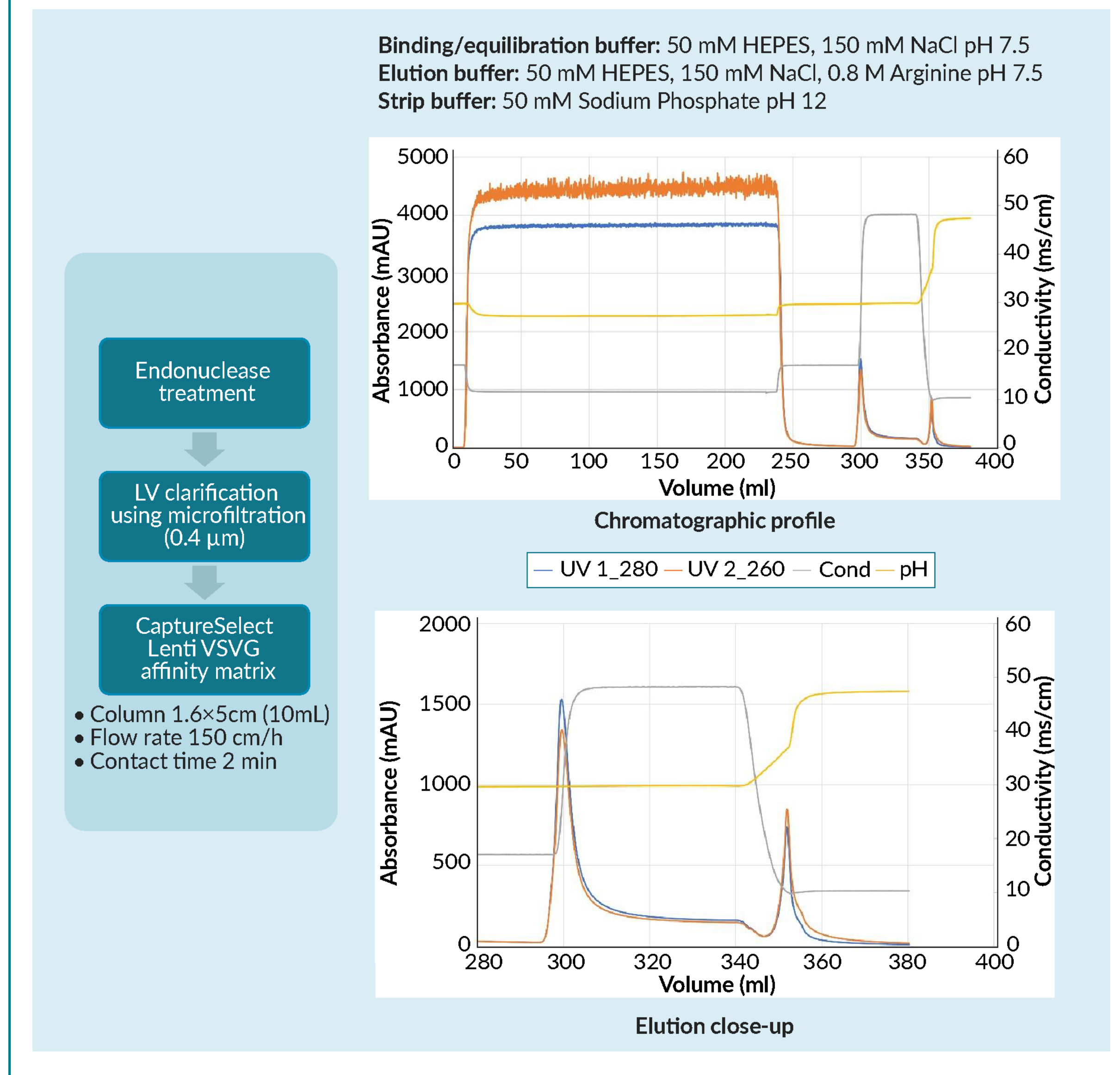
FIGURE 2

Representative chromatogram using recommended process conditions.



than 5-fold enrichment of infectious particles with a 50–60% recovery in the column eluate.

An enrichment of infectious particles is expected with the Lenti-VSVG resin due to selectively binding the VSV-G envelope protein on the capsid. The P24 and other capsid proteins will be present in non-infectious particles, whereas the VSV-G protein is present in a subpopulation of particles including the infectious particles. An 85% DNA clearance and over 95% protein clearance were also achieved.

The Lenti VSV-G ligand is immobilized onto a 65 µm highly crosslinked agarose bead. The resin has a pressure rating up to two bar with a recommended velocity of up to 200 cm per h. It is shipped in 20% ethanol and in 5-, 10-, and 50-mL bottle sizes.

ANALYTICAL TOOLS FOR PURIFICATION PROCESS DEVELOPMENT

As lentiviral vectors are used to transduce cells, they are an active ingredient in drug

substances. Drug substances must be tested for critical quality attributes (CQAs) in-process and at lot release according to FDA chemistry, manufacturing, and control (CMC) guidance. Lentiviral vector CQAs include identity, purity, strength, safety, and quality. Titer or vector concentration is an important attribute for strength, quality, and safety testing.

For lentiviral characterization and integration analysis, Thermo Fisher Scientific has recently introduced two new qPCR assays. The ViralSEQ™ Lentiviral Physical Titer Kit is a one-step real-time RT-qPCR assay for genome-based lentiviral titers, measuring physical titer in viral particles per mL. Additionally, the ViralSEQ™ Lentivirus Proviral DNA Titer Kit is a qPCR assay to measure integrated lentivirus or proviral copies in transduced cells. It can be used to calculate infectious viral titers and VCN. Combined, these two assays provide a convenient method to compare qPCR to qPCR data, for total and infectious titers, as well as for measuring VCN for analytics across the lentiviral workflow. Both assays are designed to provide robust performance and facilitate lentiviral analytics, in-process development, and manufacturing in QC environments.

The assays are just one component of the experimental workflow. Thermo Fisher Scientific provides an integrated solution to meet your needs for a complete workflow, from sample preparation to data analysis. Both lentiviral assays have been optimized for this workflow, enabling manual or automated sample preparation on the KingFisher™ Flex platform, using PrepSEQ™ nucleic acid sample preparation kit. The RT-PCR will be run on a QuantStudio™ 5 instrument, and data analyzed using the AccuSEQ software, which enables 21 CFR part 11 compliance in GMP environments. Thermo Fisher Scientific can also support other applied biosystems or qPCR instruments through instrumentation and validation.

Lentivirus physical titer kit

The ViralSEQ Lentiviral Physical Titer Kit is an RT-qPCR assay for the quantitation of genome-containing lentivirus vectors. The assay targets a conserved long terminal repeat (LTR) region in the lentiviral genome. As this region is critical to integration into cells, most lentiviral production systems have conserved LTR regions. This assay can be used across production lots and programs if they all use the same vector system. The TaqMan™ chemistry used in this assay provides high target specificity, preventing background signals from potential cross-contaminants, such as residual plasmid or host-cell DNA, from overestimating titers. The assay has over seven logs of dynamic range, from 50–10^9 copies. This actively quantifies a whole range of lentiviral yields. The kit also includes all the reagents required for the RT-qPCR reaction and comes with an RNA standard.

The physical titer kit total assay runtime, including the sample preparation, is under 6 h. The individual steps include the preparation of reagents and sample dilutions, nucleic acid extraction, a DNase treatment to remove any residual DNA, PCR reaction prep, and RT-qPCR run with data review.

TABLE 1

Comparison of total particle to infectious particle ratios.

Sample	TP/mL	IP/mL	TP/IP ratio	Recovery	HCP removal	Total DNA removal
1. Feed	1.10×10^{10}	7.98×10^{7}	138			
1. Flow through	3.25×10^{8}	8.30×10^{5}	392			
1. Elution	4.44×10^{10}	4.42×10^{8}	100	50%	99%	80%
2. Feed	1.11×10^{10}	9.00×10^{7}	165			
2. Flow through	1.28×10^{9}	5.45×10^{6}	245			
2. Elution	2.6×10^{10}	4.66×10^{8}	71	58%	97%	97%

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FIGURE 3

ViralSEQ Lentivirus Physical Titer Kit standard curve and amplification plots.

