Volume 5, Issue 12

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:

2020 vision

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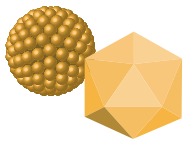
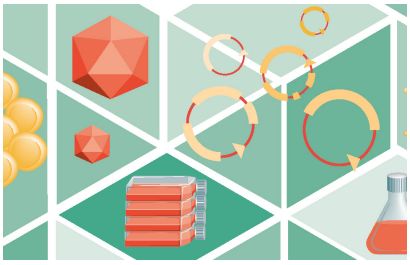


IN FOCUS:
Highlights from our Vector Channel



Assays and Titering

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2020 VISION
TOOLS AND TECHNOLOGIES OF TOMORROW

SPOTLIGHT

COMMENTARY

Vision 2020: what could come about in the next decade for biotech?

Michael Yee

Cell & Gene Therapy Insights 2019; 5(12), 1473–1481

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More than 2,000 years ago, around 400 BC, Hippocrates revolutionized medicine by describing diseases for the first time in history. In fact, he is credited as the first to believe disease was caused naturally, not by the Gods. Beyond characterizing diseases, he is also credited with establishing the earliest forms of diagnosing diseases, including acute and chronic conditions. While he is often considered the Father of Medicine due to his contributions to the field, the Hippocratic Corpus was a collection of medical works from ancient Greece.

Through another long 1,000 years man practiced various forms of plant, herbal, and other cultural medicines to attempt to treat and cure disease – mostly through trial and error.

In 1798, around another 1,000 years later, Edward Jenner used a vaccine against smallpox. Yet, it was not for another 150 years that Jonas Salk, in 1955, discovered and developed the polio vaccine, saving millions of lives against one of the most frightening public health epidemics.

While DNA was first isolated in 1869, it took 70 years for Watson and Crick to describe the double-helix molecular structure. In fact, it was on February 28, 1953 that Crick entered a noisy pub and declared to all "We have discovered the secret of life."

What he didn't realize was that while it took 1,000 years to understand and describe various diseases and another 500-700 years just to understand and develop vaccines against global epidemics, the rate of achievement and potentially the cure of many diseases would exponentially improve in the next century. It was only 60 years ago that we first understood the molecular structure of DNA. 20 years ago, we sequenced the whole human genome. Within one decade this led to a breakthrough in understanding genetic function and underlying cause for many diseases. In fact, we now know that approximately half of all genetic diseases are caused by only one nucleotide base pair error.

The recent era of innovation is moving quickly. While it took scientists centuries to understand the basics of disease and to develop vaccines and medicines to 'treat' them, it now only takes decades or less to

have major medical breakthroughs. Based on the recent breakthroughs in science–along with the efforts of the pharmaceutical industry, the critical capital from investors, and important support and oversight of the FDA–in the last 5 years alone we have gone from 'treating' diseases with toxic chemotherapy or merely adding a few months of survival to a colorectal cancer patient (Avastin), to giving CAR-T therapy and one's own engineered T cells as a treatment to wipe out DLBCL in 35% of patients. As of a few years ago, we have immunotherapy to induce metastatic melanoma patients to achieve durable remission. Now, there are over 50 different CAR-T programs in development.

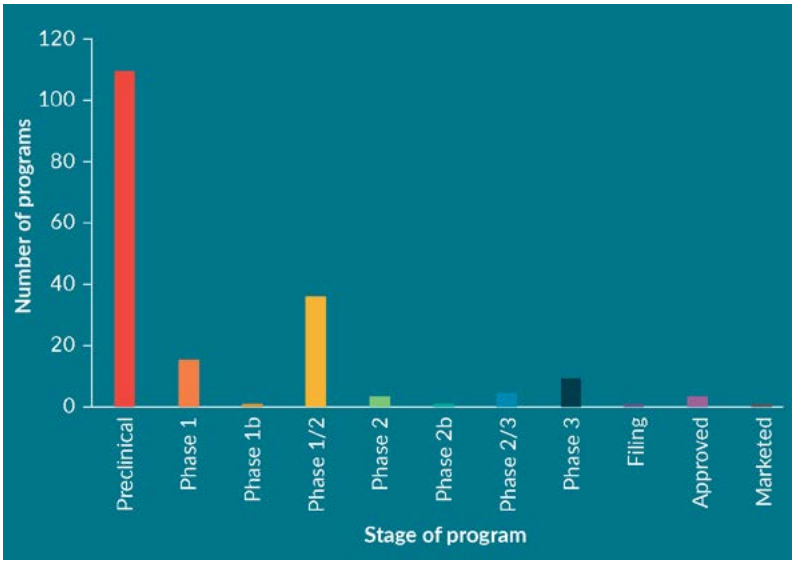
And now we have the first gene therapy approved drugs that target the genetic underlying cause of disease. These include the first FDA-approved one-time administration gene therapy drug to cure a rare blindness (Luxturna) and a gene therapy to treat babies with a fatal spinal muscular atrophy (Zolgensma). Previously, these were both diseases with no approved treatment. Now, over 100 other gene therapy drugs are in the industry pipeline (Figure 1).

Given our vision of where we see the scientific breakthroughs occurring, the FDA's willingness and motivation, and importantly, the record levels of VC and investor capital in 2018 and 2019 being deployed to fund all these projects (Figures 2 & 3), we foresee a new pace of innovation in the next 10 years, particularly in the way drugs could cure diseases. The science is moving quickly, the FDA is approving these breakthroughs, and the capital continues to come in. Over the next 10 years, this will be exciting news for doctors, scientists, investors, and most importantly, patients. We believe this rapid pace of innovation is just beginning.

Three areas of medicine we predict will see tremendous innovation for patients in the next 10 years:

FIGURE 1

Over 180 gene therapy programs in development.



Source: Jefferies research (sourced each individual company's websites).

GENE THERAPY & GENE EDITING

In 10 years (a very short period of time of development based on the historical timelines of medicine previously described) we could be curing and treating 100 more diseases that have a genetic root cause with a one-time administration of gene therapy to the patient. As evidence that the regulatory framework is evolved to help accelerate these critical breakthrough innovations, former FDA Commissioner Scott Gottlieb recently said "I believe gene therapy will become a mainstay in treating, and maybe curing, many of our most

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FIGURE 2

Biotech fund size by year.

Source: PitchBook Data.

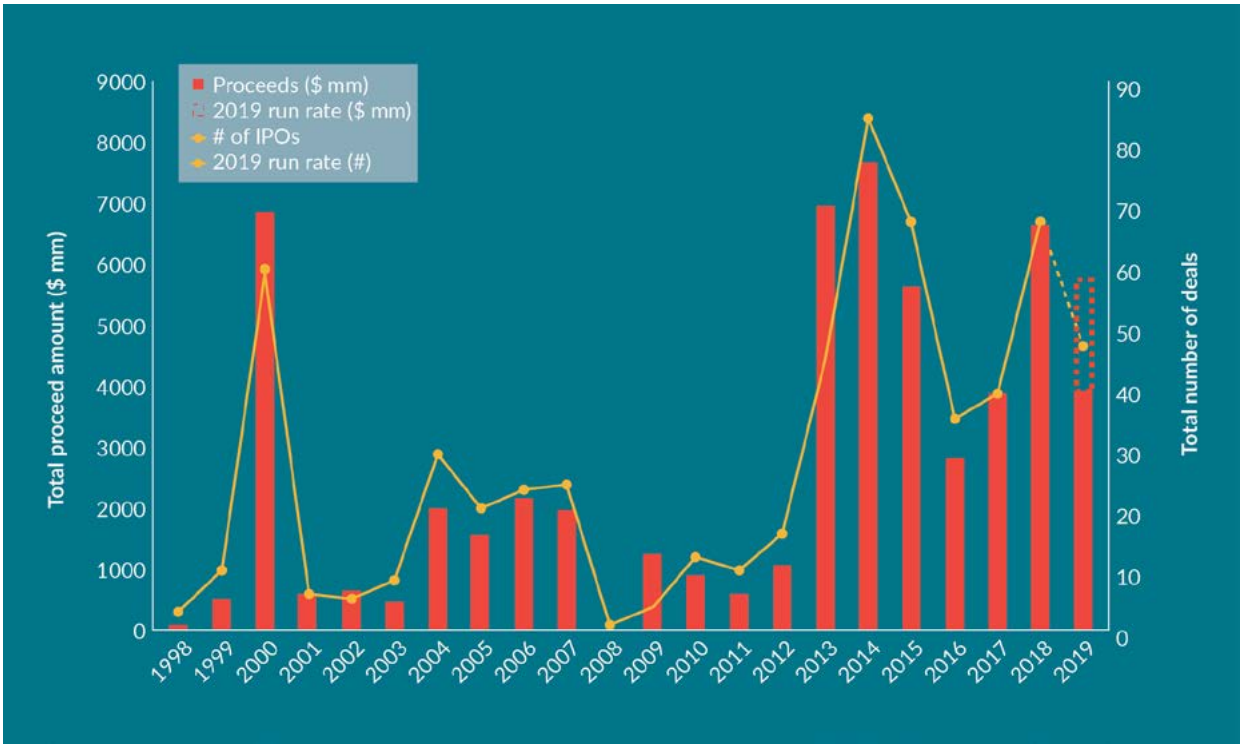
devastating and intractable illnesses…we're at a turning point when it comes to this novel form of therapy and at the FDA, we're focused on establishing the right policy framework to capitalize on this scientific opening…"

We believe the primary use of gene therapy will be to address and target thousands of (often hereditary or genetic) diseases based on a dysfunctional or missing protein. Over the next couple of years, more gene therapy drugs in late stage development have the potential to be approved, including ones for hemophilia and DMD. Importantly, over the next 10 years many more are likely to be approved as there are more than 100 programs in development for rare genetic diseases and other diseases.

Further, the development of gene editing drugs, CRISPR/Cas9-based gene and single-base pair therapies, could take this concept to another level of breakthrough.While earlier in development (the candidates are just now entering the first human studies and have only presented preliminary results versus gene therapy drugs already approved) gene editing could be more powerful because the CRISPR/Cas9 'editing' construct could 'cure' a patient by just editing the existing genes (Figure 4), opposed to delivering the corrected gene sequence that needs to be transcribed and translated in a robust manner to produce enough protein.

FIGURE 3

Total Biotech IPO proceeds by year.



Source: Jefferies research (FactSet, Bloomberg, Company reports, Nasdaq: https://www.nasdaq.com/market-activity/ipos).

At least three companies are working with CRISPR Cas9 and are in or near the clinic. This technology has the ability to potentially: (1) disrupt a mutated (disease causing) gene, (2) edit the mutated gene to be replaced with a correct copy into the genome. In addition, with 'single base pair' editing (which has broad applicability as almost half of all genetically defined diseases are caused by only one base pair error) a deaminase protein combined with a CRISPR construct can change a single base pair to introduce a stop codon, correct the erroneous nucleotide to the correct one, with possibly greater accuracy and precision. This could potentially address an even wider field of diseases than gene therapy and/or CRISPR/Cas9 editing. These types of drugs are around two years from the clinic and hence could be approved in the coming decade.

ALLOGENEIC CELL THERAPY

In 10 years, we predict there will be treatments for non-genetic diseases using off-the-shelf engineered cells as a therapeutic approach for patients. The idea to deliver 'healthy cells' such as tissues or organs isn't necessarily a novel approach, but the ability to do it in an allogeneic and robust wide-scale approach has always been a limitation to the idea. Currently, numerous companies have been able to address two primary historical issues: GMP manufacturing of these cells and immunogenicity. For the most part, this

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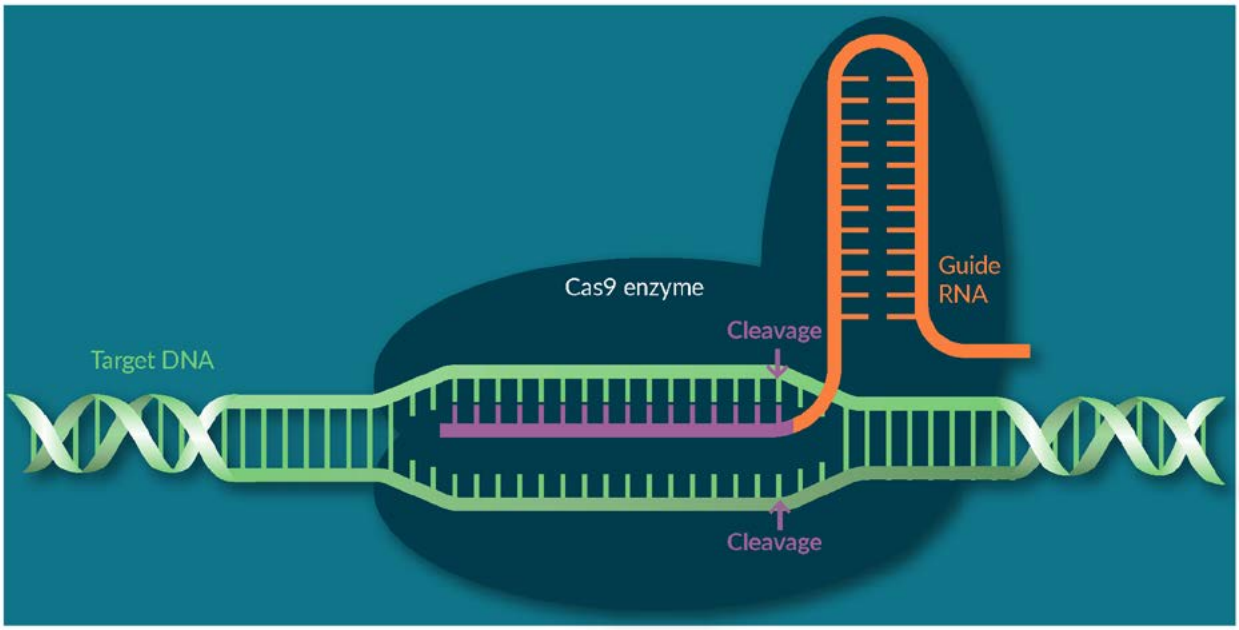
FIGURE 4

Mechanism of CRISPR Cas9 gene editing.



stems from processes that have been refined on a robust GMP scale to selectively differentiate $CD34^+$ stem cells from a donor or use induced pluripotent stem cells (iPSC) to make a specific cell of interest (neuron, cardiomyocyte, islet beta cells, etc.) as shown in Figures 5 & 6.

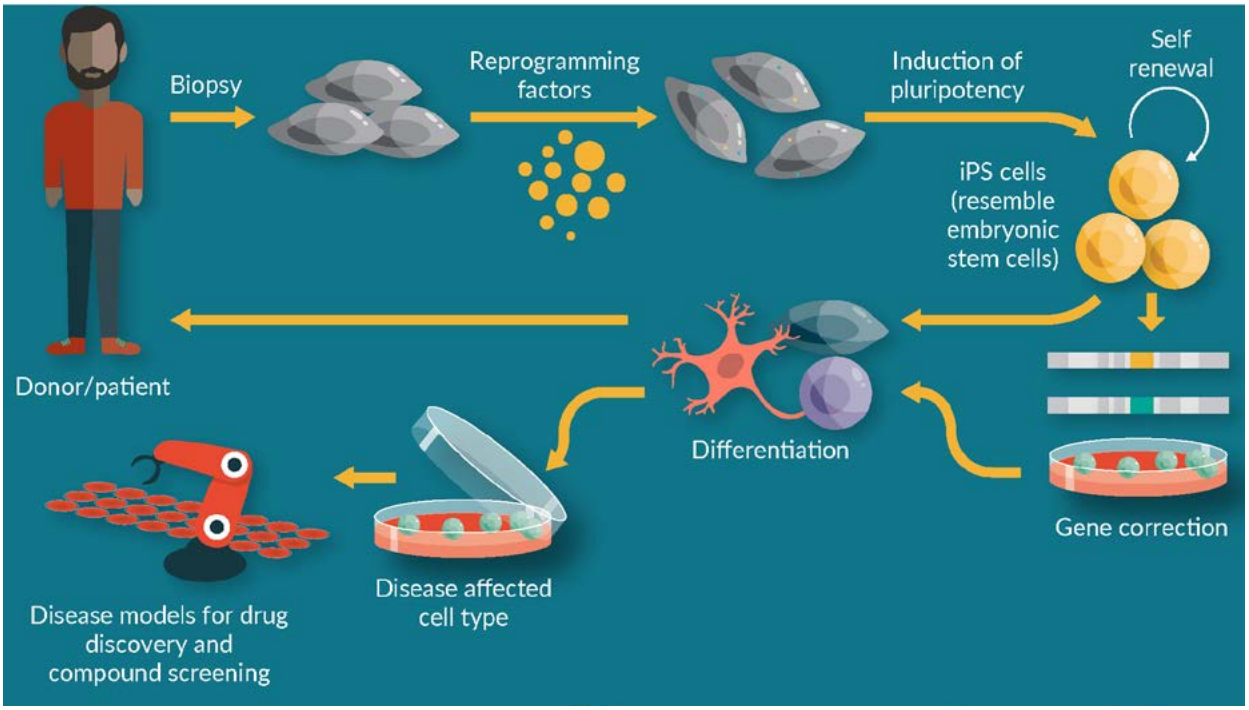
Although still early and just going into the first clinical trials, we predict that in the next decade we will be delivering fully functional cells of interest as a treatment for a disease (Figures 7 & 8). For example, in development are iPSC-derived dopamine-producing neurons to treat Parkinson's disease, cardiomyocyte cells harvested and engrafted to treat heart failure, and insulin-producing islet beta cells to treat Type I diabetes. Additional companies are working on delivering iPSC-derived cells in micro-encapsulated spheres that can be implanted into the gut. One company is using engineered allogeneic red blood cells as a platform to deliver proteins of interest inside the cell, or to deliver an antigen of interest on the surface of the red blood cell to treat autoimmune diseases. And in oncology, there is a company now testing the ability to deliver iPSC-derived NK cells to fight various cancers.

We may no longer need to deliver a small molecule or antibody drug to help the body fight disease. Rather, for a disease caused by a dysfunctional protein we will actually be making new healthy cells to get at the root of the disease, such as enabling diabetes patients to have insulin producing cells again. This could possibly offer a functional cure to the disease.