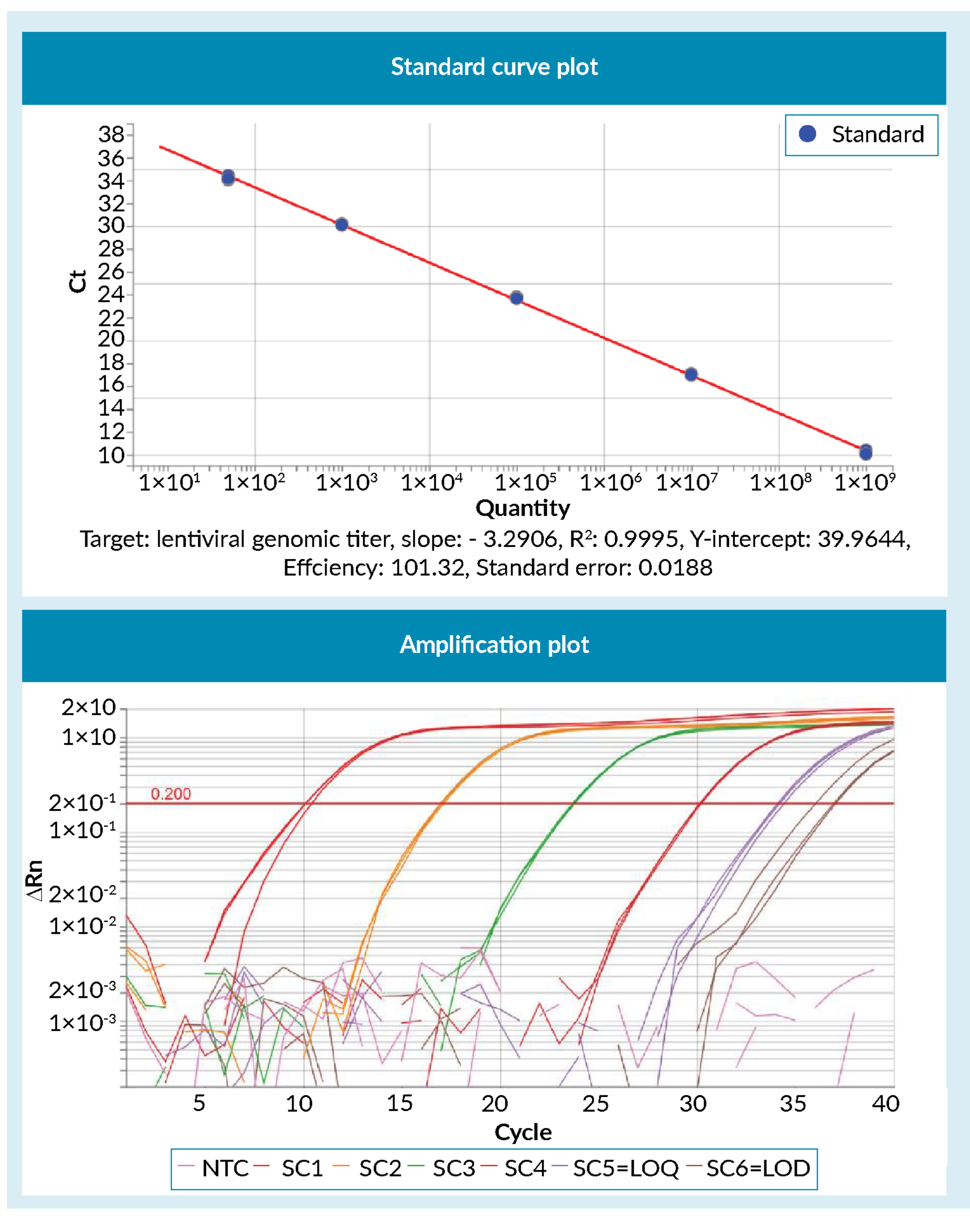


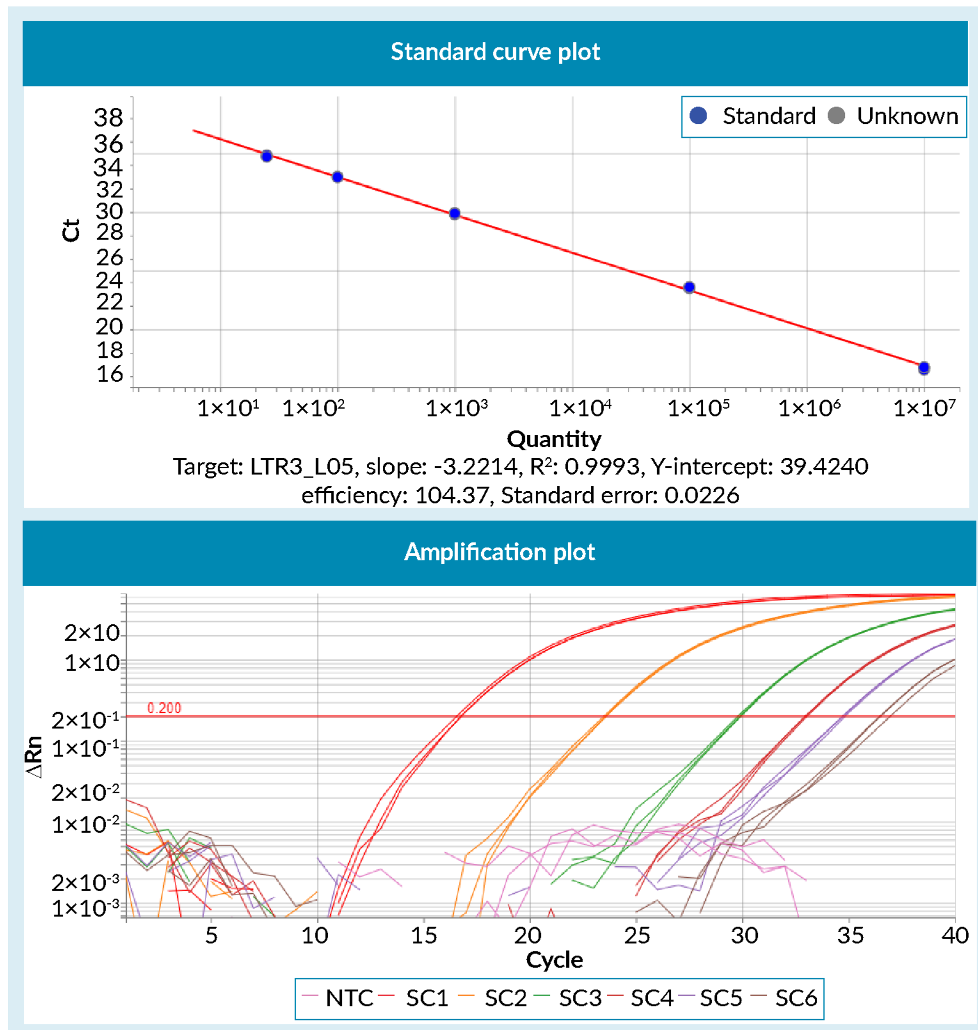
Figure 3 shows a representative standard curve plot with a PCR efficiency of 101% and an R^2 of 0.999. The amplification plot shows the amplification curve for the standard curve point across the assay range, from $50–10^9$ copies per reaction.

Lentivirus proviral DNA titer kit

The ViralSEQ Lentivirus Proviral DNA Titer Kit is a qPCR assay that also targets the LTR region in the integrated vector copies for transduced cells, making it suitable across lentiviral programs that use the same system. Data from this assay for proviral copy numbers can be used to calculate the lentivirus infectious titers and the VCN for transduced cells. The TaqMan™ chemistry provides high target specificity, and the assay range enables proviral copy number for a range of transduction efficiencies. The assay has excellent sensitivity with a limit of quantification of 25

FIGURE 4

ViralSEQ Lentivirus Proviral DNA Titer kit standard curve and amplification plots.



copies per reaction. This kit comes with all reagents required for the qPCR and a DNA standard control.

The proviral DNA titer assay takes ~5 h including sample preparation. The workflow includes the preparation of reagents and serial dilutions, sample extraction, qPCR preparation and run, and final data analysis. The representative standard curve plot in Figure 4 shows a PCR efficiency of 103% and an R^2 of 0.999. The amplification curves for the standard curve points across the assay range from 25–10^7 copies per reaction.

ASSAY DEVELOPMENT & VALIDATION TESTING

There are many regulatory expectations regarding the characterization of lentiviral vectors, transduced cells, and the validation of analytical methods that are used for quality testing of this type of product. There is a need for validated assays in each specific process that manufacturers perform as part of their CMC filing. Therefore, Thermo Fisher Scientific offers assays with verification and internal validation testing to ensure that these assays perform to the high standards required to meet validation criteria and regulatory expectations.

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As part of development testing, bioproduction and cell culture matrices have been evaluated to mimic the representative sample conditions customers will experience and qPCR data is correlated against orthogonal titer methods. For internal assay validation, multiple assay lots have been tested with multiple operators across multiple sites, to ensure robust, reproducible assay performance. Both manual and automated sample extraction have been compared, and two different qPCR systems have been used.

Internal validation for the proviral DNA titer kit

To measure site reproducibility, data was generated by different operators run on different instruments for Site 1 and Site 2. As shown in Figure 5, both sites performed similarly for standard curve metrics for PCR efficiency, R^2, Y-intercept, and slope. The assay shows good site-to-site reproductivity for standard curve performance.

Assay precision was evaluated across multiple variables, with DNA controls from three different kit lots at standard curve concentrations. The percentage coefficient of variability (CV) criteria (less than/equal to 30%) was achieved for all data points.

Assay specificity was evaluated by testing for any cross-reactivity using a panel of DNA from potential cross-reactants, such as process-related impurities including HEK293, *E. coli*, baculovirus, as well as plasmids containing ampicillin or kanamycin resistance genes. The assays were tested in two separate runs, and no cross-reactivity was detected for any of the species tested.

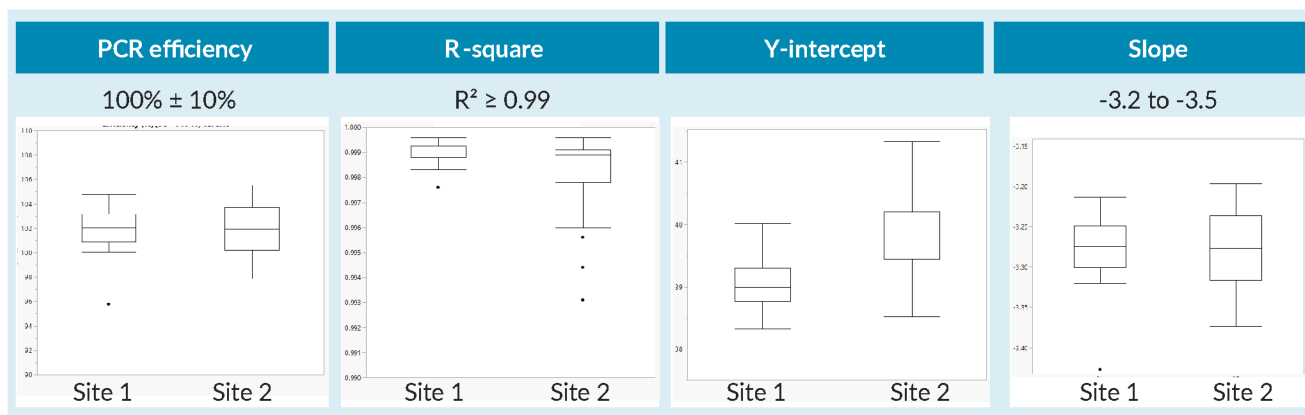
To evaluate sample preparation recoveries, the DNA control from the kit was spiked into a test sample matrix at 500 and 2.5×10^6 copies. To mimic cell culture conditions, a test matrix of 50% cell culture medium with HEK293 cell lysate at 10^5 cells was used. Extraction was then performed using the PrepSEQ™ nucleic acid extraction kit on the KingFisher™ Flex automated platform, as well as manually. The criterion for recovery was set to 70–130% and the data obtained from the KingFisher Flex platform and the manual extraction were all within this range.

To test the performance of the proviral DNA assay with a representative transduced cell sample, two cell lines that have been transduced with lentiviral vectors were sourced from our collaborators. The qPCR copy numbers were determined with the titer kit and a corresponding VCN was calculated. The data points were compared to an orthogonal test method, and the two methods showed a good correlation.

In summary, the ViralSEQ lentiviral titer kits provide a rapid, robust, and reliable

FIGURE 5

Proviral DNA titer kit: PCR performance.



solution for measuring genomic and proviral copy numbers for lentiviral characterization. Both assays quantitate based on the LTR region and facilitate analytics across the workflow from lentiviral vectors to integrated proviruses. The assays have been internally validated to support customer validation at user sites as per regulatory expectations.

Q&A

David McCall, Editor, BioInsights speaks to (pictured left to right) **Chantelle Gaskin,** Field Application Scientist, Viral Vector Purification and Downstream Process Development, Thermo Fisher Scientific and **Suzy Brown,** Senior Field Application Specialist, Pharma Analytics, Thermo Fisher Scientific

Q Can the affinity resin be used for other pseudotypes other than VSV-G?

CG: We have not generated any data yet, but the ligand was developed for specificity against the VSV-G enveloped protein.

Q In my opinion, a 25 mL max load for a 1 mL column sounds low. Is there a way to increase the capacity?

CG: Lentivirus is around 100 nm in diameter, so if you consider the difference in size between a lentiviral vector versus an AAV vector or a smaller molecule like a monoclonal antibody, you can expect the accessibility of that molecule to the surface area of the resin is going to be lower.

Typically, to increase capacity, we recommend increasing residence time or optimizing your pre-chromatography unit operations. Customers might want to start with a two-minute residence time initially, considering a balance between maximizing recovery and maximizing process productivity.

Q How do I know these lentiviral titer assays will work for our recombinant lentiviral platform?

SB: The two ViralSEQ Lentivirus kits have been designed against one of the conserved regions of the LTR sequence of the plasmid delivering your transgene.

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We have tested that sequence *in silico* and found that it is compatible with over 200 lentiviral transfer plasmids available, including a few that have self-inactivating modifications. If you are using a conserved LTR, our assay will work with most transfer plasmids.

If you have made specific modifications in the LTR sequence, then the best way for us to address this is to contact us directly so we can check the compatibility of your sequence with our primers.

Q What is the base bead of the lentiviral affinity resin? Is there a POROS™ bead backbone available?

CG: This resin has been years in the making. The newly launched resin is based on highly cross-linked agarose, but the team did initially develop different resin prototypes on both the agarose and POROS base matrix. The crosslinked agarose matrix had a better performance profile, so that was chosen to move forward.

Q Do you have any prepacked columns with the lentiviral resins?

CG: Right now, we only have bulk resin formats available: 5-, 10-, and 50-mL bottle sizes. We are currently working on prepacked columns and are trying to get customer feedback to see what column formats or column dimensions would be most useful.

Q Can the physical titer assay distinguish between plasmid and viral genome?

SB: The physical titer assay does not distinguish between plasmid or viral genomes. However, to mitigate any residual plasmid in your sample, which could lead to titer overestimation, we have included a DNA removal step as part of the workflow for that kit. You will first extract total nucleic acid, then perform your DNase treatment, which will remove any potential residual plasmid DNA and any host-cell DNA that is carried over. Following reverse transcription, during the qPCR, your primers and probes will then specifically bind to the cDNA and amplify the target LTR sequence there.

We are not able to directly distinguish between plasmid and viral genome, but we have taken steps to address any potential residual plasmid during the workflow.

Q Can your physical titer assay be correlated with transduction assays or bioassays?

SB: In terms of the correlation between total virus particles versus infectious titer, the two assays that we have discussed can be used in parallel, and then the

data can be compared. This means you are correlating results between qPCR methods. It is much easier to correlate two qPCR methods than one qPCR method and another, such as flow cytometry.

The correlation that we typically see is a 2–3 log difference between the two titers. We have heard from customers that the expectation is that for every one particle that may be infectious, there may be 100 that are not infectious. This will depend on the quality of your lentiviral vectors and the purification process being used.

Q How many times can the lentiviral resin be re-used?

CG: This is a newly launched resin, so comprehensive applications data packages are not yet available. We do have a few internal studies ongoing to be able to answer that and other questions.

We know that an effective cleaning strategy is critical to the reusability of the resin. So far, phosphate at pH 12 is our recommended strip buffer based on our data.

Q Will the lentivirus qPCR assays work on digital PCR platforms?

SB: The two lentivirus titer kits have been developed and optimized for a qPCR system only. Although we now offer a digital PCR instrument, the QuantStudio™ Absolute Q™ digital PCR system, and we have done some initial feasibility work on this, we cannot specify the performance criteria for digital PCR at this time.

Q Can I use other qPCR instruments for the assays than those presented in the validation study?

SB: Yes – all the analytical kits either use TaqMan or SYBR™ Green chemistry and can be run on any qPCR system. If you have the correct channels to detect the fluorescent dye, or label being used, you can use them.

The reason we talk about the QuantStudio 5 and the 7500 Fast instruments is that we have shown they are validatable and can be utilized with our AccuSEQ software. This software, as well as enabling the 21 CFR part 11 compliance, has been designed to support these specific assays with in-built templates, automated calculations, and presence-absence calls.

BIOGRAPHIES

CHANTELLE GASKIN is a Field Applications Scientist, specializing in protein and viral vector purification and downstream process development. She held leadership positions at Applied Genetic Technology Corporation and Brammer Bio, prior to joining the Thermo Fisher Scientific Bioproduction Division in 2020. With over 10 years of experience in gene therapy,

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Chantelle has accumulated comprehensive knowledge of standard industry practices and regulatory standards, applying this knowledge to advance development of therapies for a variety of indications including ocular, CNS and systemic disease. Chantelle holds a master's degree in Chemistry from University of Florida and a Bachelor's in Chemistry from Smith College.

SUZY BROWN is the Senior Field Application Specialist for Pharma Analytics, supporting customers in the UK and Ireland. She has been with Thermo Fisher Scientific for over 5 years where she provides training and implementation of solutions for contaminant and impurity testing designed for the cGMP environment. Prior, she worked in the biopharmaceutical industry as an Analytical Development Scientist for 3 years at Allergan Biologics, within a Molecular Biology team and has experience in molecular methods and impurity testing. Suzy holds a PhD in Developmental Biology as well as a Bachelor's in Cell Biology from University of Manchester (UK).

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Thermo Fisher Scientific

ThermoFisher
SCIENTIFIC

PRODUCT USE STATEMENT

ViralSEQ: For research use only. Not for use in diagnostic procedures.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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These kits provide a convenient method to quantitate and correlate qPCR data for total and infectious LV particles, facilitating analytics in process development, optimization, and manufacturing quality control (QC).