In oncology the same concept of delivering engineered donor T cells to fight cancer is now well into clinical trials. Only five years ago it was unclear if we could safely and effectively deliver a patient's own engineered lymphocytes to fight DLBCL (CAR-T therapy). But in those five years it has been proven to be possible and now multiple autologous CAR-T therapies are

FIGURE 5

iPSC differentiation overview.



FDA-approved (Kymriah, Yescarta) and several others are advancing through clinical trials. The key limitation of CAR-T has been finding antigens present on cancerous tissue only, not on healthy tissue. This has led to a massive investment in TCR-based therapies going after intracellular targets in cancer cells and a tremendous amount of capital is being invested in this area.

In the next decade we believe there will be a potential long-term shift away from autologous CAR-T therapy to allogeneic CAR-T therapy as companies have been able to engineer T cells using gene editing and other similar approaches to produce a CAR-T that can be derived from healthy donor cells. One donor could provide allogeneic CAR-T to 50 patients rather than a 1 for 1 self-approach with current autologous CAR-T. Many companies are working on this and are in or soon to enter the clinic.

INNOVATION IN TECHNOLOGY COULD TRANSFORM DRUG DISCOVERY EFFORTS IN THE NEXT DECADE

On a different note, the critical developments in computing power and AI technology could quickly find its way into benefitting drug development in this coming decade. While the traditional drug development path starts with thousands and thousands of molecules in a screening library, new drug development software that use complex algorithms and massive computing power to calculate complex physics and interactions involved with small molecules will likely lead to significantly faster and more

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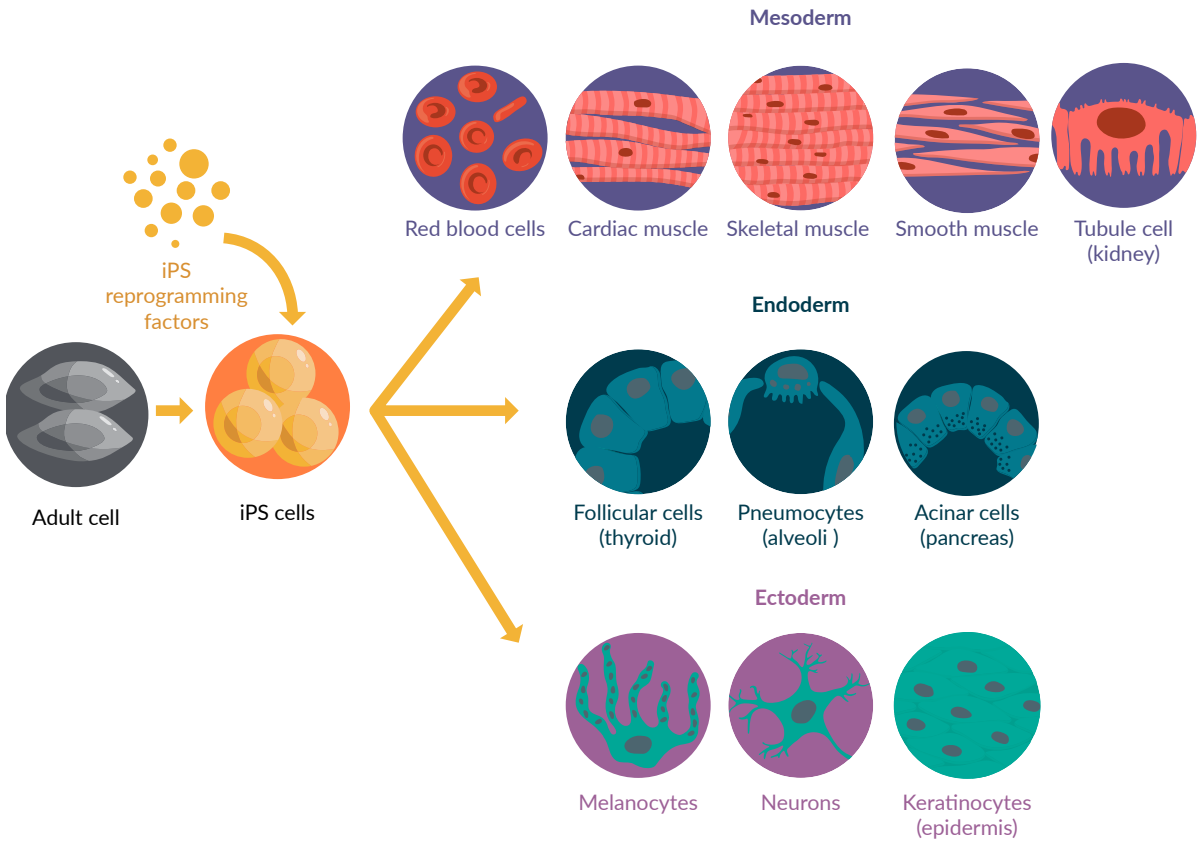
FIGURE 6

iPSC differentiation possibilities.



efficient discovery of small molecule drugs, particularly against difficult targets of interest. Bill Gates has been a major proponent of these efforts and has made significant investments in these areas in order to enable these efforts to come up with better and smarter engineered drugs in a shorter timeframe. Combined with computing power at a scale that was not even possible a decade ago, this is likely to accelerate the drug discovery process

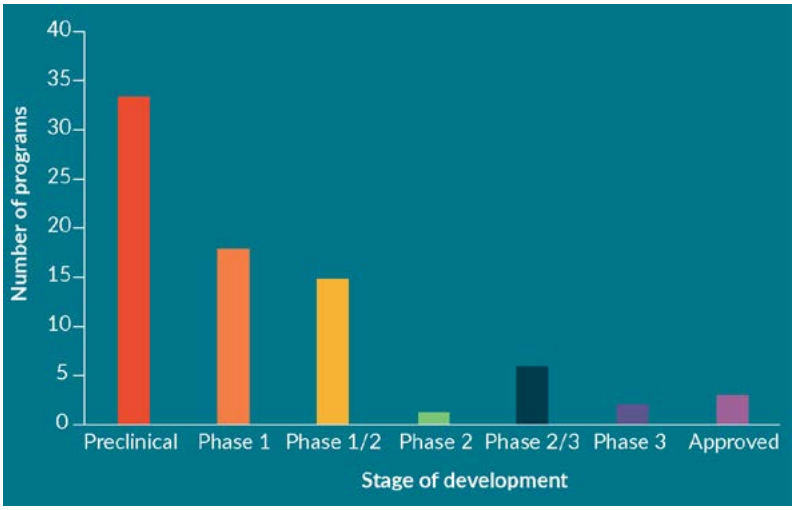
Separately, we are reluctant to predict too much about the potential applications of Big Data and the efforts of AI. Presumably if technology and AI can create self-driving cars it would seem to certainly be able to make the old school efforts of 96-well titers and drug screening exponentially more efficient along the lines of Moore's Law. We are aware of efforts by Big Pharma and Big Biotech in figuring out ways to utilize Big Data to mine clinical trial data to figure out why patients respond or do not respond to therapy, but issues such as HIPAA and privacy concerns, and inability and disinterest in sharing of clinical trial data between sponsors, are clearly gating factors.

THE RISKS TO THIS AMAZING UPCOMING DECADE INCLUDE

Continued capital investment. Mark Zuckerberg's widely touted $3 billion investment to 'cure all disease' is certainly a generous effort

FIGURE 7

Over 75 cell therapy programs in development.



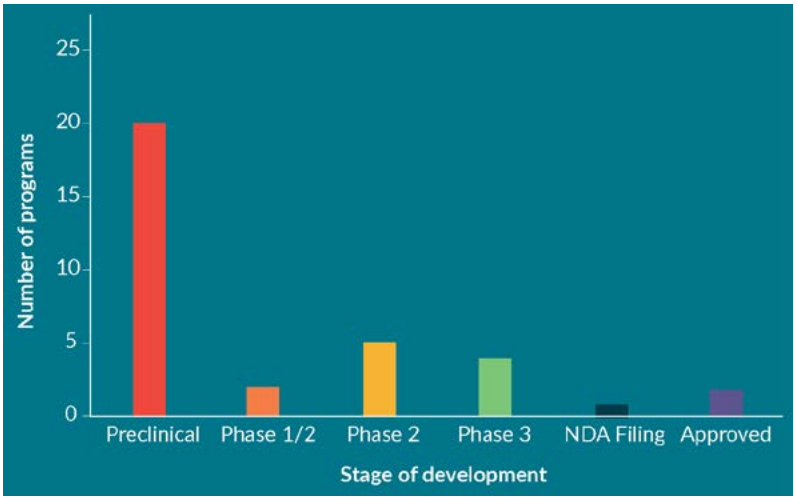
Jefferies research (sourced each individual company's websites).

that is worthy of applause. However, that's just a start – it will help fund developments, but it's not enough. To put it into perspective, the biotech industry spent $55 billion in R&D in 2018 alone and will have spent over $350 billion in the last 10 years, and they aren't saying we will cure all diseases (to be fair, the Zuckerberg initiative is built to focus on developing tools that are geared toward eradicating diseases rather than simply treating them). The VC chart shows $35 billion in funding for biopharma investments in 2018 and investment into these innovative companies has doubled in just the last few years. The good news is we think evidence suggests ROI on R&D is improving due to the scientific breakthroughs and the significant leaps in clinical benefit for patients based on the aforementioned three areas of technology. It is these scientific breakthroughs that we (and Mark) could be relying on to move the needle from treating disease to 'curing' all diseases.

A willing and able FDA that regulates and serves as the gate-keeper to any drug development in the USA. The recent Zolgensma uncoverings has led to fear about elevated levels of scrutiny and extra caution by regulatory agencies that could slow or hamper development, or become much more restrictive, which would decelerate the tremendous momentum we have.

FIGURE 8

Over 30 iPSC programs in development.



Jefferies research (sourced each individual company's websites)

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And lastly, no discussion of this amazing innovation can be had without a reasonable consensus on how society will pay for these therapies. If there's one thing that's certain to occur over the next 10 years, it will be an ongoing political and societal debate on how to pay for drugs while not slowing the pace of innovation that got us to this critical juncture in the first place – a point in time where, as we have just explained, innovation is about to significantly accelerate to create an enormous number of life-saving medicines. How will we pay? Will society accept the relative high price of drugs while understanding the high cost of R&D, or that the $350 billion in drugs in the USA is only 10% of the cost of the total healthcare spend annually in the USA? Will they understand that numerous breakthroughs have come in part as a result of investors and entrepreneurs (VC's, etc.) plowing $7 billion in IPO proceeds in 2018 and over $30 billion in capital raising for biotech in 2018 overall? These investments are not to be overlooked – they have resulted in the significantly improved benefit to patients unheard of decades ago, a time when gene therapy was a scary concept.

Our generation and the next generation will expect even more. Ten years ago, we didn't have gene therapy and cell therapy drugs approved by the FDA. We look forward to the exciting innovation on the horizon of the next decade. What genes will we have discovered? What cures will be developed? As we embark on a vision of 2020, we can only hope that the pace of innovation in biotech over the next 10 years would make Hippocrates, Dr Watson, and Dr Crick proud.

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CELL & GENE THERAPY INSIGHTS

EXPERT ROUNDTABLE

Scale-up considerations for improved yield in upstream viral vector production

SVEN ANSORGE, Director of Manufacturing at ExCellThera Inc.

Sven holds a PhD in Chemical Engineering and has led teams and managed projects within academic and industrial/GMP manufacturing environments. He is specialized in bioprocessing for the production of viral vectors, vaccines and recombinant proteins.

MIKE BURNHAM, Director of the Vector Development department at Tmunity Therapeutics Inc.

Mike has over 25 years' of biosafety and bioprocessing expertise, and is currently working to establish Tmunity's lentiviral vector manufacturing platform. Prior to joining the team, Mike worked at WuXi AppTec, serving in multiple roles in their Process Development, Viral Production/Purification and Viral Clearance departments

MICHAEL KELLY, Vice President of Process Development at Avrobio

Avrobio is a clinical stage company developing gene therapies for rare diseases. Michael has over 20 years of experience in the field of gene therapy. During this time, Mike has been responsible for various aspects of research, development and clinical manufacturing for a number of lentiviral, adenoviral and AAV-based therapeutics.

RUTH McDERMOTT, Segment Marketing Manager at Sartorius

Ruth has 20 years of business and market development experience within the life sciences industry, including roles in technology and IP commercialization. Ruth's technical knowledge has been built on a background of R&D in cell bioprocessing, stem cells and gene therapy. Ruth holds a PhD in Cell Biology and an MBA.

PETER JONES, Head of Operational Strategy at Oxford Biomedica

Peter has over 30 years' experience in the biopharmaceutical industry. He joined Oxford Biomedica in 2011, providing technical and scientific leadership. In his current role, he is responsible for overseeing strategic and operational planning to develop the Company's capabilities and capacity to support future business growth. Peter is a Fellow of the Institution of Chemical Engineers and the Royal Academy of Engineering Visiting Professor in Manufacturing of Advanced Therapies at Aston University.

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Q Can the panel define their key challenges and benefits in migrating from adherent to suspension culture systems?

PJ: Beginning with the benefits, adherent processes are based generally on academic lab processes. They use planar technologies and flatware. They are generally serum containing. They tend to be in the 1–100 liters harvest volume range. And they generally include manual unit operations: they're more laborious and involve multiple interventions, and because of these interventions, they tend to have be higher risk profiled.

This means there is a heightened risk of batch-to-batch variation, scale-up is limited, and you tend to get fewer doses – around 1–20 doses of lentiviral vector, for example – per batch. This contributes to a cost per dose that is usually very high.

Suspension processes, on the other hand, takes an approach more aligned with that exploited by mAbs and other commercial biologics in the past. Instead of a scale-out model, which would be the case with adherent, you have a scale-up model. They tend to be serum free. They have chemically defined media. They have been shown to be scalable – for example, technologies using single use systems can offer a range of 5–2,000 liters. They involve closed processing that can be automated, and there are reduced manipulations – all of which reduces the risk profile.

There are also different modalities you can use with suspension: batch, fed batch and perfusion. There is also the possibility of having inline monitoring using PAT tools, whereas with adherent, there are limited opportunities to actually monitor the process because they tend to be in monolayers.

Lastly, doses tend to be in the region of hundreds of doses or in some cases even a thousand doses per batch, meaning that the cost per dose can be much lower than it would be with adherent culture systems.

MK: I agree with all of Peter's comments. I'd just add one point, which is the potential for suspension systems to not only reduce cost but to actually support market demand. For modalities that require large numbers of doses per year, that is crucial.

SA: One technology field I would add to the mix are the fixed-bed bioreactors we've seen coming through over the course of the last couple of years. They are already being used quite extensively. I see them as being somewhere in between the two: they provide an extension to what you can do with adherent cell cultures with increased surface area, but from an operational perspective, they are also as easy to operate (or as complex to operate, depending on your standpoint) as suspension systems. They also tend to have pretty high media consumption, because of the fact the small fabrics on which the cells grow can contain so many cells, and you have to somehow keep them all alive during the production run.

MB: We took the approach of bringing vector manufacturing in-house and making it a core capability – we have new manufacturing suites coming online in Q3 of 2020.

So it's an exciting time for me and my team. We are essentially building this capability from the ground up, since there's really no true blueprint for establishing a vector manufacturing process. We're starting from an adherent process, which relates to Sven's comments: we do see an advantage in going from an adherent model in flatware to an adherent bioreactor – we don't really have to do much with media formulation or chemically defined media for that aspect of it, so we may be able to transfer our process a little bit more quickly and easily. However, when you think long-term, we're definitely going to reach a decision point around getting out of the current cell stacks-hyper stacks model into a more suitable, scalable process.

One of the challenges we're facing is designing a process with speed and flatwear does allow us to get into our clinical programs quickly. The idea is that we'll eventually move into some sort of bioreactor process for all the reasons that Peter, Sven and Michael have raised. It just makes financial sense to do so and of course, we have a fiscal responsibility to our investors to make sure we're doing everything we can to limit overall cost of goods.

MK: One further comment on the challenges side. Obviously, transitioning from serum-containing to serum-free manufacturing processes can result in product quality differences. One of the things to consider is a comparability assessment to ensure that first in human clinical data can be applied directly to follow-on clinical trials. That's something that would need to be decided upon very much on a case-by-case basis, though.

Q Can you go deeper on the chief limitations of either system type? Where specifically is further innovation needed?

SA: What we have to keep in mind is that these are basically the same processes that were developed more than half a century ago for vaccine manufacturing. Just from that perspective, it's obvious that they are rather open processes. Clearly, we're in the process of closing these as much as we can, but they tend to remain dependent on a lot of manual intervention, which is a risk.

PJ: I think there's opportunity here for innovation. We've talked about working from batch, fed batch, also perfusion and continuous processing. Now, similar to what happened with monoclonals, I can see that there will being a real drive to try and reduce the cost of goods.

I also think that there's going to be more innovation in terms of process intensification. And there's going to be an increasing use of PAT (process analytical tools) to gain a better understanding of what's going on in the bioreactors.

There's going to be a lot of work in the PAT area in particular. We ourselves currently have an Innovate UK project underway with Cell and Gene Therapy Catapult and Synthace, where we're looking at the use of Raman spectroscopy, for example, to help us monitor what's going in a bioreactor. A lot more work needs to be done in that area to increase the robustness of our processes. I think that will be the driver to gain greater control and to drive the cost of goods down.

RM: The fundamental need here is to increase the cell culture density whilst maintaining the environment, to maintain nutrient levels, and to remove all the waste products. Unless you've got some level of control over all of this, you're going to have very limited ability to do scalable cell culture.

Obviously, there are some limitations with traditional static planar vessels, which we've covered earlier. You have very limited control in those systems – no online monitoring and they are very manual.

Fixed bed culture systems, work very well for adherent cells, especially if you're harvesting a virus from the supernatant, but if you want to access and harvest virus from the cell biomass that's much more difficult. You then have

to look at optimizing some of the lysis steps and harvesting in that way.

In terms of the stirred-tank bioreactors (STRs), Peter is right that this is where you can really start looking at the control system. You can begin looking at more intensified processing and by using Process Analytical Technologies (PATs) you can understand your culture environment in much greater depth. An additional advantage is that you do have some true scale-down models, which allow you to understand whether your process at small scale is truly relevant at a larger scale. Again, you can utilize some PATs here in order to understand where and how you can achieve greater yield.