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Commercializing cell & gene therapies: a perspective from the quality function

Christoph Meyer & Andreas Wirth

The key modalities in cell and gene therapies each present unique challenges and opportunities, leading to a need for different asset requirements. The Lonza New Product Introduction Process and Lifecycle process enables a faster turnaround to manufacture and delivery of these therapies to patients. In this article, Lonza's approach to de-risking development, industrialization, and delivery to cGMP manufacture is outlined, in order to enable the development of a commercially viable process.

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INTRODUCTION

Statistical and clinical data has proven that cell and gene therapy (CGT) can be highly efficient and curative. The response rate for products currently on the market, such as Yescarta and Kymriah, is unprecedented.

Each of the key modalities in CGT – allogeneic cell therapy, autologous cell therapy, and viral vector-driven gene therapy – comes with unique challenges and opportunities. With autologous cell therapies, we deal with one patient per product batch with high efficacy but also high cost of manufacturing and usually no inventory. Here, the proximity of manufacturing to patients is key to reducing supply chain complexity. Allogeneic therapies, on the other hand, offer off-the-shelf convenience and centralized manufacturing. However, cell type diversity and the growth of living cells for a large patient pool can be an issue. Viral

vectors offer off-the-shelf models as well as centralized manufacturing.

Different asset requirements are a result due to different technologies and processes. In general, there is a lack of industrialized manufacturing processes and platform technologies. The diversity of both the underlying technology and the actual product platforms is an issue as well. The Lonza approach to developing a commercially viable process is based on de-risking development, industrialization, and delivery to cGMP manufacture. This includes establishing the baseline process, identifying the major manufacturability gaps, process optimization based on design specifications and critical quality attributes (CQAs), and all necessary steps for successful process performance qualification (PPQ), and ultimately, commercialization of the product.

Lonza is also investing in digitalization initiatives to streamline manufacturing and establish a seamless integration between manufacturer and customer systems. This includes the MODA platform, the digitization of chain of custody, and electronic batch records.

QUALITY CHALLENGES

Quality challenges in CGT include accelerated clinical development, which compresses timelines for chemistry, manufacturing and controls (CMC) activities. Complex biological starting materials impact the manufacturing process, while chain of custody of donor tissue can present further issues. The manufacturing processes often requires a large number of manual manipulations with potential aseptic risks, and long process days requiring a high number of trained operators. It is understood that when dealing with cells, sterile filtration along the product pathway is not possible, so increased emphasis needs to be placed on aseptic processing and associated operator training and qualification including ongoing supervision and improvements. Segregation and prevention of cross-contamination is key, including adequate facility and heating, ventilation, and air conditioning (HVAC) design with along with cleaning & disinfection efficacy studies covering a representative panel of microorganisms and viruses.

Furthermore, excipients are often novel and not yet of an adequate grade to support good manufacturing practice (GMP) manufacturing. Manufacturing comparability evidence must be supported by fit-for-purpose analytical methods and linked to clinical evidence. Labeling requirements and data privacy rules must be adhered to. Small batch sizes lead to challenges in applying existing guidance around visual inspection, reference/retention samples, and the representativeness of sterility samples. The short shelf life of fresh product leads to challenges in the batch release process, since not all critical data may be available at the time of batch disposition and dosing. Analytical test methods are often difficult to qualify and validate to meet the required parameters. Last but certainly not least, selection of the relevant potency assay is key for demonstrating the product's clinical benefit.

HOW TO TURN CHALLENGES INTO A PROACTIVE APPROACH?

The Lonza New Product Introduction and Lifecycle process ensures that these challenges can be managed in a most proactive manner. An early assessment of the potential risks for the manufacturing process, sterility assurance, the employed facilities and equipment, analytics, raw materials, and the supply chain is key for a successful technology transfer and fast-track commercialization. This also requires an efficient approach to the integration of CMC activities along the product lifecycle, and of all functions involved.

The need for early development of a fit-for-purpose potency assay that evaluates biological activity linked to the mechanism of action must not be underestimated. For open and manual process steps, early assessment of inherent risks is critical, including

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the development of mitigation actions, as this will support reliable delivery for operations in the clean room and improve sterile conditions of the product at hand. To address the variability of starting materials, the inclusion of studies to assess the potential impact of variability on the robustness of the process is important.

QUALITY SYSTEM

Both the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) accept that, as a product moves through the clinical development phases, the level of process and analytical control increases as knowledge accumulates. However, manufacturing standards that assure the safety of CGTs apply equally to products for first-in-man studies as they do to products for commercial supply.

There are unique key challenges for the quality system with CGTs. The short shelf life of some fresh autologous products may be only a few days. Thus, the data normally considered critical for disposition decision may not be available prior to infusion. A product-specific release strategy must be in place for swift actions in response to potentially positive sterility or mycoplasma test results.

The criteria for a successful tech transfer include a raw material and equipment list, a process description, established analytical methods, an in-process and final product sampling plan and acceptance criteria, manufacturing batch records and standard operating procedures (SOPs), risk log, number of tech transfer runs, and acceptance criteria and deliverables. Particular consideration must also be given to the biological starting materials in CGT. These may be of variable quality and thus, can impact the manufacturing process differently.

Lonza uses a structured technology transfer process to achieve clinical readiness, PPQ, inspection readiness, and commercialization. Again, it is important to note that, from an ethical and regulatory compliance standpoint, general GMP requirements apply throughout the whole product lifecycle.

FACILITIES & EQUIPMENT

One key challenge is the multiple aseptic operations required during manufacturing, given the lack of a terminal sterile filtration or sterilization process step along the product pathway due to the nature of the product. These open operations must take place in an ISO 5/Class 100 (in operation) or Grade A area. The minimum standard is that the background to aseptic operations must be ISO 7 in operation, or Grade B. It is important that smoke studies are performed during facility qualification and simulated aseptic operations and are repeated following any significant facility changes. Unidirectional personnel and material flow are preferable, with special care taken with waste versus product flow.

Environmental monitoring (EM) limits are defined in the EU GMPs for Advanced Therapy Medicinal products (ATMPs), EU GMP Annex 1 and FDA Aseptic Guide. Non-viable particle monitoring is required during aseptic operations. Monitoring should take place close to the point of fill to generate meaningful data on the likely risk of contamination of the aseptic operation. Viable environmental monitoring is required during aseptic operations. It is expected to use active quantitative air sampling and settle plates because they can be exposed for longer periods of up to 4 h, and are well-suited to capture transient contamination events during aseptic operations. In addition, personnel and surface monitoring are standard. The required disinfection frequency should be justified based on a regular assessment of the EM data and respectively based on the area classification. Disinfection efficacy studies are expected to cover the microbiological spectrum and as applicable, a significant and representative virus panel. Non-product contacting items present in Grade A areas are preferably sterilized or

else treated with a validated sporocide/virucide. For transfer of materials to grade B, the use of vaporized hydrogen peroxide (VHP) transfer hatches is recommended.

PRODUCTION

Production of clinical or commercial CGTs must adhere to the principles of GMP to ensure that the required quality attributes are within validated process ranges and meet specifications. The manufacturing process requires performance of operations and manipulations in an aseptic manner and thus, requires operators to be well trained in the performance of such aseptic manipulations. The maintenance of asepsis is critical as most cell therapy products are not able to be sterile filtered or otherwise treated to remove or inactivate microbial contaminants. Employees must be trained in all aspects of GMP requirements as per site specific training programs. It is key to have a robust quality awareness program in manufacturing supplemented by a quality 'on the floor' program to provide oversight over aseptic processing activities. An aseptic gowning qualification program should include classroom training on the theory of cleanroom gowning, followed by gowning demonstrations, monitored gowning and viable personal monitoring sampling at several pre-identified locations.

In CGT in general, it is required to assure aseptic conditions during product manufacturing end to end. In addition, special attention should also be given to critical upstream aseptic processing activities such as tissue dissection, cell washing, re-suspension, and media exchanges. Most current CGT manufacturing processes are associated with adherent cell lines, where cells grow when attached to a surface. In the event of a suspension culture, Lonza offers scalable 3D bioreactor platforms. As the cells in the cell culture vessel grow, the growth medium becomes depleted. Depleted growth media is removed from the culture vessel periodically and replaced with fresh media. Culture vessel preparation, cell inoculation, media removal, and media addition steps represent high-risk operations when performed in an open manner, where the cell culture vessel must be open to the environment to perform the transfer. Such operations must be performed in the ISO 5/Grade A/Class 100 area for maintenance of sterility.

Alternatively, these transfers may be performed in a closed manner via use of sterile tube welders, designed to join segments of sterile tubing together in a way that facilitates fluid transfer and maintains component sterility. However, closed system operations can be routinely performed in the ISO 8 environment (Grade C).

The execution of an aseptic process simulation (APS) or media fill for all products produced by aseptic processing, regardless of product phase, is required to evaluate the capability of aseptic processing activities, challenge the aseptic process for microbial contamination vulnerabilities, and demonstrate compliance with current GMP. The GMP requirement is for the repeat of media fills twice per year and for individual operators to participate in at least one media fill per year. Manufacture of CGTs often requires a high number of such manual aseptic manipulations throughout the production process.

Modular APS programs covering the maximum number of the same manipulation through operator APS and in combination with product specific process APS are possible and help to simplify the approach. In line with current regulations, APS periodic revalidation should be repeated twice a year for each aseptic process and for each filling line. The new Annex 1 version, which was published recently, includes more stringent APS requirements. Further discussions are ongoing with regard to the practical interpretation for CGT products.