MK: Coming to the manufacturing facility itself, operators are a key point of focus for future innovation. Today, it's a very inefficient, manually intensive process. Looking forward I can see robots, robotic interactions, taking care of basic manufacturing steps. I think if that's done correctly, it can really increase the capacity of a facility on a footprint basis, because you can remove some of the restrictions around people walking through facilities and the like. I think it would also improve aseptic control.

Obviously, there's a way to go in that area, but I do see a lot less manual intervention and a lot more robotic activities in the future.

MB: Downstream purification is my passion, so I tend to look at where a lot of these technologies will feed into downstream purification processes. And I think about comparability: how are these new technologies or applications from the tool providers going to help comparability from vector to vector? Experience has taught me that the transgene has the ability to create havoc in terms of how a vector behaves with the platform process you are developing. For me, that's the sort of thing we have to think about: what's the right model for looking at different vectors to ensure they fit a platform?

Q What are the keys to success when approaching scale-up of suspension culture system, particularly in terms of maximizing yield?

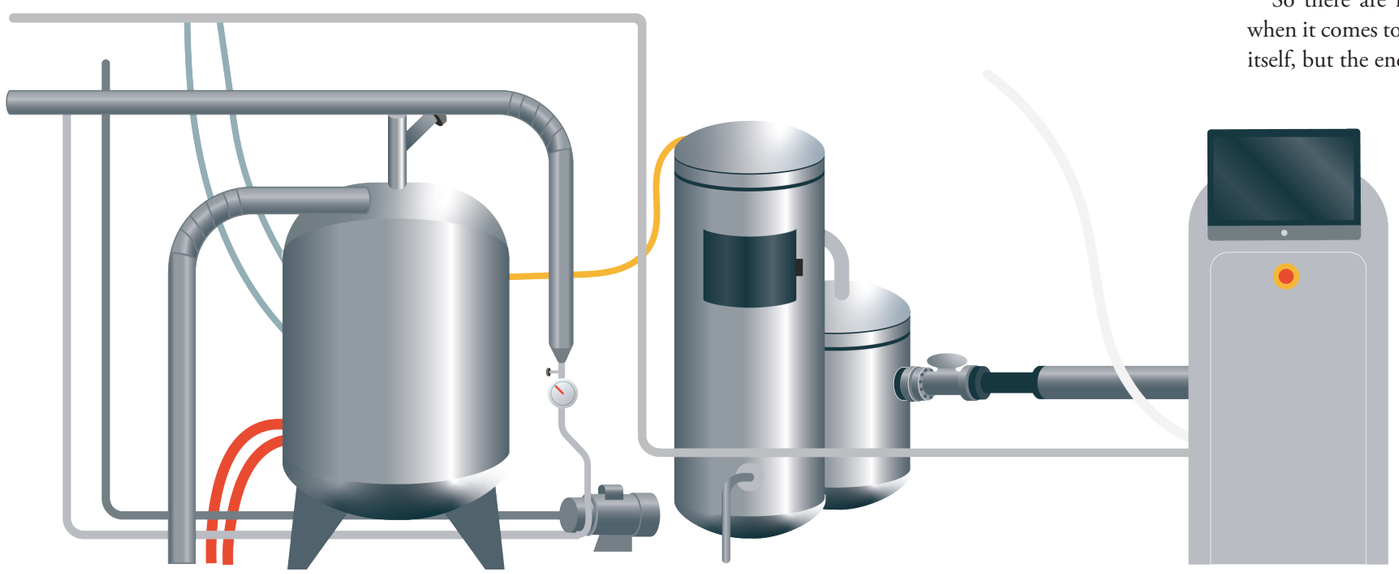
RM: It's important to understand that scale-up is not solely about increasing numbers – neither of the cells or the viral vector particles. It's really a case of looking at every single step of any given process and how that impacts your critical parameters..

We've mentioned that there is a difference between adherent and suspension systems in terms of scalability and your choice of which system to use depends on the batch size you ultimately need for your target indication(s). But again, I think you need to clearly understand exactly what you mean by scale. For example, does a four-fold increase in scale mean the same increase in surface area for an adherent cell system? Does it mean four times the volume? Does it mean four times the cell density? When you're looking at scaling-up, are you really looking at the geometric similarities across all the different scales to ensure it's a truly scalable system?

You really need to understand all the key parameters and critical factors you're looking at. Some of them might be scale-independent – for example, media composition might not be relevant to the scale – whereas others might be scale-dependent. For example, in a suspension culture system, mechanical stress and agitation rates can play a significant role. Viral vectors and viruses are very susceptible to sheer stress, so what's OK for your host cells isn't necessarily OK for the virus. In which case, to start de-risking your entire process, you can look at the agitation rates, ideally in a fully representative scale down model. This would allow you to understand the relationship between each scale.

I would add that we've seen in the outcomes of recent conference workshops and literature that there's a general expectation we'll see a mix of production technologies in viral vector manufacturing moving forward. However, we think suspension-based upstream processes will probably become the industry standard, and that a trend towards continuous bioprocessing approaches will help transform the field in terms of significantly reducing manufacturing costs.

PJ: I'd just like to widen it slightly. You also have to take into account the actual initial vector design and its optimization: the viral serotype and pseudotype as well as the transgenic sequence composition and size can also affect your titers. In the past, we've also found that the amount of vector produced upstream can vary depending on the transgene encoding, especially if the active protein expressed in the production cell line is constitutive, or if a leaky tissue-specific promoter is employed. So it's not just the actual optimization of the bioreactor and conditions – you also have to look further upstream at the vector design as well as at optimizing your cell line, especially if you're going to work towards a scalable producer cell line.



> "It's important to understand that scale-up is not solely about increasing numbers – neither of the cells nor of the viral vector particles. It's really a case of looking at every single step of any given process."
>
> - **Ruth McDermott**

Of course, you also have to consider the downstream bioprocess. As we've discussed, adherent processes involve serum, which can lead to problems because it co-purifies – it comes down with your vector and presents some difficulties as you have to make sure it's subsequently removed during processing.

As Ruth mentioned, you've got issues with shear sensitivity, especially with lentiviral vectors, but you also have to deal with temperature sensitivity, product stability issues, and freeze-thaw sensitivity. And you can only operate within a very narrow salt range, because that can affect your virus as well.

So there are lots of different issues and considerations when it comes to scaling. It's not just the suspension culture itself, but the end-to-end process you have to think about.

SA: I want to highlight the current general lack of process and product understanding in the field, which is maybe not limiting us yet but will do in the future. We have a lot of knowledge about upstream processing and how to scale-up from an equipment perspective, but we certainly do not yet understand how we can improve these processes in terms of what is happening at the molecular level. What do the cells actually need in terms of media and media composition to produce, and to do so

robustly? We're also all familiar with the challenges in generating stable cell lines. When I think about the concept of developing these processes and of at least moving towards more continuous ways of manufacturing, these are the things that I think will limit us. But I think that even interim tasks, such as reducing or removing the number of manual operator interventions, are not yet possible with today's processes because, to put it bluntly, the only thing we currently understand is the amount of vector we're supposed to be making at a given point in time. Why we're doing so is not necessarily clear to us.

Another point on the purification side. One of the challenges I see in the field is not what you make, it's what you keep at the end of your purification.

I think we all struggle with nonspecific binding of particles to matrices during cell screen purification steps. I think we need to look a little closer at how we purify, and how we have unintended loss of material. Because I think if we're getting a 30% yield, which most people would be happy with, that's obviously reducing the productivity by three-fold.

So I think we need to start looking at chemical interactions with matrix components in our purification schemes.

RM: Just to reiterate what Peter was saying, whatever upstream process you optimize and choose to use will have an impact on your downstream processing. And I think a lot of people, certainly in the early development stage, don't understand that if you optimize one it may not be optimal for the other. It would help if there was more sharing of information and for process scientists and engineers to understand a bit more of the basic research, and vice versa, for the bench scientists to have a better appreciation of what's happening at a commercialized production scale.

So as Sven mentioned it's just making sure there is a good understanding of what is happening. The interaction with different materials etc… so it's a very important point to understand the impact of each of these different steps on each other.

PJ: Can I just add something else. I think what you're finding, and we mentioned it a couple of times now, about improving our process, product understanding, and I think there needs to be a lot more fundamental work carried out, really understanding how vector production and the mechanics of vector production and how vectors are produced and then secreted or produced within the cell.

We at Oxford Biomedica are spending some time on this. We're looking at using proteomics, transcriptomics, metabolic flux analysis, to really understand what the limiting factors are either in the cell or in the vector design which means that we try then to get an understanding of what metabolites are important, to understand what's important in our media and also in our feed design, and whether or not we can actually make a better influence of that, a fundamental layer, in terms of making our bioreactors upstream more productive. And that's where I think a lot more understanding is required.

> "For us to build sustainable, scalable manufacturing platforms and processes, we must be able to take the high degree of technical art out of the equation. Otherwise, I think the necessary cost of goods reduction and associated automation of manufacturing processes cannot be realised."
>
> – **Mike Burnham**

MB: Mike Kelly made a very interesting point about the art versus the science of viral vector manufacturing - I think that's true. I also agree with the comments relating to the amount we don't yet know - I think that's a fair reflection of where the field is today. For us to build sustainable, scalable manufacturing platforms and processes, we must be able to take the high degree of technical art out of the equation. Otherwise, I think the necessary cost of goods reduction and associated automation of manufacturing processes cannot be realized.

Q Building on the cost of goods topic, how can we optimize cost control when it comes to viral vector manufacturing?

MK: Most if not all of the panel members are currently working in the clinical development setting and traditionally, we as an industry typically don't worry too much about cost of goods during clinical manufacturing. However, if you look ahead to commercialization and the pressure that the payers are under in terms of reimbursing these novel biologics, in order to have a feasible business we have to be able to make them at a reasonable cost. You just can't keep increasing the cost of novel drugs because the cost of manufacturing is high. Obviously, that burden becomes even larger with higher doses. As we've already established, in order to try and increase process productivity, we have to increase our understanding of the biology of vector production.

I think that one of the big advantages of suspension systems is that you can increase cell density per volume. We've clearly got to work towards that. We've also got to try and improve how much vector we keep through the purification process.

I would also return to Peter's comments about vector design. We develop therapeutic strategies very early in the drug development lifecycle: we choose capsids, envelopes, promoters that are designed to deliver drugs. But I think we probably don't spend as much time thinking about how that design will result in a feasible business opportunity downstream. I think one of the areas we should focus on is how to reduce the dose we need to administer by being more efficient at targeting - by choosing different envelopes, different capsids in the case of AAV, and by looking for more efficient routes of administration. The latter is key from both efficacy and safety standpoints, of course.

MB: Being a start-up company, we have to be very mindful of dollars spent. Tracking and developing a process from the ground up is certainly going to involve some spend, but how do you do that in a very controlled manner, and how do you design a process without a significant impact on your overall budget?

One of the aspects that comes to mind is speed to market. That's something we're trying to optimize here - establishing a process in less than a year and a half and having it ready for manufacture is a pretty significant undertaking for us.

Another important consideration for cost of goods is what you in-license – for example, there are commercially available cell lines out there, but they come with a hefty price tag. Trying to strategize around quickly establishing a process whilst taking such cost-related factors into consideration is certainly challenging.

SA: Manufacturing costs are a clear issue for industry but quite honestly, they were even more of a concern in my previous role when I was wearing a government hat. We spent a great deal of time debating the extent to which healthcare systems such as ours (Canadian) could accommodate products with the price tags we are seeing right now.

However, when it comes to manufacturing cost control, we have the example of the mAbs area to encourage us. It did take decades for the mAbs to get there, though - I'm hoping we in cell and gene therapy can be quite a bit faster.

PJ: Regarding lentiviral vectors and other vectors used in cell therapy, I think it's important to note that while we're very much looking for forward momentum in terms of trying to optimize titers and the productivity of our vector processes, there is also a degree of reliance on the cell bioprocessing side to optimize in terms of the amount of vector they require. There is, or should be, a push-pull mechanism at work in this regard. Again, it brings us back to the importance of looking at the whole process end-to-end.

RM: Where are most of your costs coming from? It will depend on the scale of your manufacturing, of course. Are most of the costs coming from the large GMP facilities that you may need, or is it the buffer, media, in your upstream processing? If it's the latter, you need to weigh up that cost against the benefit you might be receiving in the form of more efficient production. Going back to PATs, they can help optimize your buffer and media usage.

In other words, i's a case of balancing all of the elements that impact cost of goods. Facility design is another important consideration: understanding how a production process

is going to work and what yields are achievable at different scales so you can optimize your footprint usage.

MK: Continuous manufacturing (CM) strategies have been quite successful in the biologics space. It's not an area in which the gene therapy world has really invested as yet, and I think that's something we should be looking at. However, I do think that CM is something that's more amenable to future cell lines rather than transient transfection.

I would also echo Peter's comments: I do think we can do better on the cost of vector, but in the ex vivo gene therapy world, especially with autologous products, it's only one component of the high cost of patient-specific cell-based therapy manufacture.

I think a lot of the vector bioprocessing approaches we've taken as a field are not immediately scalable, and so we may need to think a little bit differently moving forward about how we start.

My final point is that everyone is trying to scale-up today to meet market demand. However, given the one-time administration of cell and gene therapy products, often for rare indications, there is the potential to go quite rapidly from treating the prevalence population where demand is high to the incidence population where demand is significantly lower. In other words, while the short-term struggle is to scale-up, if we're successful in that endeavour we may well find a situation in the future where we'll have to scale-down again. I think it is important to bear that in mind as we validate our processes.

PJ: I agree. I think the other thing you have to take into account is how to design your process into your facility. You can use a sort of overlapping process. You can use a continuous cell build approach. So there are many ways of actually increasing productivity and improving your overhead recovery.

A further aspect is the development of rapid analytics with their potential to minimize development and scale-up times, thereby helping to reduce costs. There's also the possibility of automating analytics so you get a higher throughput, reduced turnaround times, and therefore cost savings per sample.

So again, there are many levels to trying to drive costs down, including reducing the cost of quality and trying to reduce the timeframe for product release within the manufacturing environment.

Q Can the panel talk some more about the key process analytical considerations for scale-up?

> "I think what you will see over the next few years is a much greater emphasis on trying to develop more rapid analytics, not only to monitor the process but also for product release."
>
> – **Peter Jones**

MB: For me, the main goal is titer – that's been the primary driver in establishing our vector process. Obviously, that's while keeping considerations around purity and safety well in mind - we don't want to develop a process that's just high titer, but results in a terrible final product. In order to have that in place, we need a strong analytical development team to drive the development of the robust assays to support us along the way.

There's a lot of ways to get to your final titer. What is that final titer and what does it mean? You have a (genome copies) titer, you can have a full assay, or a cell culture-based titer with a PCR end point, or a functional titer. You have to think about the characterization around the cell line and how that leads to the robustness of the assay. And then how do you tie everything together? I think that's the struggle I have as a non-analytical person: understanding the difficulties that our analytical team have to face with getting these assays up and running. Then there is training operators and standards, too.

These have been the key considerations that our analytical team has been trying to resolve as rapidly as possible to support us. So I'm putting a lot of emphasis on process development based on titer, with purity and safety in mind, and I'm placing a lot of trust in the assay as it's still being developed in order to drive us towards a manufacturing process.

> "...we're lacking the methods to provide real-time information about how much virus is coming out of your cell - maybe this is where Raman or other technologies can assist in future."
>
> – **Sven Ansorge**

RM: I'd just add here that there's a difference between what you're looking at during the process and what you require at the end for your QC. If you've got better analytics throughout the process, it can help you in some ways to 'fail early' – in the event that your process isn't working, if you understand what your readout is saying, you can then optimize or change things in that particular process' parameters. But the parameters are different for QC testing and QC testing is looking at product definition rather than process information.

The other key thing to consider is using specific reference standards if available. This goes across the board: 'what is a cell count?' 'what is a viral titer?' 'is your viral titer the same as someone else's?' 'how do you compare processes, including across sites, especially if they are using different analytical equipment?'

PJ: I do think the current analytical toolkit is lagging behind where we need it to be from the industrial viewpoint. Where we are now is that we've developed specific platform assays which in many cases are compendial(safety) assays covering endotoxin, bioburden, sterility, mycoplasma (absence of RCL and absence of adventitious agents). We have more specific product and impurity assays such generic DNA detection method (PicoGreen®), residual plasmid DNA (KanR qPCR) and residual host cell DNA (18S qPCR) etc. Then we've got the product-specific assays, including potency and identity. What we're trying to do is to also bring in automation, because we need to bring in higher throughput as I've said and reduce the cost of analyzing each sample. We also need to use automation to reduce our operator-to-operator variability, and to improve our reliability and reproducibility. However, that also opens up another issue and area for discussion: it's all well and good running all these analytics, but we then need to do something with the data. We need to streamline our data analysis.

I can foresee a time when we'll probably see more involvement of mass spectrometry (MS) as (an analytical) tool, next generation sequencing as well. And I think what you will see over the next few years is a much greater emphasis on trying to develop more rapid analytics, not only to monitor the process but also for product release.

SA: For me, rapid analytics means real-time monitoring of processes. I think the work Peter mentioned earlier on Raman spectroscopy is a good example of this.

We have done work on using capacitance, which provides good information on what's happening at the cellular level. However, we're lacking the methods to provide real-time information about how much virus is coming out of your cell - maybe this is where Raman or other technologies can assist in future. Obviously, we'd have to combine that with rapid analytics, because you probably cannot think about doing process analytics without having a cell-based assay in place. That would ideally be faster than what we're doing right now.

Regarding the data processing and analysis challenge, I think we've seen in other fields where these sorts of technologies are more established that this is not insurmountable.