A successful PPQ will confirm process design and demonstrate that the commercial manufacturing process performs as expected. The successful completion of a PPQ provides the evidence necessary to demonstrate

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that a manufacturing process is ready for commercial production using cGMP conditions. Batches combining facility, utilities, equipment, and trained personnel with the commercial manufacturing process, control procedures, and other components to produce commercial batches are required. For PPQ readiness, a particle control and visual inspection strategy, buffer hold time studies, process intermediate stability studies, consumable leachable/extractable studies, and container-closure integrity studies may be performed prior to or during the PPQ. The raw materials list, the critical process parameter list, and the in-process and final product release testing is finalized prior to PPQ start. The process failure mode effect analysis (FMEA) identifying critical process parameters, along with non-critical attributes of the process and criticality justification, is also finalized for PPQ start. Process characterization studies are usually performed before to understand the capability of the process, and to understand the impact of operating at the outer limits of the process specifications. To verify process consistency and product quality, there should be a scientific and risk-based rationale for the number of PPQ lots to be selected. Typically, a minimum of three PPQ lots run at commercial scale is required to demonstrate process consistency. Proper and fit for purpose analytical characterization methodologies must be in place for analyzing the PPQ batches.

LABORATORY CONTROLS

The product development stage and intended use of the test method should be taken into consideration when determining the level of test method qualification/validation required. Prior to the execution of validation studies, a test method must be sufficiently developed so that it is scientifically sound and capable of providing reliable results. Standard/compendial test methods do not require validation; however, they should be verified to ensure suitability under the actual conditions of use. For investigational products, test methods related to the safety of the clinical trial subject should be validated before first-in-human trials. Release testing of finished Cell & Gene Therapy products, for example, must include sterility, mycoplasma, endotoxin, identity, purity, potency, and dose testing.

Particular challenges may arise from potency testing. Even though the potency assay may align with the proposed mode of action, many other factors may impact the clinical outcome, such as patient factors, treatment factors, and the collection of the leukapheresis product.

MATERIALS

Apart from cells themselves, a range of ancillary complex biological materials are commonly required, including products derived from plasma, biological extracts, cytokines, growth factors, toxins, and feeder cells. Sourcing cells from humans presents transmissible disease risks to the patient, and the relative immaturity of the sector means that finding sources of these materials suitable for GMP manufacture can be challenging. Sterility of materials is sometimes not properly declared or is only tested by the manufacturer. Most CGT processes are dependent on single-use product contact materials that are purchased pre-sterilized. Compliance with sterility and endotoxin requirements must be ensured. These components have a major impact on the quality of the product, both in terms of sterility assurance and the potential for particulate contamination.

The selection and control of single-use consumable materials is essential. Where possible, the preference should be for the use of licensed medical devices. At a minimum, assessments which provide assurance over the safety of the consumable, such as sterility and compliance with endotoxin specifications, should be completed before first-in-human studies. Leachable and extractable studies as well as container closure integrity testing (CCIT) are typically required prior to PPQ. The sterility

of critical in-process containers is essential to assure the safety of the finished drug product and ultimately, patient safety. As part of the qualification of a new sterile material, confirmatory sterility and endotoxin testing of the final product container should take place. The impact from potential microbial contamination of cells, media, and additives used in CGT manufacture on patient safety can be significant, whether the material is present in the finished drug product or not.

The criticality of each starting material should be defined based on basic criteria relating to the likely impact of the material on the quality, safety, and efficacy of the finished drug product. This risk assignment can be refined during the product lifecycle. The manufacturing site and supplier for each critical material should be identified. Full traceability of the supply chain should be known to identify any potential entry routes for adulterants or contaminants. For starting material which are declared sterile the conditions of manufacturing and the sterilization method need to be known. The assessment of the material against documented selection criteria to assure suitability for use is a must. The preference should be for chemical-defined, non-human/animal sources of materials. Tests are required to characterize respectively assess the identity, purity, functionality, and safety of the material. Specifications for each starting material should be developed according to principles of quality risk management. Some complex materials may require functionality testing before a new lot of material is approved for use.

Material sampling and testing is key, as there is a GMP requirement to confirm the identity of each batch of a starting material that forms part of the drug product. As many excipients used are purchased as sterile ready-to-use preparations, the conventional prospective sampling and testing of containers of excipients is likely to introduce an unacceptable risk to sterility. Therefore, an acceptable approach can be the retrospective testing of the residual of sterile material following its use in the process. For materials that do not form part of the finished drug product (non-excipients), such as media and buffers not present in the final formulation, the requirements of ICH Q7 are followed as these materials are analogous to those used in the manufacture of an active pharmaceutical ingredient. However, evidence of manufacturing under aseptic conditions must be proven. The requirements for testing of raw materials depend on the material criticality and stage of usage in the product lifecycle. An assessment of the criticality of each raw material must be in place as early as possible in the product lifecycle. A critical material is defined as having a direct impact on the quality, safety, or efficacy of product. For excipients, full specification testing should take place.

EU regulation 1394/2007 lists some specific requirements for CGT for supply to EU that must appear on the packaging. CGTs are classified in the USA as biologics; therefore, 21 CFR 610 Subpart G outlines the requirements for container and package labelling. Specific requirements exist in US and EU GMP for the control of packaging operations, which apply fully to ATMP manufacture. For the manufacture of autologous products in particular, strict controls over label generation, issuance, and packaging are essential. Data privacy versus patient traceability is also to be considered: EU and US regulations detail that all materials must be traceable through sourcing, manufacturing, packaging, storage, transport, and delivery.

SUMMARY

The Lonza New Product Introduction Process and Lifecycle process is applied throughout the product lifecycle from development to commercialization including technology transfer, pilot and engineering runs, clinical production, and PPQ lots **(see Figure 1)**. It allows for pre-approval or pre-license readiness and finally, commercialization of the product. Through this process, shortened timelines and a faster turnaround to manufacture and delivery to patients is possible. De-risked product introduction is achieved via robust fit assessment

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to ensure alignment to Lonza Quality requirements, which helps to ensure compliance and avoid delays and rework.

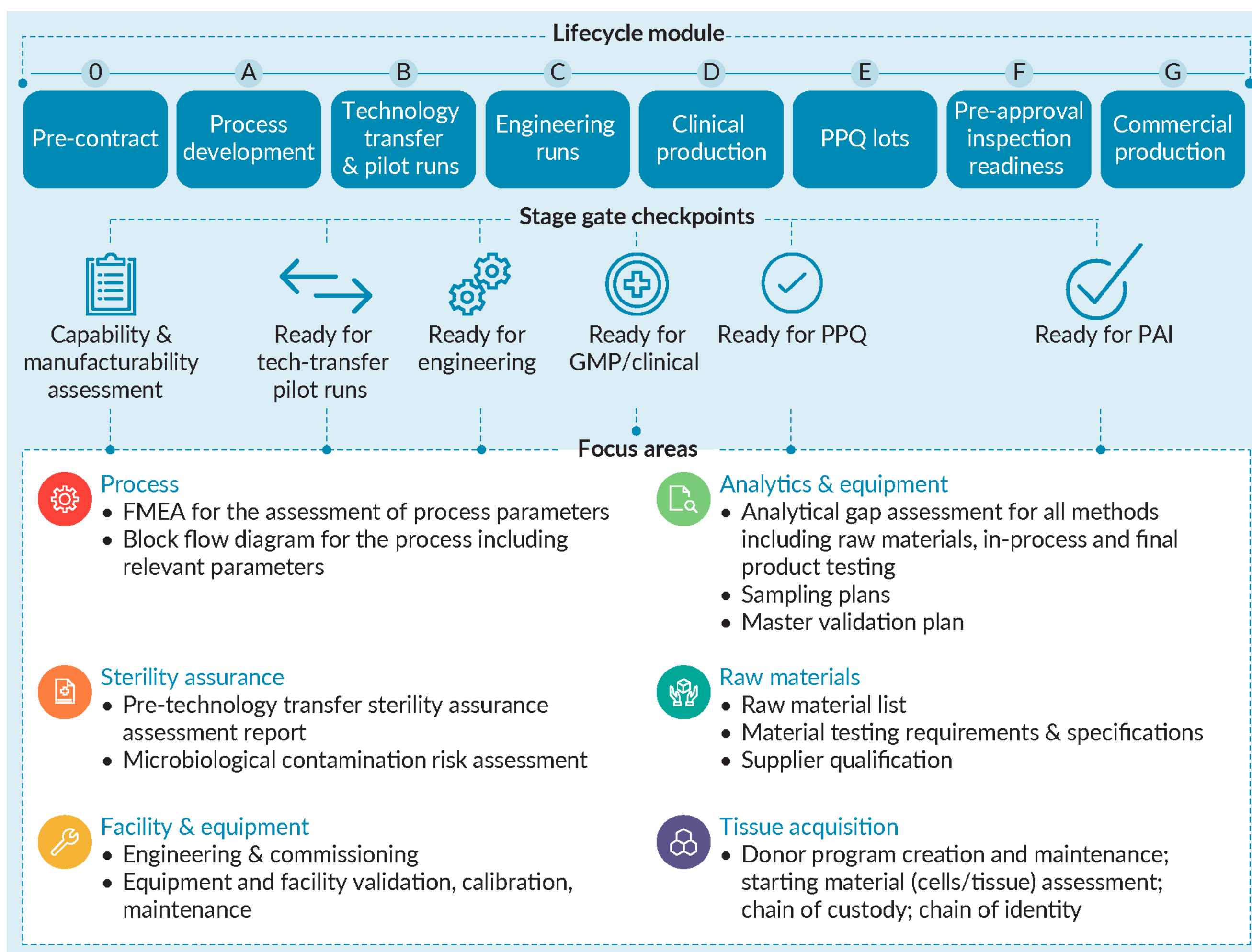
One key enabler is the standardization across all relevant areas, including tissue acquisition, donor program creation and maintenance, starting material assessment, and chain of custody. Also required is an FMEA for the assessment of process parameters, raw material testing, and specifications including supplier qualification, proper sterility assurance assessment reporting and microbiological contamination risk assessments, and a focus on equipment and facility validation, calibration, and maintenance. Analytical gap assessments for all methods are needed including raw materials, in-process and final product testing, sampling plans, and a master validation plan.

Turning a properly defined process into manufacturing and filing success is possible via clearly defined deliverables at each stage gate, with adequate checklists and standardized technology transfer across all sites and customers globally.

The Lonza approach assures that quality is part of the business strategy which will allow for reducing variance and variability throughout the product lifecycle and will ensure a successful commercialization of the product.

FIGURE 1

The Lonza New Product Introduction Process.



GMP: Good manufacturing practice; FMEA: Failure mode and effects analysis; PAI: Pre-Approval Inspection; PPQ: Process performance qualification.

Q&A

David McCall, Editor, BioInsights speaks to
Christoph Meyer, Global Head of Quality Control, Lonza Cell & Gene
and
Andreas Wirth, VP, Global Head of Quality, Lonza Cell & Gene (pictured left to right)

Q Are the EM limits given in Annex 1 Manufacture of Sterile Medicinal Products and the EU GMP, Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products, fully aligned and harmonized? Are the EM limits as given in EU GMP for ATMPs specifically the same as those given in Annex 1?

CM: We reviewed the revised version of Annex 1 and European Union good manufacturing practice (EU GMP). The EU GMP for Advanced Therapy Medicinal products (ATMP) is a hybrid between the currently enforced Annex 1 and the revised Annex 1. In terms of viable contamination, all these documents are largely the same – however, there is a slight difference. Annex 1 includes the maximum permitted total particle concentration for classification for ≥5 µm/m^3. For routine monitoring the EU GMP ATMP guidance includes the definition for ≥0.5 µ/m^3 and ≥ 5 µm/m^3, which is aligned with the revised Annex 1.

Q To what extent are aseptic process simulations or media fills required in the production of investigational medicinal products for clinical trials?

AW: Aseptic process simulations must be applied throughout, from initial clinical trials onwards. Regardless of the phase, these programs need to be in place and maintained. As process knowledge grows, this would have to be included in subsequent programs, but the initial requirements for first-in-human trials are clearly spelled out.

Q In the case of starting materials, can a manufacturer rely on the results of mycoplasma tests performed by the material vendors, or should they conduct this testing themselves?

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CM: This is dependent on a few things. Firstly, if you obtain a test result, it must be from a properly qualified test laboratory and the method must be properly validated. Secondly, when dealing with third parties, all these things need to be properly manifested in a quality agreement. And thirdly, if you accept the vendor's mycoplasma test result, then you need to ensure a proper chain of custody to reduce risk.

Q Can you provide examples of 'phase appropriate' GMP?

AW: This is a broad question, but I will focus on a few aspects where 'phase appropriateness' is valid, and in our experience, also accepted by regulators.

In the beginning, as there is not much history of a product yet, for a visual inspection test kit, a generic approach can be applied. This generic approach should at least be specific to the container type, provided there is a good justification of coverage of turbidity of the various products in scope, and contingent to the transparency of the container. We have good experience with this setup. Then, over time, test kits can be changed as more knowledge is gained – specifically, before process performance qualification (PPQ) and commercialization.

In general, some inert materials should be used to mimic the product in a proper way. We have a service at Lonza to provide such test kits – then, the factors of test kit qualification and operator training must be considered. Another example is smoke studies, which have also been discussed above for CGT applications. Our approach is to initially cover the basic interventions with the knowledge available at an early stage, and ensure there is a matrix or a bracketing approach. Prior to PPQ, a detailed smoke study should be in place with the appropriate justifications for all the interventions involved in a phase appropriate and safety-focused manner.

Looking at analytical methods, it is clear that robustness studies are required at least prior to PPQ. In our experience, robustness studies, including elevation studies and then later validation, should be started much earlier because this can become a roadblock in PPQ and filing. In terms of phase appropriateness, it is clear that human safety must always be ensured first.

Q What measures are put in place to avoid cross-contamination risks during parallel processing?

AW: Measures include concepts and procedures to ensure correct material handling and personnel flow. Other important factors include the adequacy and size of the room to allow proper separation between different runs of the same product. In-process labeling requirements, including the associated reconciliation, are key. Another key area is around material identification prior to the addition to the product pathway for the specific run, and the assignment of the batch-specific traceability. In other words, ensure that the materials have been identified and that they belong to the respective batch they are assigned to. Having

electronic batch records linked to a materials management system could work, although simple technical solutions are also possible to ensure traceability and batch-specific consumption.

Q Should all new sterile materials undergo confirmatory testing?

CM: Patient safety is at the center of everything we do. The risk of contamination in GMP materials is significant, so must be avoided. An important thing you must consider is that cell-containing products cannot be sterilized at the end of the manufacturing process. The sterility of the final product container and of all other critical in-process containers is essential, so for the qualification of a new sterile material will require confirmatory sterility testing.

Q Is there justification for bypassing sterility testing to shorten vein-to-vein time for autologous products? Is there an appetite from health authorities to adopt such an approach?

CM: Process durations have been shortened, so there may be newly established processes that enable fast-track processing. The testing is a time-critical step, so you do need to be sure the testing is not sacrificing all the advantages you get from a fast-track production process.

If you produce fresh products or cells, due to short shelf life, you must reinject the product to the patient as quickly as possible. There are now processes that allow for fast track infusion of the product into the patient by making use of rapid sterility testing, but even fast-track sterility testing may not be fast enough with respect to the shelf life of the product.. The EU GMP guidelines on ATMPs specifically mention the option of infusion before all tests results are available due to medical need ('may not be possible to wait for the final result of the test before the product is released due to short shelf-life or medical need').

Q What are the main challenges regarding change management at the process verification stage?

AW: Due to the short chemistry, manufacturing and controls timelines that come with these types of products, you need to think about the challenges that could arise. There is not necessarily sufficient experience in the field in this regard as yet. Through continuous process verification, some changes may be required. The subsequent filing and reporting category would have to be agreed with authorities and based on company judgment.

Another aspect that is common is the variability and availability of critical starting materials that may not be in continuous supply. A backup supplier should be established. You need to know the extent to which a material is characterized in terms of the impact on quality of the product itself.

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FURTHER READING

1. 9 CFR 113.53 Requirements for ingredients of animal origin used for production of biologics.
2. 21 CFR 11 Electronic Records, Electronic Signatures.
3. 21 CFR 211.170 Reserve samples.
4. 21 CFR 600.13 Retention samples
5. 21 CFR Part 610 General Biological Products Standards.
6. 21 CFR 1271.145 Prevention of the introduction, transmission, or spread of communicable diseases.
7. 21 CFR 211.84 Testing and approval or rejection of components, drug product containers, and closures.
8. 21 CFR 211.130 Packaging and labeling operations
9. 21 CFR 211.165 Testing and release for distribution.
10. EMA. Reflection Paper on classification of advanced therapy medicinal products. May 2015.
11. EP 5.1 Sterility.
12. EP 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products.
13. EP 2.6.7 Mycoplasmas.
14. EU GMP Annex 1 Manufacture of Sterile Medicinal Products.
15. EU GMP Annex 11 Computerised Systems.
16. EU Regulation 1394/2007
17. EU GMPs for ATMPs.
18. FDA Guidance: Homologous use of Human Cells, Tissues and Cellular Tissue-based Products. October 2015.
19. FDA Guidance for Industry: CGMP for Phase 1 Investigational Drugs
20. FDA Good Tissue Practice Guidance, December 2011.
21. FDA. Guidance for Industry Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice
22. FDA Guidance for Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs); Guidance for Industry).
23. Federal Register / Vol 58, No 197.
24. Guidance for Industry, INDs for Phase 2 and Phase 3, Studies Chemistry, Manufacturing, and Controls.
25. FDA. Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice.
26. ICHQ5A Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.
27. ICH Q5D Derivation and characterisation of cell substrates used for production of biotechnological/biological products.
28. ICH Q7 Good manufacturing practice for active pharmaceutical ingredients.
29. ICH Q10 Pharmaceutical Quality System.
30. USP <1> Injections and Implanted Drug Products (Parenterals) – Product Quality Tests.
31. USP <63> Mycoplasmas.
32. USP <790> Visible Particulates in Injections.
33. USP <1043> Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
34. USP <1046> Cell and Gene Therapy Products.
35. USP <1079> Good Storage and Shipping Practice.
36. USP <1116> Microbiological Control and Monitoring of Aseptic Processing Environments.
37. USP <1211> Sterilization and Sterility Assurance of Compendial Articles.
38. USP <1790> Visual Inspection of Injections.
39. PICS PI007-6 Validation of Aseptic Processing.
40. WHO Technical Report Series, No. 961, 2011 Annex 9 "Model guidance for the storage and transport of time- and temperature–sensitive pharmaceutical products.