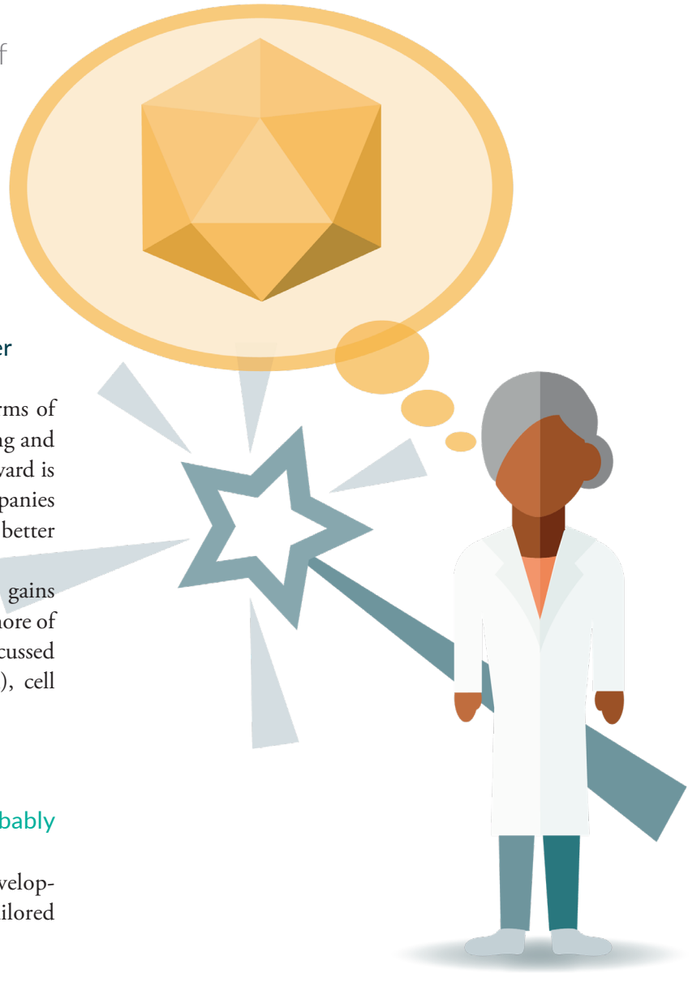
MK: I agree with everything I've heard. One of the things I worry about is that the amount of time from manufacturing to release of a viral vector is somewhere between 3 and 6 months. I'm not sure exactly what the industry standard is, but obviously, that's a significant issue.

In addition, the actual result is something of a black box. I think the lack of in process controls (IPCs) to really understand what's going on during the manufacturing process is an issue – our ability to predict and anticipate failures is really not where it needs to be. There's work to be done there.

I think we all have this vision of an artificial intelligence-managed, automated robotic process. For that to be a reality, though, the analytics have to be better – more accurate and precise. And we have to have more real-time monitoring capability. We're not close to that today. When things take a long time, that usually means the cost is high as well, so I think we have to do better there.

One other point on the analytics side: we obviously all know about accuracy and precision, but also some of the methods are very inefficient and require huge amounts of vector. We need to have analytical methods that don't use up the majority of a batch. I think the average right now is about 25% of a batch is used for dosing patients, the rest is used for characterization. There's a clear pathway to reducing cost by reducing the amount of vector product lost to non-clinical uses.

Q What would be at the top of your personal wish-lists in terms of novel innovation in the vector bioprocessing area?



PJ: A lot of the technologies we're currently using are based on 'old school' biologics – on understanding gleaned from the wider biologics area, such as the vaccines field.

The tool suppliers are beginning to step up in terms of trying to come up with better technologies for making and purifying vector. I think what you will see going forward is a lot more collaborations between suppliers and companies who are producing a vector to try to come up with better ways of actually making the vector in the first place.

I think we will make continue making marginal gains towards an end-to-end, holistic approach. I see a lot more of that work going on in all the different areas we've discussed today: vectorology (vector design and optimization), cell line development, process innovation and analytics.

SA: My wish-list is easy to say but probably rather difficult to achieve!

I'd be very interested in seeing investment in developing better cell culture media and feeds, specifically tailored to viral vector production.

> "I think we need to look at it from an end-to-end perspective. But I would like to begin with the end in mind, which means requiring less vector per patient."
>
> – **Mike Kelly**

The second point is I'd like to see stable cell lines that produce not just for a couple of days, but for weeks at a time – that would truly enable continuous manufacturing.

My third point is devices that are specifically tailored to viral vectors. For example, perfusion devices, or perhaps completely different downstream processing unit operations to those we currently employ – tools that help us to really view and treat the vector as a product. This is probably more important for lentiviral vector because of its poor stability.

Fourthly – and this one is probably impossible to achieve, but we've touched on collaborations today and I'd like to see an open, collaborative forum that would allow people to discuss what their current problems are without IP constraints. I know we are not going to get there, quite, but maybe we can take baby steps because I truly believe that none of the organizations that are currently in this field will be able to fix all of these problems alone. The more discussions we can have around this, the better it will be for the entire field.

MK: The burden of manufacturing is incredibly high, so I would like to see the vectors that we're using being more efficient.

In other words, I think we need to work on this from both ends: I think we need to improve scalability and everything else to do with vector production, but I also think we need to try to find ways of reducing the amount of burden

a. for manufacturing costs; and
b. for patient safety.

I would like to see better designs and more efficient ways of delivering vectors. I wholly agree with Peter - there's no one switch that we can turn on that will solve our problem. I think we need to look at it from an end-to-end perspective. But I would like to begin with the end in mind, which means requiring less vector per patient.

MB: Sven stole my thunder there a little bit on collaboration! I was thinking about the various consortia in the monoclonal antibody industry, having participated in those from the CDMO side. I think that a free-to-share information network for the cell and gene therapy space would be very nice to have.

I also think about rapid analytics. You can only get so much information out of a p24 ELISA kit – having real-time monitoring available to help you understand how well your scale-up process really works would be great. You put a lot of time and effort into the small-scale model, do the engineering runs to make sure it's suitable for transfer into manufacturing, and then you start your manufacturing campaign, but you don't really know anything until you've got that bulk drug substance tested. And a lot of that test material goes out of the door, as Michael mentioned. That effectively means a potential patient isn't getting treated because of the analysis that goes into the final product, which is a very hard thing to reconcile.

WATCH THE FULL ROUNDTABLE NOW

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CELL & GENE THERAPY INSIGHTS

2020 VISION
TOOLS AND TECHNOLOGIES OF TOMORROW

SPOTLIGHT

INNOVATOR INSIGHT with Frank Corbin, Vice President, New Venture Technologies at Terumo BCT

"Automation will be the field of enabling technology that will do most to drive the broad adoption of cell therapy."

Automation in cell and gene bioprocessing: challenges, opportunities and what lies ahead

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INTRODUCTION: EVOLVING 'SYSTEMS THINKING' TO DRIVE AUTOMATION OF CELL & GENE THERAPY BIOPROCESSES

Since Terumo BCT's inception in 1964, the company has focused primarily on applying 'systems thinking' to solve customer problems across all fields of medicine.

This approach fundamentally consists of seeking to understand the actual customer environment in which an issue occurs, while recognizing it as part of an integrated set of experiences and, ultimately, of solutions.

For example, in the blood collection space, the application of systems thinking to the challenge of how to optimize operations and better utilize the precious pool of donors led to the development of a game-changing leap forward in automation – the Trima Accel® Automated Blood Collection System. This solution enables the collection of any combination of red blood cells, platelets and plasma based on the individual donor's physiology to best address the inventory needs of the blood center. Software was developed in tandem to provide not only Good Manufacturing Practices (GMP) documentation, but also data that could be analyzed to improve operations.

> "In the blood collection space, the application of systems thinking to the challenge of how to optimize operations and better utilize the precious pool of donors led to the development of a game-changing leap forward in automation..."

Similarly, systems thinking played a major role in the development of the Spectra Optia® Apheresis System. In this example, the goal was to automate therapeutic apheresis, making this critical process safer and more comfortable for patients and more efficient for operators. The result was an apheresis system that executes a consistent and high-quality apheresis procedure with minimal nurse/operator involvement, so the apheresis nurse can pay greater attention to the patient's clinical needs. This leads ultimately to a better overall outcome for that patient.

A third example is the Quantum® Cell Expansion System, which builds upon Terumo BCT's extensive fluid management systems expertise. The aim here was to provide a well-controlled, automated and closed microenvironment in which to grow cells by making the process more user-friendly and repeatable – again, leading to better eventual outcomes.

In essence, systems thinking blends systems engineering skills developed over years with a strong focus both on understanding the client's individual problems and on the patients themselves. It is through this lens that this article assesses the ongoing challenges and opportunities in continuing to automate highly manual cell and gene therapy bioprocesses.

PRIORITY TARGETS FOR FURTHER AUTOMATION OF CELL & GENE THERAPY BIOPROCESSES

It is important to recognize that the cell and gene therapy sector is still in its relative infancy. While solutions to industrialization challenges are beginning to be found, it may take years to develop the broader applications required for optimal commercial manufacture of advanced therapy products. It is widely recognized that automation will be a key enabler on this journey, prompting the question of where next to focus innovation efforts in this sphere.

Upstream bioprocessing

The majority of starting material cells for cell therapies are collected by apheresis, with the aforementioned Spectra Optia as the go-to system. However, regardless of collection method, the cells must then undergo a purification step. It is here in particular that there is room for improvement in terms of innovative, automated front-end bioprocess solutions.

Starting material cells are often collected and frozen at a single location before being transported to a manufacturing site for processing. Unfortunately, cryopreservation of the raw material often leads to clumping, which complicates the subsequent separation of specific cells of interest. A logical approach to this issue would be to drive starting material purification and cell selection further upstream. Automation technology in a closed environment could make the process more efficient and consistent, while also providing data to help continually improve the process. Various novel and emerging separation approaches are currently in development, including centrifugal, microfluidic, acoustic and immunomagnetic particle-based systems. All share the same goal: improving the quality of starting material earlier in the process for downstream benefits.

Recent advances in bioreactor technology – e.g., the emergence of hollow fiber bioreactors (HFBs) – are also driving significant improvements in upstream bioprocessing. An increased ability to dictate and control the cells' microenvironment differentiates these bioreactors from the more static suspension culture systems, for example.

Downstream bioprocessing

As previously established, the systems thinking approach demands recognition of bioprocessing pain points. For cell therapy manufacturers in

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> "Recent advances in bioreactor technology … are also driving significant improvements in upstream bioprocessing. An increased ability to dictate and control the cells' microenvironment differentiates these bioreactors from the more static suspension culture systems."

the early commercial stages, arguably the greatest of these is the final washing and concentration of the cells following expansion and their final preparation for cryopreservation. This step in the downstream process tends to be both tedious and time-consuming, creating a significant bottleneck and ultimately leading to delays in getting therapies to patients, which they can ill afford.

The various fill and finish steps provide an additional opportunity to address through automation challenges related to contamination risk, product variability/viability and process consistency. For example, the Terumo BCT Finia® Fill and Finish System is a first-generation solution that allows placement of final cells in the correct media, the addition of cryoprotectant and allocation into multiple containers for freezing in a closed system. Importantly, key variables including time and temperature may be managed throughout, reducing the risk of potential product loss, which can have devastating repercussions for patients.

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Automating manual steps can help alleviate many of the challenges in upstream and downstream bioprocessing. Steps can often be eliminated as a result, which saves precious time. Automation also helps ensure processes are followed consistently, helping drive quality improvements. Both lead to two further, vital benefits: cost savings and increased scalability.

However, in order to capitalize on these potential advantages, it is of paramount importance to first ensure the right processes are in place, together with the means to capture data in order to help troubleshoot and improve processes and products on an ongoing basis. It is equally vital to bear in mind that automation is not a replacement for human decision-making – it can only improve the capacity of a person to make decisions and carry out certain tasks.

The next generation of advanced therapies will encounter myriad challenges relating to consistent manufacturing, quality control and stable outputs. Delivering them will require the sort of strong background in systems engineering and experience in applying it to process control, automation and data management that Terumo BCT possesses.

CONCLUSION

The perennial question for cell and gene therapy biotechs of when and where to invest in bioprocess automation technology may be simply answered: as early as possible, financial constraints allowing.

> "The perennial question for cell and gene therapy biotechs of when and where to invest in bioprocess automation technology may be simply answered: as early as possible, financial constraints allowing."

It is recommended to focus initially on those elements of the process that involve biologically modifying the cells, such as genetic modification and cell expansion. These are the most complex elements of the process, and improving these first will lead to greater process control, which ultimately leads to therapies reaching the market faster.

When embarking upon process development for a novel cell therapy, it is advisable to consider manufacturing automation beginning at the cell collection stage. The fluid processing steps, such as wash and concentrate, fill and finish, can lag behind somewhat. However, automation of all of these steps should ideally be in place by the time pivotal clinical trials commence.

It is also important to select a long-term, strategic partner with the capacity to help evaluate the risks, develop the processes and apply the right technologies at the right time – all are key to maximizing the value of automation.

Automation will be the field of enabling technology that will do most to drive the broad adoption of cell therapy: without automation and process control, these very complex medicines will never reach wider patient populations. Ultimately, stakeholders across the industry will need to come together to enable a delivery system that makes cell and gene therapy more accessible to a greater number of patients. Terumo BCT is working hard to do its part, leveraging more than 40 years of experience with blood and cells and automating manual processes to help bring novel advanced therapy innovation to life.

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TERUMOBCT
Unlocking the Potential of Blood

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SPOTLIGHT

COMMENTARY

New frontiers: cellular immunotherapy beyond cancer

Matthew Brook, Joanna Hester & Fadi Issa

Cell-based immunotherapies have the potential to revolutionize our approach to patient care. The first steps towards routine administration of cell therapies are being taken with early clinical trials underway in transplantation and in patients living with autoimmune conditions such as Type 1 diabetes and inflammatory bowel disease. Here, we consider the need for cellular therapy in the clinical setting; the current position, with a specific focus on transplantation where significant steps forward are being taken, and what the next decade may hold in this rapidly developing field.

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INTRODUCTION

The successful adoption of cellular therapeutics into the clinic has created a paradigm shift in medicine. Cytotoxic T-cell therapies are at the forefront of this revolution, having become an integral part of the approach to the treatment of challenging infections and cancer. In this article, we consider the role that regulatory cellular therapies could play in preventing or controlling the pro-inflammatory state of transplant rejection.

The emergence of cellular therapy as a therapeutic option in these clinical settings is the culmination of decades of dedicated work unequivocally demonstrating their potential in experimental models [1]. Much of the focus of these efforts

has been on demonstrating the role that regulatory T cells (Tregs) play in the control of immune responses. The power of Tregs is highlighted by the immune dysregulation that results from their absence. A delicate balance exists between effector immune function and its regulation that results in appropriate immune activity that does not harm the host. Tregs play a key role in this balance, by preventing an overshoot of immune responses or autoimmunity whilst ensuring effective pathogen clearance and tumour surveillance.

THE PROBLEM

Current clinical practice for transplantation is centered on the administration of a variety of broad immunosuppressive medications with a spectrum of effectiveness and associated off-target toxicities. To facilitate successful transplantation many centers use an immunosuppressive regimen that includes the administration of an induction agent that typically results in a profound systemic depletion of leukocytes (e.g. anti-thymocyte globulin (ATG) or alemtuzumab), alongside life-long treatment with medications that primarily function through inhibition of lymphocyte activation (calcineurin inhibitors) and proliferation (mycophenolate, azathioprine). Such agents may also be combined with low doses of glucocorticoids to further dampen the immune response. The resulting broad immunosuppression results in an increased susceptibility to life threatening infections and malignancy as well as an increased risk of diabetes, cardiovascular disease, and nephrotoxicity. In many cases patient survival is curtailed not by the original pathology requiring transplantation, but by the off-target effects of immunosuppression. This is an approach which has not changed significantly in the past 40 years. Very few modern medical therapies demand this degree of compliance in the face of such serious side effects.

Cell based therapies have the potential to revolutionize the care of transplant patients [2]. Clinical trials in kidney and liver transplantation are now leading the way in bringing cellular immunotherapy into the non-cancer clinical setting. Demonstration of safety and efficacy in the transplant setting will contribute to the growing body of evidence and help pave the way for more widespread introduction of cell-based therapies in the field of autoimmunity.

A strong body of pre-clinical evidence and advances in manufacturing have meant that cellular therapies are now able to be produced to a standard acceptable for clinical use [3]. The field of transplantation has been quick to adopt this emerging possibility. Between 2010 and 2017, the EU FP7-funded ONE Study consortium explored the potential for cell therapy in live donor kidney transplantation [4]. Using a unified approach, each team assessed the use of a specific regulatory cell population together with an immunosuppression regimen standardized across the consortium. Cell types assessed included Tregs, antigen-reactive Tregs, regulatory macrophages (Mregs), tolerogenic dendritic cells (DCs) and Tr1 cells. The comprehensively designed study sought to explore the safety of cell based regulatory therapies and included immune monitoring studies to assess the impact of treatment

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on the immune phenotype and reconstitution of recipients. While a number of regulatory cell types were assessed within the framework of the ONE Study, we have opted to pursue polyclonal Tregs for further clinical assessment. Building on our experiences as investigators in the ONE Study, we have now commenced recruitment to the Transplantation Without Overimmunosuppression (TWO) Study, an MRC DPFS-funded Phase IIb randomized controlled trial of autologous polyclonal regulatory T-cell therapy with controlled immunosuppression reduction in living donor renal transplant recipients as a means to assess efficacy. The TWO Study includes thorough immune characterization to ensure that any changes in phenotype as a result of therapy are detected with appropriate power and depth for interpretation. In a clinical field where early patient outcomes are often excellent, a clear understanding of the changes in immune phenotype over time may be the best measure of a successful outcome [5].

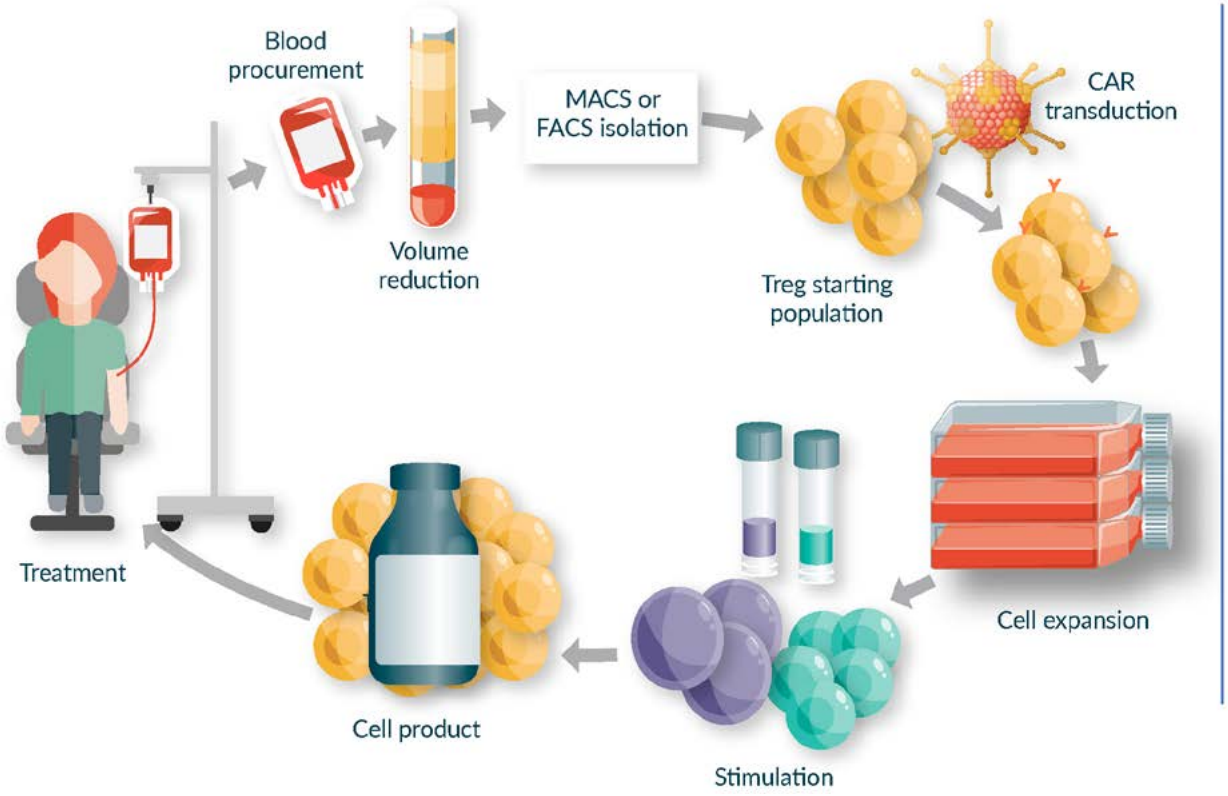
TREGS AS A CELLULAR THERAPY

Treg have the ability to suppress pro-inflammatory effector T-cell responses and in doing so facilitate long-term transplant survival in the absence of immunosuppressive medications. In our experience, Tregs can be easily isolated from a single unit of peripheral blood taken from kidney transplant recipients [6]. We have used a magnetic bead separation system to preferentially select a $CD4^+CD25^+$ T-cell population that is enriched for $FOXP3^+$ Tregs. Once isolated, Tregs can be expanded polyclonally using anti-CD3 and anti-CD28 bead stimulation together with recombinant human IL-2. The addition of rapamycin to the culture medium helps reduce contaminant growth. Others are exploring the use of flow cytometric cell sorting as a method to improve pre-culture purity [7]. The resulting cell product is a significantly expanded autologous polyclonal Treg population with high expression of Treg associated phenotypic markers and potent suppressive function in *in vitro* assays **(Figure 1)**. The cell product may then be cryopreserved for future use.

Naturally occurring Tregs isolated from peripheral blood, as used in our studies, are polyclonal in nature and thus raise the possibility of the development of a detrimental non-specific immunosuppressive effect. However, we anticipate that these somatic cells exist peripherally as part of the physiological repertoire that appropriately suppresses inappropriate effector responses that may, for example, lead to autoimmunity. Accordingly, our expectation is that the risk of undesirable non-specific immunosuppression is low. It is suggested that up to 10% of the immune repertoire is potentially alloreactive through the unique mechanisms of direct antigen recognition that occur in transplantation and through cross reactivity. This phenomenon is exploited to provide protection against rejection of a transplanted organ through a simple boost of Treg proportions without compromising overall host immunity. Nevertheless, the attraction of antigen-specific immune regulation remains strong and is likely to be a focus of attention over the coming decade.

FIGURE 1

Schematic of clinical-grade Treg production methodology.



Tregs are isolated by MACS or FACS from volume-reduced whole blood or leukapheresis products. Isolated Tregs are then expanded directly or transduced with a CAR before expansion. Cells are stimulated with anti-CD3/anti-CD28 coated beads or allogeneic antigen presenting cells in the presence of rapamycin or all-trans retinoic acid to enhance Treg purity and proliferation. The final cell product may be cryopreserved for later intravenous administration or infused directly following expansion.

ANTIGEN-REACTIVE TREGS

Donor-specific Tregs were manufactured and trialed for the first time in kidney transplant patients as part of the ONE Study. Donor and recipient leukocytes were cultured *in vitro* in the presence of co-stimulatory blockade resulting in expansion of a donor-specific Treg pool. This approach would likely permit a donor-specific treatment alleviating any fears of a non-specific suppressive effect and therefore provide therapeutic efficacy at a lower cell number than required in the context of polyclonal Tregs [8,9]. However, there is concern as to whether manufacture of sufficient cell numbers is possible given the smaller precursor frequency of cells able to respond to donor antigen compared to polyclonal stimulation. Furthermore, the more challenging culture conditions required to stimulate in an antigen-specific manner may further limit the ability to scale up such methods reliably to consistently manufacture a reliable cell product. Finally, donor-specific Treg have been shown to have greater suppressive capacity than similar numbers of polyclonal Treg in response to alloantigen. However, this is likely to be a reflection of increased precursor frequency to

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respond to donor antigen amongst the donor-specific Tregs rather than being attributable to an intrinsically increased suppressive capacity. As such, whilst it may be possible to achieve a therapeutic effect at a lower cell number using donor-specific Treg, whether donor-specific Treg confer any benefit over a polyclonal Treg population given at a higher number remains to be seen.

GENETIC MANIPULATION FOR ANTIGEN SPECIFICITY

Recent breakthroughs in the clinical use of chimeric antigen receptor T cells (CAR-T) in patients with hematological malignancies have raised the enticing prospect of generating a CAR-Treg cell product for use in transplantation and, potentially, autoimmunity. CAR-T cell therapies act by promoting an immune response against specific tumor antigens, and trials to date have revealed this to be a promising therapeutic option. However such treatments come at a cost: Kymriah, a CAR-T cell product used to treat patients with large B cell lymphoma has a list price of £282,000 per treatment. Moreover, there is evidence that CAR T cell effector activity may result in harmful systemic inflammatory responses. Although this should be less of a concern in the context of CAR-Tregs that are designed to suppress inflammation, any instability in Treg phenotype could prove detrimental.

CAR-Tregs are manufactured by isolation of Tregs from the proposed transplant recipient and modification of their genome such that they express a CAR on their cell surface. The CAR is designed to recognize an antigen exclusively found on the donor organ leading to preferential homing to and local activation of the Tregs within the target organ of interest [10,11]. Transplantation lends itself to this approach as the HLA mismatch between donor and recipient provides a clear source of donor-derived antigens to which a CAR receptor can be directed. The past 2 years have seen the emergence of two commercial companies dedicated to the development of CAR-Tregs for clinical transplantation: TxCell/Sangamo Therapeutics and Quell Therapeutics. The initial focus is on the generation of CAR-Tregs capable of recognizing the HLA class I antigen HLA-A2, with each company using a proprietary CAR. This is an opportune target as up to 50% of donors may be HLA-A2 positive, meaning that the likelihood of an HLA-A2 positive organ being given to an HLA-A2 negative donor is high.

While an exciting prospect, it remains to be proven that CAR-Tregs have a benefit beyond that seen with polyclonal Tregs. Although such cells may have increased antigen specificity, stability and associated increased suppressive function this may well be explained by their high alloantigen-reactive precursor frequency. Whether the anticipated significant costs of CAR-Treg therapy translate into a clinical benefit remains to be seen. Progress towards commencing clinical trials of CAR-Tregs is therefore underway and the highly anticipated results of these are likely to emerge in the coming decade. Should initial trials of CAR-Tregs prove successful then this may pave the way for a future in which 'off the shelf' CAR constructs targeted against any HLA mismatch could be transduced into recipient Tregs, permitting widespread application to all

transplant recipients. In living donor transplantation such treatment could be planned in advance and administered at the time of transplantation, whereas for deceased donor transplantation in may be possible to administer treatment in the post-transplantation period once the donor mismatch in known. Finally, specific CAR constructs could be manufactured that recognize any number of known antigens contributing to autoimmune disease [12]. There is clearly the possibility of a readily available cellular therapy accessible to a wide range of specialties and with the potential to revolutionize the field of medicine [13].

TRANSLATION INSIGHT

Despite encouraging progress over the past decade, a number of fundamental questions remain unanswered in the field. Firstly, the dose of cells required to provide efficacy whilst maintaining safety is currently unknown. In the ONE Study, we infused patients with polyclonal Tregs at doses of $1x10^6$ cells/kg to $10x10^6$ cells/kg using a traditional 3+3 dose escalation design. Such doses represent a boost of around 5 to 8% of the total endogenous Treg pool. Whether this is sufficient to demonstrate a clinical benefit is yet to be proven, particularly given data from animal models where a 100% boost in Treg numbers is often used. At Northwestern University, $5x10^9$ total cells were administered to kidney transplant recipients without any reported complications [14]. Whilst this is the current situation for polyclonal Tregs, those pursuing antigen-specific or CAR-Tregs will likely require lower doses to achieve the same clinical efficacy.

Another unknown is the impact of timing of administration. This is particularly relevant for transplantation, where different clinical scenarios provide the potential for pre-transplant, peri-transplant, early or late post-transplant administration. In the ONE Study we initially treated patients with polyclonal Tregs 5 days post-transplantation. In the TWO Study we have introduced lymphocyte depleting induction therapy with alemtuzumab (anti-CD52) and therefore delayed administration of the Treg cellular therapy product to 6 months post-transplantation when studies suggest there might be more conducive environment for Treg function [15]. Such an approach, if proven beneficial, will likely be more widely acceptable to the transplant physicians who might otherwise be reluctant to give up induction immunosuppression with its proven benefits to transplant outcomes.

It is also unknown whether a single administration of Treg therapy will be adequate. While we envisage that a single dose would alter the immune repertoire permanently, it may be that further doses of Treg are required to achieve this, although there is very little experimental evidence to support a multiple dosing strategy.

CONCLUDING REMARKS

Over the past decade we have made a smooth transition from pre-clinical studies to Phase I and II clinical trials of Tregs in transplantation. It is likely that the next decade will bring answers to the most important questions of Treg efficacy, safety and dosing strategy. In parallel, early phase trials will assess personalized

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gene-edited regulatory cell therapies. Given the many unknowns at this early stage of development and the high costs of producing these treatments, it is essential that those of us taking the first steps are quick to report our experiences and share with others collaboratively. Such an approach will facilitate the redesign and optimization of emerging clinical trials to take into account current data. Beyond the current stage of development, later phase clinical trials may need to employ composite outcome data that includes robust immune monitoring to give the best chance of assessing efficacy and safety.

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SPOTLIGHT

INTERVIEW

Oncology and beyond: looking to the future of CAR-T and autologous cell therapy

STEPHAN GRUPP is the Chief of the Cellular Therapy and Transplant Section, Director of the Cancer Immunotherapy Program, and Medical Director of The Cell and Gene Therapy Lab at the Children's Hospital of Philadelphia (CHOP), as well as the Yetta Dietch Novotny Professor of Pediatrics at the University of Pennsylvania Perelman School of Medicine. Dr Grupp graduated from the University of Cincinnati after completing the MD/PhD program with a PhD in Immunology. He completed pediatric residency at the Boston Children's Hospital, followed by a fellowship in Pediatric Hematology/Oncology at the Dana Farber Cancer Institute and postdoctoral work in Immunology at Harvard University. He then joined the faculty at Harvard University until 1996, when he came to CHOP. His primary area of clinical research is the use of CAR T and other engineered cell therapies in relapsed pediatric cancers. He led the pediatric ALL trials of CTL019 (now approved as Kymriah), including the largest and most successful engineered T-cell therapy clinical trial conducted to date, as well as the global registration trial for CTL019. As a result of this work, he presented the Clinical Perspective at the July 2018 FDA ODAC meeting, at which reviewers voted 10–0 for recommendation of approval for Kymriah in pediatric ALL. His primary laboratory interest is the development of new cell therapy treatments for pediatric cancers. Dr Grupp was elected to the National Academy of Medicine in 2019.

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Q What are you working on right now?

SG: I run the cancer immunotherapy, cell therapy and transplant programs at Children's Hospital of Philadelphia (CHOP). We're a free-standing children's hospital as well as being academically part of the University of Pennsylvania, where I am a Professor of Pediatrics.

There are two major areas that we're working on currently. One is to deal with the increasing number of patients that need access to this therapy – both at my own institution and around the world, as cell therapies for leukemia and lymphoma roll out in many countries. That takes up a great deal of my time at the moment.

Then from a cell therapy R&D point of view, we have a major interest in engineered stem cell therapy – for instance for thalassemias, hemoglobinpathies and the like. We are seeking to extend what we're already seeing with CAR-T in oncology. Then specifically in the CAR-T world, we're looking to expand its reach to other types of leukemia, such as AML. And like everyone else, we are hoping to see some action in the solid tumor area.

Q Can I ask you to look back on the momentous decade for cell & gene therapy that is just drawing to a close? What for you were the most significant successes (and failures) which have brought the field to its current state of partially realized potential and continuing promise?

SG: Heading into 2010, it was not at all clear that CAR-T was anything to shout about, or even that it was going to work. There had been a lot of failed studies over the decade prior to that, and going even further back – a lot of observations that you could make cells that looked good at the end of manufacturing, leading to cell therapies that were non-toxic but with no efficacy whatsoever.

What's still amazing to me is the step change that occurred around 2010 from nothing working to at least a small handful of things working amazingly well. That caught all us by surprise, to be honest – that was an incredible thing to see happening. What we saw was the first few patients with both astonishing efficacy but also very significant toxicity that we didn't understand at all at the beginning. Trying to figure that out on the fly was an extraordinary experience: having patients critically ill as a result of CAR-T cell therapy, figuring out what was actually going on from a pathophysiologic point of view and then implementing blockade of interleukin 6 as a way of controlling cytokine release syndrome.

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"I want to see way more immuno-oncology – CAR-T and other engineered cell therapies in the oncology sphere – and … I'd like the reach of engineered cell therapy to have extended beyond oncology."

We started to see what the rules might be, learning something from every new patient. That led to us embarking upon a 5-year lead up from the first ALL patient to FDA approval of the first CAR T cell immunotherapy product ever, which I think was a pretty impressive timeline.

Q Reflecting on your journey at CHOP/UPenn over this same period, how has your own work and focus evolved in step with progress in the wider field?

SG: From a lab guy's point of view, what's been amazing in my own personal journey – in addition to seeing these incredible clinical responses in the kids we take care of with uncontrolled leukemia – is trying to implement this in the larger world. It's a different ballgame, trying to understand the policy implications and to convey the science to lots of different stakeholders who are seeking to understand this brand-new field of medicine.

Thinking about issues like insurance and policy had never been in the purview of anything I personally had to worry about previously. Thinking about the safe rollout of these highly novel, very powerful therapies to multiple hospitals around the world. Being involved with the first international registration trial for a cell therapy product.

What we have done is go from an initially small number of successes, followed by building the scope: building the scope in our own setting at CHOP; building the scope across multiple institutions and multi-site trials; building the scope of rolling this out in multiple hospitals worldwide.

It's really astonishing to me just how quickly this is being adopted.

Q As the decade turns, what would you pick out as the most important challenges facing the cell therapy field as a whole today, and how might they be best addressed moving forward?

SG: In terms of CAR-T, I think the obvious question is 'how do we make this stuff work in solid tumors?' We don't have the recipe for that yet. We've got multiple possibilities for how that's going to work, but we have yet to experience that same step change moment in solid

"I think the next thing is really how do we implement the tools we already have? For instance, how do we deal with the fact that the current marketed therapies are autologous and so you have to make a product for every patient?"

tumors that we had in hematologic malignancies. So that's something we're all fascinated by and working on, and I have every confidence we're going to start seeing some answers to those questions in the next couple of years.

I think the next thing is really how do we implement the tools we already have? For instance, how do we deal with the fact that the current marketed therapies are autologous and so you have to make a product for every patient? How do you deal with the expense and the length of time associated with making such products, which can be anywhere from 17 to 22 days? Can we reduce that timeframe? And can we make it cheaper? Can we do things in a more automated fashion?

I would really like to see the drug companies that are pushing CAR-T therapies into the market right now show their ability to invest, innovate and completely automate cell manufacturing. I think that for the cell therapies we have now, that's an absolute requirement. And in future, if anything works in a more common tumor type than the leukemias and lymphomas where we've seen CAR-T work to date, these issues of scaling-up and doing thing faster, cheaper, in a more automated fashion, are going to be the whole game. Because you can maybe do this for a thousand patients with current processes and technologies, but you can't do it for fifty thousand.

Q More specifically, where do you see a particular need for further cellular immunotherapy innovation in the pediatric oncology sphere – what would you like to see the field focusing on in this regard?

SG: The first thing to get approved in CAR-T was for a pediatric indication – for pediatric and young adult patients with ALL.

So let's not forget the kids as we move forward. For one thing, immuno-oncology obviously includes not just CAR-T but checkpoint inhibitors as well – however, checkpoint inhibitors have activity in almost no pediatric diseases. There are just a couple of exceptions to this rule at the moment. To me, that just makes it even more imperative that we keep developing

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CAR-Ts for pediatric indications. I think you can make a business out of it, but beyond that, it's just really important that the field doesn't forget: the kids brought us here in the first place.

Looking further afield in cell therapy than CAR-T, I think engineered stem cell therapies for a variety of monogenic disorders are going to be a huge part of the story over the next 4 or 5 years. I already mentioned haemoglobinopathies and thalassemias, but there are other metabolic and neurologic disorders that are coming through quickly. There are in-born errors of the immune system to be corrected, too.

To someone like me – a bone marrow transplanter – all of these things carry the potential to replace allogeneic bone marrow transplant, with its toxicities including graft versus host disease, with autologous transplant that is going to be much, much safer.

You can look to do these things in adults but we're really looking to cure these disorders earlier in life, and that obviously means kids. I think engineered cell therapy is going to be a big part of pediatric healthcare and diseases in general moving forward, not just oncology.

Q Can you expand on your expectations in terms of specific cell & gene therapy technologies and modalities coming to the fore over the 'twenties' to come?