BIOGRAPHIES

CHRISTOPH MEYER has worked at Lonza since 2021 as Global Head of Quality Control Cell and Gene Technologies. Prior to this, he was at Novartis for 16 years and occupied several leadership roles in the fields of QC, Development, Quality, and Operations. Christoph was awarded a PhD in Analytical Chemistry from University of Tübingen (Germany).

ANDREAS WIRTH is Lonza's Global Head Quality of Cell and Gene Technologies, Personalized Medicine. He has 30 years of experience in the pharmaceutical industry across different functions such as Manufacturing, Quality, Clinical Development and Regulatory Authority.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

INTERVIEW

Exploring the potential of insect cell lines for efficient AAV production

Yusuke Tomioka, Senior Engineer Upstream Biomanufacturing, Manufacturing Sciences and Technology, Merck Ltd. Japan, speaks to **Tsuyoshi Teshima,** Director and General Manager, and **Mai Sasaki,** Researcher, Research & Development Department, Gene Therapy Research Institution Co., Ltd.

TSUYOSHI TESHIMA is the Director and General Manager of R&D at Gene Therapy Research Institution Co., Ltd. (GTRI), and is in charge of manufacturing process development, non-clinical studies, and regulatory affairs of AAV vectors. After earning a Master's degree in pharmacy from Tokyo University of Science, he worked at Japan Tobacco, Banyu Pharmaceutical, UMN Pharma, and IHI in R&D of small molecule drugs, vaccine marketing, clinical development, business development, and corporate planning. Before joining GTRI, he was active as the president of UNIGEN, a Biopharmaceutical CDMO.

MAI SASAKI is a researcher in the Research and Development Department at Gene Therapy Research Institution Co., Ltd. (GTRI), and is in charge of the development of AAV vectors using suspended insect cells. After earning a master's degree in Life Sciences from Hokkaido University, she worked at WDB Eureka Co., Ltd. and engaged in basic research on middle molecule drug discovery.

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Q Considering the risk of contamination of processes, such as by adventitious agents, what safety measures does your company take regarding adeno associated virus (AAV) production? How important is the quality and safety of raw materials, including cells, to your company's pharmaceutical manufacturing?

TT & MS: Unless it's impossible, we try to incorporate fully closed systems. In addition, adopting single-use materials such as bags, assemblies, sensors, etc., reduces the risk of process contamination. The quality and safety of raw materials are extremely important because they are linked to the quality and safety of the product itself.

It is essential to be able to obtain the information for providing sufficient explanations when applying to health authorities such as Japan's Pharmaceuticals and Medical Devices Agency (PMDA) and US Food and Drug Administration. Regarding requests to raw material suppliers, it would be helpful if they could respond flexibly concerning information provision that is difficult to disclose, such as by registering it in the master file (MF).

In addition, of course, consistency and stability of raw materials are also important for stable in-house manufacturing.

Q Have you encountered any regulatory concerns that have arisen from cell lines for AAV production?

TT & MS: There is concern about rhabdovirus when using Sf9 cells. Since this may be the first time Sf-RVN® will be used for commercial production, we are currently receiving questions from PMDA about the method for generating Sf-RVN® cells and the test method for the absence of rhabdovirus.

We are in the stage of incorporating information on rhabdovirus test methods, results, and cell line history conducted by Glycobac, for the consultation document with PMDA. There have been problems with animal cell lines that use serum for culture due to suspected contamination by serum-derived viruses. Chemically defined cell culture media supplied by Merck can definitely help solve these points.

Q What are the main reasons for your company to select insect cells for AAV production?

"The high cost of plasmids and transfection reagents as a percentage of the total cost is a major challenge for production using HEK cells, but insect cells can greatly reduce this cost, which is an advantage."

TT & MS: The high cost of plasmids and transfection reagents as a percentage of the total cost is a major challenge for production using HEK cells, but insect cells can greatly reduce this cost, which is an advantage.

It has also been confirmed that insect cells produce higher AAV yields and full capsid ratio for many pipelines. We believe that AAV production using insect cells is advantageous from an economic perspective.

Q Why did your company select Sf-RVN®? Is safety against contamination one of the reasons for choosing Sf-RVN®?

TT & MS: It was possible to allay in advance any concerns about rhabdovirus, which requires a clearance test, and it was selected because it is expected to greatly shorten the development time.

Currently, cells with associated bioethical concerns are still widely used in the drug development field. On the other hand, Sf-RVN® is derived from insects, so we believe that such concerns will be unlikely in the future.

Q What are the main advantages of having a good manufacturing practice (GMP) cell bank and rhabdovirus-negative cell lines that have been tested for adventitious agents?

TT & MS: If GMP cell banks and adventitious agent tests have been completed, it is possible to reduce characterization tests when master cell banks and working cell banks are created in-house, and even considering the cost of annual licenses, we believe that it is worth it for the advantage of making the development period shorter.

We have multiple pipelines, but many of them target rare diseases for which there are currently no fundamental treatments, so we need to develop products quickly and deliver them to patients. So the shortening of the development time is very important.

Q Why did your company select EX-CELL® CD Insect Cell Medium?

TT & MS: The medium shows good performance in both low and high cell density conditions, and compared to other media on the market, the yields and ratio of full capsid of produced AAV in various serotypes are equal to or better than those when using the Sf9 platform. Therefore, we selected this platform based on a comprehensive judgment.

Q Have you compared the actual culture performance of both Sf9 and Sf-RVN® as applied to the Sf-RVN® platform?

TT & MS: We have made the comparisons.

There were no significant differences in doubling time and peak cell density. The cell diameters were larger with Sf-RVN®, and we observed a bit of a difference between Sf-RVN® and Sf9 in the cell expansion and polyhedron formation following baculovirus infection.

Although it depends on the gene of interest and serotype, we believe that it is necessary to evaluate the stability of baculovirus while confirming the differences from Sf9.

Q Are you satisfied with using the Sf-RVN® platform for AAV manufacturing?

TT & MS: We are satisfied with the concept, but there are some points for operation that should be evaluated. Currently, Sf-RVN® is only one rhabdovirus-negative insect strain that is commercially available, but it would be a 'nice to have' if the cell line had variations such as different clones and different origins, which would expand the scope of drug development. In particular, we would like to be able to select the best cell lines with high productivity for each AAV serotype and each baculovirus.

Q How do you feel about the regulatory support provided by Merck?

TT & MS: Our company has not yet reached the point where products using Sf-RVN® have been administered to humans, but we plan to submit a clinical trial application this year in 2023, and the first administration is scheduled for 2024.

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Preparations for regulatory matters are still to come, but we strongly expect Merck to provide sufficient support.

Q What was your impression from your evaluation of the Mobius® Bioreactor? From the perspective of commercial manufacture, what aspects of the system do you see as an advantage?

TT & MS: We are very happy that the system is very easy to operate and intuitively understandable. Since the HMI for parameters setting and operation are easy to understand, we felt that making a technical transfer to another facility would be easy, if we contracted with a contract development and manufacturing organizations (CDMO) company.

We plan to submit a clinical trial application this year in 2023 and the first administration of a product made with Sf-RVN® cells is scheduled for 2024.

We are confident in the support that Merck will provide for regulatory matters based on the documentation and discussions with their team so far.

We expect that the introduction of new in-line monitoring technologies such as Raman spectroscopy will enable non-invasive and robust process monitoring at low cost.

Batch-to-batch differences are likely to occur because cells are living organisms, but it is thought that by having a lot of data at the time of production culture, it would be possible to quickly identify the cause of batch differences and the cause when something happens, and then to make acceptance/rejection decisions at an early stage during manufacturing.

Q Have you faced any challenges in scaling up your AAV production upstream process? What were some of the most important critical quality attributes (CQAs) in your experience and how did you manage them?

TT & MS: Scale-up studies are currently being conducted with a 3 L bioreactor and a 50 L bioreactor. Ideally, each cultivation should be able to be cultured multiple times in a smaller-scale evaluation with three or more different vessel sizes. Regarding critical quality attributes (CQAs), in the upstream process we focus on the yields and full capsid rates.

Cell conditions and the above CQAs can differ significantly between culturing in an Erlenmeyer flask and culturing in a reactor, so we would like to use a reactor as much as possible in the early process development. However, there are many challenges as it is difficult to arrange the required number of control devices due to lab space and costs, and it is difficult to prepare a huge volume of high-cost raw materials such as the baculovirus.