SG: I want to see the first gene therapy for sickle cell disease approved. There are hundreds of thousands of people affected with sickle cell disease and while allogeneic transplant is a potentially curative therapy, there are many challenges with it, including the fact that a large number of patients aren't eligible to receive it. Having a real curative therapeutic option for those patients is something that is incredibly important from my point of view.

> "I would really like to see the drug companies that are pushing CAR-T therapies into the market right now show their ability to invest, innovate and completely automate cell manufacturing. I think that for the cell therapies we have now, that's an absolute requirement."

Moving CAR-T into other kinds of leukemia that involve different targets, like AML, for instance, is another very important area. I think that in the next year or so we're going to see great data on this – we'll see how it's going to work.

And as always, we come back to the ability to successfully target solid tumors – that's what everyone wants.

Q What is your vision for oncology healthcare by 2030, particularly in terms of the overall approach to fighting cancer given the evolving therapeutic toolbox?

SG: What I desperately hope is that by then we're seeing way less chemotherapy. I'm also looking for less allogeneic transplant – as I'm a transplanter, that might be a surprising point of view to some. But I want to see way more immuno-oncology – CAR-T and other engineered cell therapies in the oncology sphere – and as we've discussed, I'd like the reach of engineered cell therapy to have extended beyond oncology.

By 2030, I see us doing much more of this sort of engineered cell therapy across the spectrum of human disease. I would like to see the field really embracing the notion and delivering on the promise of cell and gene therapy being a 'one and done' therapeutic option: that you do this once, your patient is potentially cured of their disorder and they don't need any further intervention. That for me is where the real promise of cellular therapy lies and that's what we have to deliver on.

Q Finally, what do you hope to achieve in your own work over the decade to come?

SG: I'd like to see the completion of the safe rollout of CAR-T therapy to centers all over the world – to see this therapy become even safer so that we have the potential to offer it in smaller centers and in countries that are not necessarily right on the quaternary cutting edge of medical care.

In addition to that, if we can have a couple of successes outside of oncology – as I mentioned, in sickle cell diseases and the like – then in my own work, that would represent a very satisfying next decade.

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SPOTLIGHT

PODCAST INTERVIEW with Markus Gershater,
Chief Scientific Officer at Synthace

"As an industry, we need to raise awareness that these capabilities are out there and also support people on their journey towards realizing the value from them."

Introducing flexibility to automation to unleash the power of biology

Markus co-founded Synthace after working as a Research Associate in Synthetic Biology at University College London where he developed novel biosynthesis methods using pathway engineering. Prior to UCL, he was a Biotransformation Scientist at Novacta Biosystems working as part of the industrial biotechnology group that conducted more than 90 contract research projects for over 20 clients. Markus has a PhD in Plant Biochemistry from Durham.

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Q Can you give us some brief background on what Synthace does, and why it has relevance to current trends and challenges in cell and gene therapy?

MG: Synthace is fundamentally about automation: automatic lab processes and also the data processes associated with those experiments.

The fundamental issue is that automation is inflexible. Once you have a process programmed then it can do that one thing, but as soon as you want to change something it becomes an issue. We make software that allows the flexible reprogramming of automation on both the lab and the subsequent data processing sides. This enables an escape from low throughput manual processes, which despite being arduous, variable and error-prone, are still the norm in biological R&D today.

This includes cell and gene therapy, of course, but it is not limited to it – biological R&D across the board is a lot more manual than we would all like it to be, and this is purely down to the inherent inflexibility of traditional automation. With a more flexible approach, we can bring automation into this space a lot more than was previously possible – that's the potential we are seeking to unlock.

The interesting thing is that we started out as a bioprocess development company, not as a software company. We were looking to do ever more sophisticated experiments, for which we ultimately needed automation, and so we built the software to program that automation in the lab. Our chief focus was on addressing the complexity of biology for our own ends, but we came to realize that this software could enable not just our own lab work but everyone else's, too – so it became a product its own right and now it is at the core of our business model.

> "Our chief focus was on addressing the complexity of biology for our own ends, but we came to realize that this software could enable not just our own lab work but everyone else's, too."

Q What differentiates Synthace's data message from those of other solution providers in the cell and gene therapy space?

MG: For me, this comes down to the fundamental way in which people are thinking about digitization as a whole. They tend to think, "OK, the digital output of what we're doing is data, therefore all of our digitization problems have to do with what we want to do with that data."

We see things differently. We want the digital world to reach much further into what we're doing – to play an active role in helping us to define a much more sophisticated experiment in the first place, which then produces these data.

Because the digital world is already integrated with the experiments, we can better understand the context and structure of the data that is produced. We kind of flip the whole problem on its head: it's not a question of, "we have this huge mass of data from our experiment – what can we do with it?" Instead, it's about how we are generating the data in the first place. This approach gives us much more direct insight into the biology than we would get if we were trying to piece everything back together from all the various data received from different pieces of lab equipment.

Q Tell us about some of the key common misconceptions you encounter regarding data strategy in this relatively immature sector.

MG: Firstly, I'd mention that I don't think this is limited to cell and gene therapy. Actually, the entire biopharma industry seems to be thinking about this in the reactive sense of what to do with all this data it's generated.

Currently, there are people throughout the industry stating that they want to employ AI to transform their sector. We fully agree that AI will transform the industry, but it will only do so once the fundamental basis that is the routine production of structured, beautiful data sets is in place. The data are there solely for us to gain a better understanding of biology, whether or not that's through efforts helped or augmented by AI. An issue I see at the moment is that people are seeing data and experiments in isolation, as their own things, instead of seeing the data as the natural product of experiments, which are themselves there purely for us to gain insight into the biology.

So we see the need for a much deeper digitization strategy, which boils down to removing all of the obstacles between us and the biology. These obstacles are all the manual data structurings and other pieces of manual work that are typically required. We're basically trying to disintermediate between the biologists and the stuff they're trying to work with.

This idea of automated sophisticated experiments naturally producing sophisticated data sets feeds into the future potential for things like machine

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> "Cell and Gene Therapy is a field where the therapeutic modalities are more complex by orders of magnitude than anything we've had to deal with before in the biopharma industry."

learning, which in turn will enable the design of even more sophisticated experiments which can be carried out in an automated way. We call this overall ecosystem of tools and capabilities computer-aided biology (CAB).

CAB is quite a specific route towards unblocking or unleashing all of the powers of the digital world within biology – to harness them to help us really grapple with biological complexities, especially in the area of cell and gene therapy. This is a field where the therapeutic modalities are more complex by orders of magnitude than anything we've had to deal with before in the biopharma industry.

Q What changes in both culture and enabling tools are needed to facilitate a shift to a stronger data strategy in cell and gene therapy companies?

MG: We envisage an ecosystem of tools that enable automation in the lab and data structuring – that's where we see ourselves playing. But it's important to be clear that we're not trying to do everything ourselves.

When the structured data are produced, there are all sorts of ways in which they might then be analyzed. Anything from basic statistics all the way through to very sophisticated, deep learning methodologies could be used, according to what is most pragmatic and appropriate at the time.

There will of course be lots of companies out there developing those kinds of tools. When I talk about automation in the lab, we are not actually making the hardware. We're relying on a fantastic ecosystem of physical tool providers – of automation manufacturers and analytics manufacturers. What it's really about is the seamless integration of these fantastic physical tools that are already out there, and of new ones that might come through in the future.

At the moment, where this ecosystem exists, it is very fragmented. We believe that as an industry of technology providers to the cell and gene therapy space, we need to be thinking about the best ways of integrating not just in terms of the physical devices in the lab, but also between the digital tools. This will allow a cell and gene therapy scientist to be able to use whatever is most powerful for the task they need to carry out – for example, if they have a favorite bit of data visualization or exploration software that they really want to use, then they should be able to use it. There shouldn't be walls up between these different things that will slow everything down.

> "...as an industry of technology providers to the cell and gene therapy space, we need to be thinking about the best ways of integrating not just in terms of the physical devices in the lab, but also between the digital tools."

Regarding culture, I don't expect scientists to have to change dramatically in order to use the tools. We see scientists as being very much within the loop. You sometimes hear people talking about AI as though everything is automated and machine learning drives it all. But that would work only if the machine learning somehow had all the expertise of all the different scientists coded into it somehow. To me, the optimum strategy would be to take all the power of machine learning and all the expertise of the scientists and bring it together.

This means you are creating tools that augment the capabilities of the scientists, they don't replace them. The cultural shift is therefore really one that means we're looking for people to start using these tools and start realizing what that means for how they can go about their science. It isn't some sort of fundamental thing where everyone has to learn to code, for instance (although that is another misconception one sometimes hears). While there will certainly be some parts of the industry where that will be very helpful, without a doubt, the main thing for a scientist to think about is, "OK, now I can now do these much more sophisticated experiments within the lab, what does that allow me to do as a scientist? What problems does that allow me to address?"

The real shift is one to a much more systematic way of doing experiments – moving away from a kind of stepwise exploration of a space to a much more comprehensive characterization of a particular biological system. That's what's being enabled by these kind of tools – that's the power they offer.

Q Let's talk about Design of Experiments (DOE) and its utility in the cell and gene therapy space. Firstly, how is DOE being implemented in the wider biopharma space today, and with what impact?

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MG: DOE is a somewhat unhelpful term in that it refers to something very specific, although the name doesn't really suggest that.

What it actually relates to is multi-factorial experimentation. The traditional approach to experimentation is to look at one factor at a time as you go along. For example, you might firstly look at the impact of temperature, then move on to the effect of a particular cytokine, and so on. DOE takes the alternative approach of asking what are all the different things that might affect our process, and how can you prioritize a subset in order to investigate all of them simultaneously?

For people who are less familiar with the mathematics involved, this sounds very unlikely to succeed. But it's actually a very well-developed branch of maths and it's been used for decades, although unfortunately, not nearly as much as it should have been in biology.

Biology is fundamentally an interconnected system, where you have lots of things coming together and then phenomena emerging out of the combination of lots of different simultaneous factors. What DOE allows is an unpicking of all of those different interactions in order to get to the underlying cause or causes. Getting to those causes enables you to really address the complexities inherent in biology.

In industry at the moment, that power to understand something more holistically is being applied only where it's absolutely critical that biological complexity is properly nailed down. For example, when we're producing a therapeutic, we want to know we're producing something that's going to help people and not hurt them. That process must obviously be exceptionally well understood and so the FDA demands that DOE is used as one of the methods of characterizing a biological process that's going to make a product.

But unfortunately, from my perspective, it is mainly being used as this kind of regulatory compliance tool, as opposed to a tool with enormous power not just to help you understand a biological system, but to enable you to engineer that system a lot more predictively than would otherwise be possible.

I think that cell and gene therapy has this opportunity to not just use DOE as a tool for regulatory compliance, but to wield it to help address the extremely high levels of complexity within the space. If we can get to that higher level of understanding, it will result in all of the things we are producing become that much more scalable, that much more tractable, that much more engineerable. We'll be able to roll these products out to all the patients who need them, as opposed to the few we've managed to treat as a relatively nascent therapeutic field to date.

> "...things get really exciting when you don't just use Design of Experiments and automation in isolation, but in conjunction. At that point, you can do high throughput, sophisticated experiments."

However, things get really exciting when you don't just use DOE and automation in isolation, but in conjunction. At that point, you can do high throughput, sophisticated experiments.

High throughput has been used before in the therapeutics industry, of course, for things like screening. Those are often pretty unsophisticated experiments, though – you're basically just posing the same hypothesis millions of times. DOE, on the other hand, can pose far more sophisticated hypotheses in a much more holistic way.

If we can take these sophisticated ways of experimenting and make them high throughput, then what can we achieve? Well, what's really exciting at the moment is we're just starting to see the impact within cell and gene therapy of exactly this kind of method. For example, Oxford Biomedica has been using Antha for a number of years now, and we released a joint case study where they used automated DOE to optimize transfection and transduction at the heart of their lentiviral vector production process. They got an order of magnitude increase in yield from properly addressing the different factors that might affect that transfection/transduction.

Beyond the very positive result, you could also see them starting to change the way they think about their science. So we come back to the cultural aspect: you give people new tools and new capabilities, and these things become transformative. It's quite remarkable when you first start using them to see just how powerful they can be. You often find people who haven't used DOE before become really evangelical about it, because of the step change in the amount of power it provides to address biological complexity. The next thing those individuals ask themselves is 'what other problems can I apply this to?' In this way, it becomes a part of their thinking, and without the need for a major cultural shift, these quite transformative tools become endemic within an organization.

Q Can you go deeper on where specifically you see DOE bringing benefits to the cell and gene therapy field? And what will be the key obstacles to overcome before its full potential can be realized in this space?

MG: DOE is a statistical tool. It's a general method of being able to pose lots of sophisticated hypotheses simultaneously. In that respect, it can be used extremely widely.

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> "We can use Design of Experiments to make sure that all the different components of a media are properly balanced, which in turn ensures we're differentiating robustly to a particular cell type..."

In our own labs, we use DOE whenever we have a process to optimize. For instance, if we have an analytical process that has too much variance and we need to tighten up those error bars, then we can use DOE to make sure it's just that much more robust – that the precision is really dialed in and we get high quality data from it.

You can use it on much more complex processes, too, as in the case of Oxford Biomedica. We've also used it for optimizing all sorts of molecular biology methods in the lab, as well as cell growth methods and media optimizations. We can use DOE to make sure that all the different components of a media are properly balanced, which in turn ensures we're differentiating robustly to a particular cell type, for one example, or that we can make organoids in a robust and reproducible manner, for another.

Wherever there is any complexity, then we need to be using these more powerful tools in order to be able to ask more sophisticated questions. And in cell and gene therapy, complexity is everywhere!

We can also think about how DOE can be applied in the cell and gene therapy space in a similar way to how it's already being applied within the broader biotherapeutics area – in antibody production, for example. That relates to how we can use DOE to really understand the production processes that are required to make our therapies. Once we understand the production processes that much better, then we know that even given the diverse inputs that we often encounter with cell therapies in particular, we're always going to get to a high-quality product that is suitable for the patient.

The issue with DOE is that there is a learning curve. This is a different way of thinking about science to the way we are all taught through school and university. Indeed, I think it's one of the major problems we face with the way science is being taught today: these much more powerful experimental techniques are just not 'baked in' from the start. This means there is a bit of a cultural shift to negotiate, a bit of a knowledge gap and also a trust gap. These tools sound really powerful, but I'm sure there are a lot of people reading who remain skeptical, and that's entirely correct. As biologists, we should be skeptical about things – I was profoundly skeptical before I started using them. But when you start to see the data coming out, that's when you get excited.

So there is this gap between first hearing about it and actually receiving those first data and becoming really excited about what they're showing you. It is not something that is significant, but it does need to be addressed. As an industry, we need to raise awareness that these capabilities are out there and also support people on their journey towards realizing the value from them.

Q Why is it so important to push Quality by Design (QbD) further upstream in cell and gene therapy R&D?

MG: What is QbD? QbD is basically a system, a framework in which you can think about all of the process that you're addressing, all the biology, and consider what are all the things that could contribute to that biology not working.

You start off with something called the root cause analysis. This is where you consider all the different inputs and ask which of them could vary, or what happens if the lab temperature is different from this day to that day, etc. There are lots of different things that could contribute to variability or failure within biology.

QbD therefore begins with really in-depth thinking, which I think is something we don't pursue a lot of the time – we tend to think about the things that are more immediately in front of us, as opposed to all of the things that could potentially go wrong. I guess it's quite a negative way of thinking!

But what it does give you is a list of all of these different things that could result in or contribute to problems further down the line. And we do have the tools to address a lot of these things. For example, you can then use DOE in order to explore the potential issues systematically and see which ones really matter, and which ones might not.

In our own labs, we use this kind of methodology just for routine lab tasks. For instance, you want a PCR to work every single time – well, if you actually do this kind of analysis and you do the experiments associated with it, you get that PCR nicely optimized and it will work every time. You don't have to go back and redo things. Fundamentally, what we're looking to do is build that foundation of quality, which then means we can proceed to the much more interesting and meaningful questions of how we can actually develop and produce these therapies in a really reliable and scalable manner.

Q What does the lab of the future look like to you, and what tangible steps and marginal gains can be achieved today to put cell and

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gene therapy companies on the right path to realizing this vision?

MG: I think 'lab of the future' means a lot of things to a lot of different people.

However, we think that within whatever vision people might have, there needs to be this component of automated lab processes, and then the automated structuring of the data that comes from them to make really high quality, contextual data sets. There could also be some kind of machine learning, which is usually another component of most people's labs of the future.

It's really just a subset of the overall lab of the future – there will be other technologies that are needed as well – but this computer-aided biology vision is something we're looking to define quite clearly. So in contrast to a much more expansive vision of the future, if you like, we're saying 'look, this is something that is obviously very powerful and that could also build the foundation for something a lot more exciting going forward, and these are the steps we can take to get there'.

Q Finally, can you summarize what needs to happen over the decade ahead if cell and gene therapy is to fully capitalize on the promise of automation and machine learning by 2030?

MG: I think it's actually a reasonable timeframe. I don't think it's too unrealistic. That's because there are a lot of pressing issues right now, and a lot of the sensible ways of addressing them are through the technologies we've been talking about. It's not as though we're expecting people to make a huge leap – in terms of culture, for instance, as we've discussed.

Overall, I'm pretty optimistic. I think there can be some clear arguments made that are based purely on hard-headed things like return on investment from automation, and how we can get better data integrity – higher quality data, data that is actually put in the context of the experiment it comes from. These are all perfectly logical things we want to do. Again, I don't think there needs to be a massive leap forward. In fact, when you do see people try to make that direct leap towards an AI-augmented future, they tend to spend a lot of time later making up for the fact that the foundations weren't really there in the first place.

So it's just about get those foundation things in place: making sure we're building on really high quality, automated protocols, both for lab and for data. I believe we will then get there quite naturally through the curiosity of scientists who are motivated to solve problems, because they're such important problems to solve.