> "In both the main culture process for AAV production and the process for producing baculovirus for infection, we believe that one of the causes of lot-to-lot variability is the method for cell lines control strategy during culturing."

The available minimum reactor size for a scale-up study is larger than our expectation, and the significant expenditures of time, effort, and money per study are bottlenecks in the early-stage evaluation. Regarding small-scale studies, we feel that the currently available reactors suffer from limitations when applied to AAV manufacturing process development.

Q What were the results in terms of scalability when using the Sf-RVN® culture in the Mobius® bioreactor? And what are your impressions of the scalability and safety support for AAV production from Merck? How does Merck's total solution and technical/regulatory support contribute to the speeding up and robustness of AAV production using the Sf-RVN® platform?

TT & MS: We have observed that the scalability of both Sf-RVN® cell culture and AAV production in the 50 L Mobius® bioreactor has been fully verified. Since Merck could bring the actual system to our company for a demonstration, and we could visit Merck's M Lab™ and see the actual system, it is easy to imagine the installation in a manufacturing room and have an idea how it could work, and we are very grateful for this.

We greatly appreciate the company's deep knowledge and experience concerning scalability and the technical support offered during webinars and demonstrations.

Regarding regulatory support, which will come into play down the road, we look forward to Merck's support when the time comes.

Q From the actual commercial manufacturing perspective, what do you think requires further study in terms of quality control of drug substances?

TT & MS: In both the main culture process for AAV production and the process for producing baculovirus for infection, we believe that one of the causes of lot-to-lot variability is the method for cell lines control strategy during culturing.

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Currently, we are only able to confirm the conditions before and after culturing, but we would like to be able to measure and manage cell conditions, metabolic by-products, titers, etc., in real-time.

In the manufacturing of baculovirus for infection, the titer increases until a certain period of time but then decreases rapidly after a certain period of time, so we intend to create a process in which it is possible to carry out the harvest at the time when the titer is highest. We currently do not have a sufficient observation of the process parameters that affect this instability yet, but we expect to be able to do so as monitoring technology improves. We believe that even when looking at the industry as a whole, the understanding of critical process parameters (CPPs) in AAV manufacturing is insufficient, and we would like to consider this challenge using the Sf-RVN® platform in the future.

Q When you consider adaptation to future process improvements and potential regulatory compliance, what new process optimization technologies would you like to expect from your suppliers of cell culture media and cell lines?

TT & MS: When we think about improving the speed of development, we are wondering if a simpler and less hands-on process is required. At present, we believe that if some supplements which are added during the process, such as cholesterol, have been configured as one cell culture medium product in advance, and if satisfactory shelf life and stability are pre-evaluated, it will support faster process start-up. In addition, we believe that systems that can measure multiple parameters, such as Raman spectroscopy, are very powerful for evaluation of the impact on CQAs in process development. We feel that it is very beneficial to have a package or service that has already been demonstrated for the measurement of these CPPs.

Q What new upstream process technologies for AAV production do you expect to see when considering future process improvements and next-generation technology adaptations?

TT & MS: In our current AAV production process lines, it is necessary to proceed with multiple culture lines at the same time, such as the baculovirus production operation and the main process operation. In addition, at our company, multiple drug pipelines are manufactured at different times in the same manufacturing facility, so it takes time to fumigate the facility, clean the lines, and complete the changeover.

One of our Sf-RVN® AAV formulations involves the preparation of four types of baculoviruses, then the production of three types of AAV using those baculoviruses, and then

the three completed AAVs are mixed into one product, all of which require a total of seven manufacturing cycles.

We would welcome Merck's proposals for novel facility designs that could reduce risk of cross-contamination and time expenditure in the future. In addition, we expect perfusion technology, continuous manufacturing, and the development of cells that stably express specific genes, etc.

Our goal is to lower the financial and time expenditures on the way to market, and to reach a future where products can be delivered to patients quickly and at low cost.

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AUTHORSHIP & CONFLICT OF INTEREST

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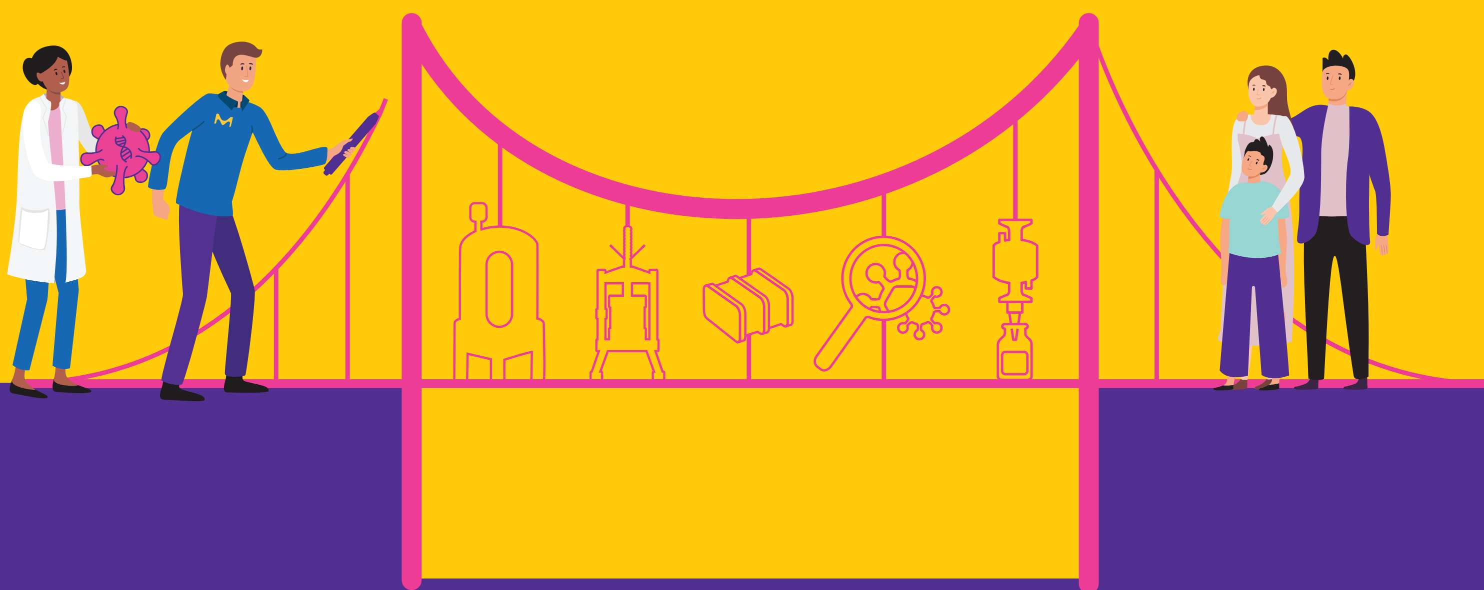

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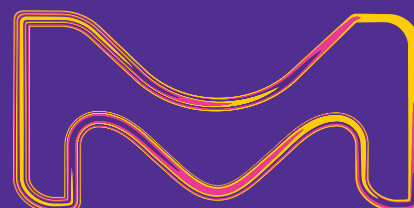

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CELL & GENE THERAPY INSIGHTS

CORRIGENDUM

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Corrigendum to: thinking ahead: developing biosynthetic blood to anticipate donor drought

Andrew S Goldman, Shane Kilpatrick, Marinna Madrid, Zhong Ri Lim, Steve Oh, Taylor Rose, Lena Patel & Barbara A Nelsen

This corrigendum contains corrections the article 'Thinking ahead: developing biosynthetic blood to anticipate donor drought' *Cell & Gene Therapy Insights* 2022; 8(11), 1561–1570; DOI: 10.18609/cgti.2022.227. In the version of this article initially published, it was stated that clinical trials for Hemarina M101 had been suspended. In fact, at the time of publication, Hemarina's HEMO2life® had just received CE approval for the preservation of kidney grafts for transplantation. The correction is listed in full below, and the amended article can be accessed here.

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In the version of this article initially published, it was incorrectly stated that trials for Hemarina M101 had been suspended. The paragraph with the corrected information is below:

Unfortunately, further development has been stifled by insufficient funding. Federal agencies have focused funding for decades on artificial (synthetic) blood substitutes based on purified forms of hemoglobin to provide oxygen carrying capacity in acute blood loss situations [23]. Despite some advances, no synthetic blood substitutes are approved for use in the US or Europe. Significant safety issues and high mortality [24], including myocardial infarction and death, have been associated with the use of these artificial blood products [24]. Some synthetic products such as Erythromer [25] are still proceeding through early-stage development. In a first for the category of oxygen carriers Hemarina's HEMO2life® just received CE approval for

preservation of kidney grafts for transplantation [26]. And while a retrospective study of ten patients showed one artificial blood product to provide an effective oxygen bridge for patients unable to be transfused with RBC [27], other case reports illustrate the significant challenges with use of artificial blood [28].

Furthermore, reference 26, has been corrected to:

26. Hemarina. The European Union recognizes the first universal oxygen carrier. Press release. Sep 30 2022 https://www.hemarina.com/the-european-union-recognizes-the-first-universal-oxygen-carrier/?lang=en.

DOI: 10.18609/cgti.2023.007