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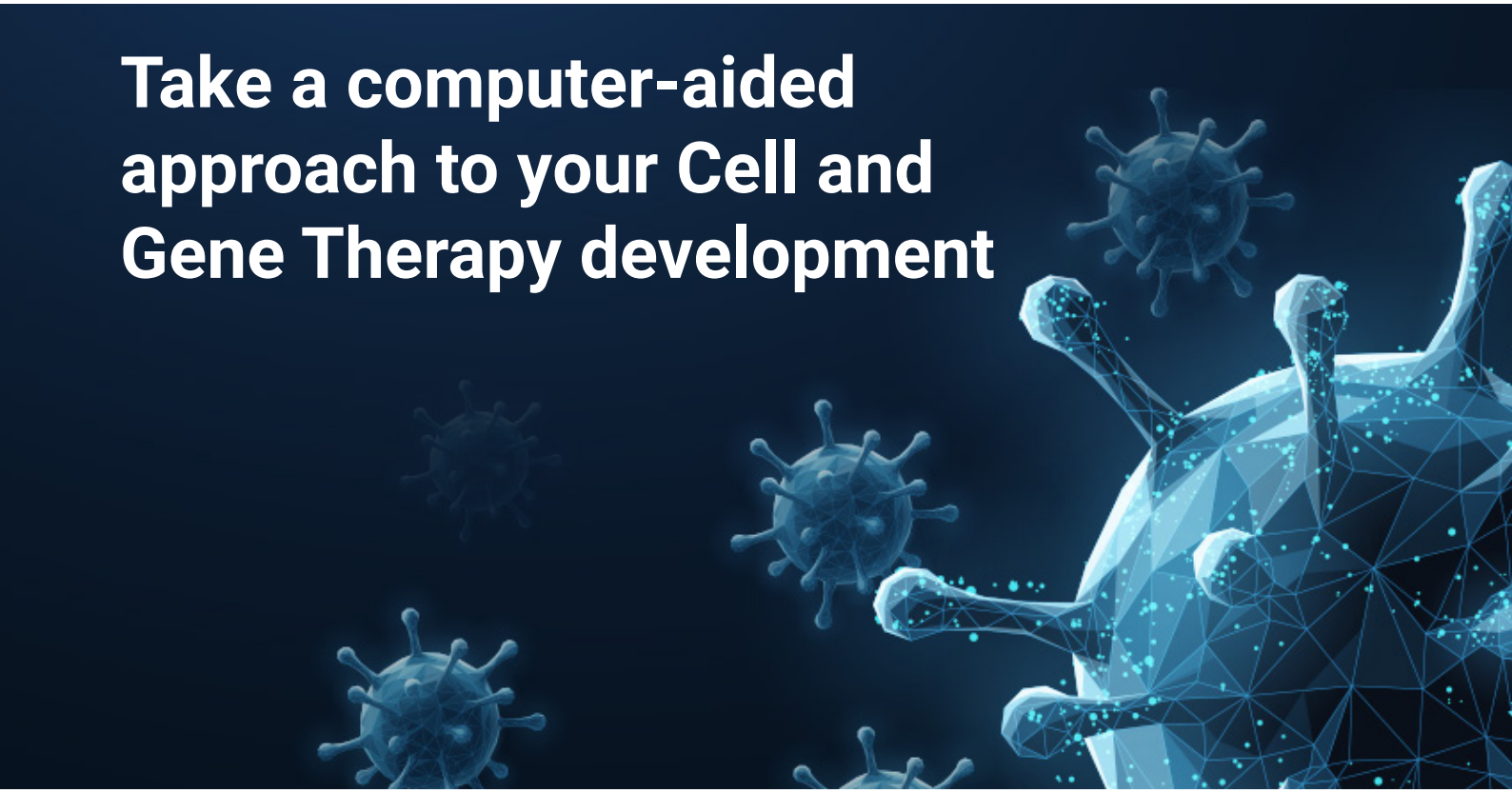

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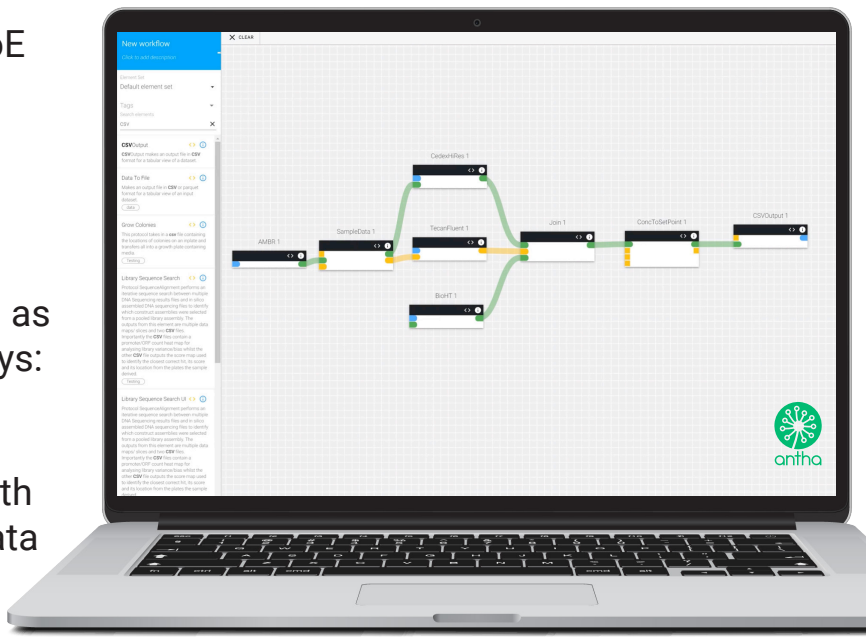

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SPOTLIGHT

INTERVIEW

Where will synthetic biology take cell & gene therapy?

TIM LU is a pioneer in synthetic biology and an Associate Professor at MIT. He received his MD/PhD from MIT and Harvard. He is a co-founder of Synlogic (NASDAQ: SYBX), Tango Therapeutics, Sample6, Eligo, BiomX, and Engine Biosciences. He has received the NIH New Innovator Award, the US President Early Career Award for Scientists and Engineers, MIT Technology Review's TR35, Navy and Army Young Investigator Prizes, and others.

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Q Synthetic biology platforms are rapidly coming to the forefront as cell & gene therapy enters a new decade – can you share your perspective on the broad benefits and advantages over other methods that they offer the field?

TL: Cell and gene therapy has experienced a real resurgence and renaissance over the last decade, especially in the last few years. However, what you're seeing with many of the current products in the clinic or approved is that they're still fairly simple.

Taking gene therapy as an example, you're engineering a vector, maximising expression of the transgene, then delivering it to patients with no control. And T cell immunotherapies are basically being genetically engineered to go after a single antigen — through a CAR, for example. But again, there's no real regulation of the therapeutic effect through engineering of those cells to enhance efficacy or safety.

There's a set of indications you can address with that sort of approach — for instance, in the case of diseases where you have a very wide therapeutic window and where some of the barriers to efficacy are perhaps lower. That's what we've seen with certain liquid tumours, such as B cell leukaemia, where one can completely wipe out the CD19 population without having to worry about dealing with barriers, such as the solid tumour microenvironment. Similarly, with *in vivo* gene therapy, people have been going after diseases where the therapeutic window is quite broad, meaning you can express a lot of your transgene without encountering significant toxicity issues. I think these approaches are great for patients and have really blown open the doors of the field.

Yet, for me, synthetic biology is the latest stage in the evolution of genetic engineering, in that it brings to the table the means by which to build the next wave of programmable medical treatments. For example, it allows us to broaden the applicability of cell therapies to solid tumour applications, where we know the solution is not going to be as simple as a single target CAR T. Equally, it can help drive in vivo gene therapy into diseases that are not rare or ultra-rare indications – or diseases where you have a narrow therapeutic window and must ensure you're either hitting the right cell type or controlling the transgene to a large extent.

Traditional genetic engineering consists of essentially cutting and pasting DNA from one place to another. However, what's really changed over the last decade, because of synthetic biology, is our ability not only to simply cut and paste, but also to write arbitrary genetic code. When you can do that, it becomes really powerful in terms of the biological programs that can be encoded.

At the same time, synthetic biology becomes more challenging, because you have a greater degree of freedom and, consequently, more ways of potentially screwing up the system. Nevertheless, I firmly believe it can provide a framework to do next-generation genetic engineering in a repeatable, robust fashion.

Q Turning to Senti's specific approach, can you give us some more background on how it works and its ongoing development pathway?

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"...what we've done is to engineer cell and gene therapies that can deliver multiple immunotherapy payloads within tumors and turn a "cold" tumor into a "hot" one."

TL: From a technology perspective, our specific approach to any therapeutic problem is to break up what cells do into one of three buckets:

- First, there's the idea of sensing – in other words, what do the cells respond to? We have technology that allows us to build artificial receptors or other sensors that can basically transduce information about a disease inside a cell, including disease biomarkers such as antigens, cytokines, etc. We can also engineer sensors for FDA-approved drugs so that cell and gene therapies can be titrated ON or OFF with the addition or withdrawal of these drugs.

- We have a second bucket of technologies around cellular decision-making – or more specifically, how you enable cells to take in multiple pieces of information, decide what to do with that information and then trigger a therapeutic output.

- The third bucket involves the actual outputs that cells produce. We think about cell and gene therapies as effective drug-delivery vehicles that can make not just one, but multiple biologically active molecules to treat a disease. For example, one output might be killing the offending tumour cells, while another could be secreting payloads, such as cytokines, that trigger a robust anti-tumor response by stimulating the tumour microenvironment.

Internally, we're very much focused on oncology applications, in which there are two major challenges we're trying to tackle that can be addressed by gene circuit technology:

The first is around dealing with solid tumours, which have many ways of shutting down the immune system. Specifically, what we're exploring is how to achieve highly potent immunotherapy locally in the tumour microenvironment.

In that regard, what we've done is to engineer cell and gene therapies that can deliver multiple immunotherapy payloads within tumors and turn a "cold" tumor into a "hot" one. To ensure that we trigger a robust anti-tumor response and stimulate the entire immune system, we're typically not just going after a single target in the tumour microenvironment. Instead, we're engineering cell and gene therapies to deliver two, three or even more complementary drugs. By doing this, we can get powerfully enhanced activity.

The second major challenge we're trying to tackle in oncology is the lack of good antigens. Many people, today, in the antibody and cellular

immunotherapy fields, are searching for single antigens that discriminate tumour from non-tumour cells. However, this remains a difficult challenge.

For one thing, tumours are heterogeneous, so they don't uniformly express a single antigen. If you only go after a single target, tumours can find ways of escaping by losing the target. For another, some of those antigens can be displayed on healthy tissue cells, meaning you can have off-target effects.

To tackle this problem, we are engineering cell and gene therapies that can make a tumor versus non-tumor decision based on multiple pieces of information. For example, we can build gene therapies that sense two cancer-specific signals and employ an AND gate to trigger a therapeutic response only when both those signals are present, thereby enabling greater precision. Or we can employ a NOT gate in which the gene circuit will only kill tumour cells expressing antigen A, yet not affect healthy tissues that may express antigen A, but also express a healthy tissue antigen B (the NOT signal).

Thus, by using gene circuits, we can enhance therapeutic efficacy against solid tumors by programming cells and viruses to deliver targeted combination therapy within tumors. In addition, we're using logic gates to create therapies that are highly selective for cancer so that we can address tumor heterogeneity, while minimizing off-target effects.

Q What are Senti's chief priorities and goals for the coming 12-24 months?

TL: First, we have built out an internal pipeline of really exciting cell and gene therapy programmes that we're translating into the clinic for oncology. Over the next 12 to 24 months, we anticipate being in the clinic with our first trials and starting to collect data demonstrating the utility of our gene-circuit-enhanced products.

"by using gene circuits, we can enhance therapeutic efficacy against solid tumors by programming cells and viruses to deliver targeted combination therapy within tumors. In addition, we're using logic gates to create therapies that are highly selective for cancer so that we can address tumor heterogeneity, while minimizing off-target effects."

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Second, we're quite passionate about continuing to invest in the platform. We want to ensure we stay at the forefront of what is a very fast-moving field and continue to build the leading technologies for programming biological systems.

And third – which is really important to us – is exploring how we can use these tools and technologies in areas outside our internal pipeline. We're very flexible and interested in partnering with companies that have disease-area expertise – such as in autoimmune diseases, neurodegeneration and regenerative medicine – or that have powerful delivery vehicles, like specific cell types or viral vectors. By joining forces, we can create transformative therapies for patients and accelerate these products into the clinic.

So, just to summarise, our three major goals are: continue to advance our pipeline, invest in our platform, and seek partnerships on a broad basis.

Q Could you go a bit deeper on some of the other therapeutic areas and cell and gene therapy types synthetic biology could impact in the relatively near-term?

TL: I think probably the nearest neighbour for us in oncology is the autoimmune disease area. Just as we are trying to stimulate the immune system for cancer applications with highly specific combination therapy, we can also suppress the immune system for autoimmune disease applications. You can actually make the argument that the need for synthetic biology is even greater here: Many of these diseases are chronic and without the high mortality rates of cancer.

In the context of autoimmune disease, it's very important to be able to shut down the immune system in a very controlled and targeted way, so that the effect is localised to the areas of inflammation, and you have an optimal safety profile. If you think about diseases such as lupus, inflammatory bowel disease and multiple sclerosis, many are intermittent. They involve flares, so you may not want a chronic therapy that is turned on all the time. If you can build a cell therapy or a gene therapy product that only turns on at the right time, or that can be titrated, or that only has local activity, then that would offer a significant benefit.

Turning back to the expansion of AAV vector-based gene therapies into diseases with larger patient populations, I think it's very important to be able to control the expression of these payloads. For example, with diseases such as certain epilepsies of the brain, we know we can't just over express the offending gene. We need to control that gene expression in the right

range and, in many cases, within the correct cell types. That is something that requires more sophisticated genetic engineering.

Q Can you speak to some of the challenges you and others will need to work through to fully realise the potential of synthetic biology for the cell & gene therapy area?

TL: As with any drug development, there are always the general translational challenges to address – such as, making sure we're going after diseases where we have a reasonable way of characterising the activity of our medicines in some sort of preclinical model before moving into human trials.

"I would expect there might need to be some functional testing introduced into the QC process for products with gene circuits in them."

In terms of challenges more unique to synthetic biology – especially as you start thinking ten, twenty years into the future – you first need to figure out how to manufacture and to develop quality control (QC) procedures for these types of therapies. After all, we're talking about therapies that can change their behaviour once they go *in vivo*, that can be tuned in vivo, or that can make decisions on their own.

So, how do we make sure those cell and gene therapy products are working really well during the manufacturing process? For starters, I would expect there might need to be some functional testing introduced into the QC process for products with gene circuits in them.

It's also very interesting to think about the regulatory path downstream. As these therapies become more and more sophisticated, they are going to be increasingly customisable and precise – more autonomous, basically. So how do we run a clinical trial across five different patients, where the therapeutic product we're putting in is behaving differently in each one, because we've programmed it to do so? That raises interesting questions in terms of how to ensure uniformity of the patient population, and the size of a patient population you might be willing to study.

Q As one of the field's pioneers, what is your vision for the next decade in synthetic biology – what are your hopes and expectations for what this technology area can achieve over this period?

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TL: This is a field that hasn't just appeared overnight. The first papers on synthetic biology were published in 2000. So it's been almost two decades of development.

However, we've gone through a lot of ups and downs. The initial applications for synthetic biology were very heavy in biofuels, for example, and, actually, they had good technical success. It's just that the commercialisation of these products was challenging, especially when competing with cheap oil.

But it's always been a personal dream of mine, and for a lot of other people in the field, to see this technology translated into human therapies, because there's a very compelling case for its use. You see, we all want drugs that are smarter, more powerful and more controllable in the body. So my hope and expectation is that we're going to see the first impact of these sorts of therapies within the next few years – most certainly in the next decade – and that will open up a whole new field of medicine.

The type of medicine we're mainly employing today is still primarily dominated by non-living therapeutics comprised of classical small molecules and biological drugs. But I think there's a whole range of complex human diseases – cancer, neurodegenerative disorders, inflammatory disorders – where we can't cure them with that sort of traditional approach. At best, we're sort of putting a Band-Aid on them with current approaches.

To really drive transformational cures, I believe we need completely different approaches that match the complexity of disease biology with sophisticated, programmable drugs. And I think synthetic biology can play a big role there.

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SPOTLIGHT

COMMENTARY

Clinical development of ATMPs: hospitals as an exemption?

Martin Hildebrandt

The ATMP landscape continues to be characterized by the fact that academia represents the major source of innovation. Undoubtedly, academic institutions do not possess an industry-like capacity to vigorously pursue the full developmental pathway to market authorization. At the same time, Industry has brought the first products to marketing authorization and defined novel modes of interaction with academia regarding the procurement of starting materials, manufacturing steps including storage of the product, clinical application and performance of trials. With ATMP development set to continue attracting and also challenging clinicians and scientists, this opinion papers aims to discuss logistical, financial and regulatory issues that might help to reshape the academic environment and to resuscitate some concepts that may have contributed to the original idea of the Hospital Exemption Clause.

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INTRODUCTION: ATMPS & ACADEMIA

ATMPs are medicinal products based on gene therapy, somatic cell therapy or tissue engineering. Regulation (EU) 1394/2007 has been designed to ensure the free movement of ATMPs within the EU to facilitate their access to the EU market and to foster the competitiveness of European pharmaceutical companies while guaranteeing the highest level of health protection for patients [1].

As ATMPs pose specific challenges to developers, manufacturers,

regulators and clinicians, Articles 4 and 5 of Regulation 1394/2007 have asked the European Commission to establish distinct sets of rules for these innovative and complex cell-based medicinal products:

- Good Manufacturing Practice (GMP) Issues related to Advanced Therapy Medicinal Products have been addressed in a separate Part IV to EudraLex Volume 4 [2]. This document has received serious criticism outside Europe [3] but offers noteworthy flexibility in manufacture, quality control, choice of reagents and release of products, among others;

- Good Clinical Practice (GCP) issues have been addressed in a stakeholder consultation [4]. Although of critical importance for academic centers in particular, this consultation has not addressed the qualification of clinical trial sites and investigators. Ethics Committees confronted with these innovative therapies will require the expertise to consider centers and investigators as being qualified in terms of having the experience and infrastructure to process and apply the ATMPs, and the therapeutic portfolio to deal with potentially toxic therapies and hitherto unknown side effects [5].

HOW CAR-T CELLS CHALLENGE ACADEMIA

Chimeric antigen receptor (CAR)-transduced cells appeared as a game changer to the ATMP arena, giving a boost to clinical attention, public demand and technical exploitation [6,7]. While two CAR-T Cell products have already been authorized for marketing in the USA and the EU, and with at least a third approved product on the horizon [8], there are more than 40 other CAR-T cell products currently in clinical development [9] and almost 400 trials employing CAR-T cells are currently recruiting [10]. The development of CAR-T based therapy has become an example of how ATMP concepts have reached beyond academia and into market authorization by major pharmaceutical companies.

The current models of CAR-T cell manufacture, distribution and application have major implications a) on existing resources and b) on the position of academia in the ATMP landscape. It may be assumed that the clinical infrastructure needed for CAR-T cell therapy, whether it be for late-phase clinical trials or for authorized products, is at least as complex as for early-phase clinical trial units, mandating a structured, interdisciplinary approach as described [7]. In addition, apheresis units and tissue procurement units need to be established, authorized and sustained. Pharmaceutical industry can benefit from the existing academic structures to pursue both early- and late-phase clinical trials and to provide authorized products in a more or less standardized environment, and FACT-JACIE standards as well as accreditation processes by the companies themselves serve to better define the qualification of sites. Even so, the major burden of investment and costs has remained with public funding, leaving a major fraction of high-risk investment with the academic institutions.

Following the establishment of a manufacturing platform for the

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early stages of ATMP development and initial clinical trials, academia is now challenged with providing the GMP environment needed for both early phases of ATMP development and for final stage preparation of products tested in late-phase, industry-sponsored trials, as well as for authorized products. The position of academia as described [11] is now at the front and at the end of the trajectory of clinical development, in both cases bringing academic institutions to their limits in terms of logistics, infrastructure, personnel, regulatory expectations and costs.

De Wilde *et al.* recently described the strengths of and hurdles for academia in ATMP development [12]. What might be considered in addition to these is the availability of a qualified GMP environment with clean room capacity that often is not used to its full extent. This contrasts strikingly with a global shortage in clean rooms that is projected to dramatically increase in the coming years [6]. Are clean rooms in academia a gold mine that academic institutions are not aware of, whilst efforts to maintain these GMP capacities are challenged by costs that appear high from within academia only?

REGIONAL ATMP COMPETENCE CENTERS: A SOLUTION FOR ACADEMIC HOSPITALS?

European research institutions have begun to network available GMP facilities at an EU level to promote academic-led 'first-in-man' gene therapy trials by linking the available expertise, GMP production facilities and human skills. This is with the aim of providing a proof of efficacy for a range of technologies. At this point, the technology could be transferred to the private sector, which would then undertake further development having taken advantage of academic knowledge and know-how [13]. However, the extent of this effort is underestimated and the capacities within academia to handle this work are limited.

It has been proposed that regional centers of excellence could offer a localized approach to therapy delivery, as an alternative to a global generic rollout [14]. This perception cannot be maintained entirely in light of experiences with those ATMPs that have been authorized for marketing to date. However, the concept still prevails that regional centers, with their collected experience in cell therapy, early-phase clinical trials, and serious and rare diseases, could provide an ATMP platform with the required GMP and GCP environment. Especially in the CAR-T field, this becomes evident as manufacturing devices and technology platforms become available that pose similar challenges (and opportunities for collaboration) to all users, including academic institutions. Key issues to be addressed here include defining the standards to qualify these centers as 'ATMP Competence Centers' and challenges relating to clinical trials as recently discussed [5]. However, there is also an opportunity to define potential recipients of funding by sponsors and health care insurers where the costs related to ATMPs such as CAR-T cells have to be tackled by public health care systems and by society.

The landscape described above brings us to the questions of how the regulatory framework for ATMPs could support such an Academic Platform, and whether the Hospital

Exemption Clause was perhaps designed to serve this very purpose?

Article 3 [7] of the EU Directive 2001/83/EC has been adapted to include an exemption from central authorization for ATMPs which are "prepared on a non-routine basis and used within the same Member State in a hospital in accordance with a medical prescription for an individual patient". Member States have been requested to lay down rules for authorizing these products by the national Competent Authority whilst at the same time ensuring that relevant Community rules related to quality and safety are not undermined. It was initially hoped that 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" [15]. However, it has not been defined how this relates to 'development' as part of clinical trials. The usefulness of the Hospital Exemption Clause has found limitations not only in the extent to which the Member States have recognized and interpreted the provision, but also in the fact that the Hospital Exemption clause is outside the arena of Advanced Therapy Investigational Medicinal Products (ATIMPs), raising the question of how parallel systems can be merged when no regulator overlap seems possible?

The implementation of Part IV to the EU GMP Guideline [2] has brought substantial flexibility to many facets of manufacture, including qualification of materials, clean room environment and release. The need to consider two independent sets of rules (i.e., Part I and Part IV of the EU GMP guideline) can be dealt with through creative solutions. However, the academic centers might benefit instead from a process-driven approach: manufacturing licenses could be linked to a certain process or technology in a generic fashion, while the authorization for a clinical trial could then build upon a) the generic process, and b) the specific manufacturing license for the investigational product to be tested. Taking CAR-T cells as an example, the manufacture of such genetically modified cells to express a certain receptor would be performed to GMP compliance with a generic technology, and the vector encoding for a certain receptor would define the investigational medicinal product as part of the clinical trial. A generic manufacturing license could also be seen as attractive for sponsors, who could use these manufacturing sites based on the existence of the generic process performed under GMP compliance.

CONCLUSION: ARE ACADEMIC HOSPITALS AN EXEMPTION FROM THE RULE?

Hospitals, especially those affiliated with academic institutions and university medical schools, are the source code for ATMPs. They have an inherent focus on complex and orphan indications, and their specialized clinicians and scientists integrate best patient care with pathophysiological, fundamental and therapeutic research in multidisciplinary teams. This truly academic and curiosity-driven research, combined with access to patient cohorts and their clinical samples, provides an inspiring and creative environment for the design of innovative cell therapies.

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Driven by promising preclinical data, a large number of academic institutions have constructed cell therapy manufacturing facilities that are compliant with Good Manufacturing Practice (GMP) guidelines over the past 15 years. Recent estimates suggest there are approximately 50 GMP cell therapy facilities in academic institutes in each of the USA and Europe, providing wide geographic coverage [12]. The difficulties faced by these institutions are enormous - not only in terms of funding but also because GMP is not a standard part of academic curricula (other than in divisions of Pharmacy and Transfusion Medicine) and timelines for grants, publications and limited contracts are often more familiar to academics than the timelines needed for drug development. However, academic GMP manufacturing capabilities and capacity is available, is recognized by Competent Authorities, and strives to have an independent standing in front of (and in collaboration with) industry. Academic GMP manufacture is not exempt from drug regulation, but finds itself in a continuous struggle with its role as a junior partner in constantly changing and varying models of interaction with the pharmaceutical industry.

Successful future collaborative business models between academia and industry will need to integrate the academic and hospital roots of the product into their value creation. A focus on generic, point-of care manufacturing (non-industrial may truly be the appropriate term) processes, independent and standardized platforms, and a structured GMP/GCP environment could all help shape the position of academia and to better define the different but interdependent roles of academia and industry in ATMP development.

A proportionate regulatory framework will by necessity involve the voices and perspectives of patients [16], and the academic and hospital sectors are arguably better positioned than industry to establish such dialogues and partnerships. As long as academic institutions and hospitals manage to hold on to their identity as representatives of a public stakeholder community, collaboration with industry will continue to present an opportunity for more involvement, transparency, and ultimately, therapeutic success.

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CELL & GENE THERAPY INSIGHTS

2020 VISION
TOOLS AND TECHNOLOGIES OF TOMORROW

SPOTLIGHT

INTERVIEW

Regulatory insights and lessons learned for a new decade in cell & gene therapy

JOYCE L FREY-VASCONCELLS PhD is considered one of the foremost regulatory experts regarding cell therapies, combination products, gene therapies, tumor vaccines, and tissues and brings extensive regulatory expertise and experience for this unique group of products. Prior to starting Frey-Vasconcells Consulting, Dr. Frey-Vasconcells served 6 years as a regulatory consultant for Pharmanet. Prior to joining Pharmanet, she served more than 12 years at the FDA as the Deputy Director, Office of Cellular, Tissue, and Gene Therapies (OCTGT) with the Center for Biologics Evaluation and Research (CBER). She was instrumental in developing many of CBER's science and public health policies regarding the regulation of cells, tissues, gene therapies, tumor vaccines, and combination products (tissue engineered products). In 2001, Dr. Frey-Vasconcells was named the Regulatory Expert for Cell Therapies at FDA. Since starting Frey-Vasconcells Consulting, Dr. Frey-Vasconcells has continued working with industry on an individual basis and with organizations whose mission is to foster product development in these unique areas of medical science. She brings extensive regulatory expertise and experience for this unique group of products.

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Q What are your overall reflections upon the past decade in cell & gene therapy?

JF-V: It's been extremely exciting to watch the field grow over the past few years. There were so many years where it seemed

"...I think in the past everyone was pretty open to discussing successes, problems and failures. However, that's generally not the atmosphere in big pharma. I worry that the openness is going to start decreasing. And I think that's sad, because I believe openness has greatly benefited this space, both industry and regulators."

like nothing was coming forward – it's not unusual for a new field to take several years to reach the stage we're at now, with more and more products coming forward showing really exciting clinical results.

It has reached the point where the interest of the larger industry players has been piqued and they're now getting into the game. My fear is that I really think that's going to change the dynamic of the field.

Cell and gene therapy surfaced and became established in academia and through the work of entrepreneurs at small companies, and I think in the past everyone was pretty open to discussing successes, problems and failures. However, that's generally not the atmosphere in big pharma. I worry that the openness is going to start decreasing. And I think that's sad, because I believe openness has greatly benefited this space, both industry and regulators.

Q You have been fundamentally involved in shaping US FDA regulatory guidelines and legislation relating to cell & gene therapies. Can you firstly comment on the key elements of that evolution that have proven to be most beneficial to the cell & gene therapy community?

JF-V: I was at FDA when this field was getting started and I think probably the biggest thing was we made a really strong commitment to try to be as accessible to industry as possible.

We admitted to industry that we didn't know everything and that we were learning along with them. It was an education for FDA reviewers but also for many in the industry, because as I said previously, the people developing those early products weren't part of big pharma and they didn't know what it took to move a product forward. So there was a lot of interaction and learning on both sides, and I think that was probably the number one aspect that benefited industry in the early days.

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"... there's a generation of these products that have absolutely stellar clinical data, but the manufacturing is a mess."

I would add that one of the things we did when I was at FDA was to recognise that some of the testing, particularly for gene therapy, was very expensive to do. As a result, we kind of backed off on some of the requirements for manufacturing. I do think that now, FDA is finding that in some ways that actually hurt industry, because they let CMC slide - now there's a generation of these products that have absolutely stellar clinical data, but the manufacturing is a mess. So there was give and take. I think it was necessary to get the field going, but I think FDA waited too long to start increasing the requirements on the manufacturing side.

I think the other thing that benefited the field goes back to the atmosphere of openness: I think there's been a lot more harmonisation between countries because of all the early discussions that took place. I remember what it was like with the small molecules and monoclonal antibodies when ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) first started - it was painful to try and harmonise between countries. But with cell and gene therapy, there's always been a certain level of harmonisation – the field hasn't had to endure the same growing pains in that regard.

Q Are there any particular areas of international regulatory divergence which concern you at the moment, though?

JF-V: When it comes to CMC characteristics, I think there are a lot of areas of harmonisation.

I believe, for example, the EMA and FDA talk on a quarterly basis - they discuss specific products that they both have in front of them. I do think they are really trying to take into consideration what other agencies are saying - I know that if you have a meeting with, say, EMA and then you go to FDA and the FDA find out you had a meeting with EMA, FDA will actually ask you to submit the EMA advice to your IND, because they want to see it. They want to make sure they're not diverging so significantly that it makes it difficult for companies.

I would say where there is still divergence is with the approval pathways. FDA probably has the most restricted approval pathways - they only have two, whereas EMA has four or five. Japan now has a type of conditional approval pathway – something that still does not exist with FDA. As a result, I think a lot of companies are now going to EMA or Japan to get

approval based upon phase 2 data prior to ever approaching FDA, because they know the FDA won't accept it.

I think there's also some divergence in the requirements for the quality of reagents, as well as donor eligibility requirements. With donor eligibility, there's even divergence between individual EU countries. It would be really nice if at least the donor eligibility issue could be solved, because that has become a real problem for cell therapy.

Q Can you go deeper on the most significant pitfalls or issues that you see cell & gene therapy developers struggling with today?

JF-V: I have several! One of them is actually a lack of understanding of what the requirements are for approval. I was really hoping that when the larger pharma companies got involved, they would bring the necessary regulatory expertise. However, what I'm finding instead is they're just trying to cut corners like everyone else is, and that's not the way to do it.

I think another issue is a lot of companies in this space are rushing through development. Many development plans today basically consist of doing a small phase 1 followed by a jump to a pivotal registration study. Well, those developers do not have a clear understanding of their product or their process, or even how the therapy is working. I think there are a number of clinical trials out there that are failing because they didn't do proper product development and they rushed.

I'm not saying that the old traditional 'phase 1, phase 2, phase 3' clinical development model is a must, but the breadth of knowledge gained through that process is necessary for success. As I touched on earlier, I think a lot of companies are not keeping their CMC on track with clinical. Last year, the Center Director for CBER said they had five BLAs in front of them where, clinically, they were done - the FDA did not need to see any more clinical data for either efficacy or safety. But in each case, the CMC was such a disaster that the FDA could not approve the product.

" I was really hoping that when the larger pharma companies got involved, they would bring the necessary regulatory expertise. However, what I'm finding instead is they're just trying to cut corners like everyone else is, and that's not the way to do it. "

This leads me to another issue, which is that a lot of people want to move to automation of

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manufacture. Now, I think automation is going to help two aspects: firstly, the current lack of contract manufacturing capacity in both cell therapy and gene therapy; and secondly, the lack of skilled CMC labour available. The problem comes in trying to move from a manual to an automated process when you don't understand your product or your process - that move is a major manufacturing change and it's going to be very difficult to make it if you don't have that understanding in place.

Those are the key pitfalls and issues I'm seeing right now that I really think are stalling the field.

Q It is clearly a huge challenge for regulatory authorities to stay abreast of such a broad and rapidly moving technology area – can you identify any particular bottlenecks or areas of regulatory development that you feel could be prioritised moving forward to further enable patient access to novel cell & gene therapies?

JF-V: One of the major bottlenecks I see from a regulatory perspective is just the interaction with the regulatory agency. As I said earlier, when I first got into this field and got into regulation, we were constantly meeting with people, we were constantly on the phone with them. And we had no problems having informal conversations.

Well, informal conversations just don't happen anymore. Not only that, but I'm finding the FDA is even limiting the number of interactive telephone meetings, let alone face-to-face meetings. I'm even seeing companies go in for an end of phase 2/pre-phase 3 meeting and the FDA isn't granting them a face-to-face. For me, that is one of the most important meetings and I think they should be face-to-face at that stage of the game. I do completely understand FDA has a huge resourcing issue, but it really is a major problem and of course, the new accelerated designations are causing even greater concern there.

"Well, informal conversations just don't happen anymore. Not only that, but I'm finding the FDA is even limiting the number of interactive telephone meetings, let alone face-to-face meetings...I do completely understand FDA has a huge resourcing issue, but it really is a major problem"

Other regulatory areas that are inhibiting the progress of cell and gene therapy products? I think there needs to be another look at endpoints for cell and gene therapy clinical studies. I don't think you can always apply traditional

"The other thing that I think is getting really frustrating for industry - and I'm hoping that FDA come out with something on this - is really defining what is a new product. Many of these companies are trying to treat orphan indications and they don't know if somebody is going to be able to block them..."

endpoints in this field, but all the regulatory agencies continue to follow what was done for recombinant proteins and small molecules.

I would also return to what I think is perhaps the biggest thing, particularly for cell therapy, which is really just defining the product and understanding its characteristics. These are living entities that change depending on the environment - how do you determine that you have a consistent product when it is changing? And every day we're finding out more and more about different cells. MSCs is a prime example - an MSC from fat is not the same as an MSC from bone marrow. However, the tools to really define those differences are just not there yet.

The other thing that I think is getting really frustrating for industry - and I'm hoping that FDA come out with something on this - is really defining what is a new product. Many of these companies are trying to treat orphan indications and they don't know if somebody is going to be able to block them, because FDA has not put out guidelines on what they are going to recognise as being the same product.

Added to this, there are still not a lot of precedents in this area, meaning people just don't know what it takes to get to approval. I think all these things build on top of one another.

Q What do you expect the future of expedited development pathways to look like, particularly as the cell & gene therapy field grows larger and more competitive?

JF-V: I think expedited development pathways are here to stay - they are helping the field.

I do think they have changed the dynamics of what a phase 1 study is. Many phase 1 studies are now larger, because the expedited pathways require clinical data and it has to be somewhat convincing.

I do worry that as the field continues to grow and becomes more competitive and less open, these development pathways will put more and more strain on the regulatory agencies. I'm not sure how sustainable that will be in the longer term.

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Q Many expect the 2020s to see allogeneic cell therapy rise to prominence and widespread clinical application, driven by enabling technologies such as iPS cells and gene editing platforms – what is your view on this, and what would be the related key considerations or concerns from your perspective?

JF-V: I actually don't think it will move as fast as people will want it to. Gene editing iPS cells seem like it could be very beneficial, but the problem is there are still a lot of unknowns about the gene editing aspect.

With gene editing, the biggest risk is its specificity and I think agencies are going to want to roll out products very cautiously - to have a good idea of what the risks are before they open up products to the general population.

It seems as though every time a new gene editing platform comes out, people say it's perfect and there are no undue off-target effects. Well, if you really dig deep, I don't think there has been one yet that doesn't involve some off-target activity. And it's that off-target activity that will continue to cause a lot of angst amongst the regulatory agencies moving forward, because it needs to be controlled.

Q Finally, can you distil for us the most important principles of cell & gene therapy development as you see them from your experience as both a regulator and regulatory consultant?

JF-V: I would say most companies don't have their end goal in sight beyond simply wanting an approved product. For me, one of the biggest principles is: write your label. How do you want your product to be administered? You need to plan for success and a big part of that is really understanding what the end goal is. I think if you do that and you understand the approval process and what is needed there, things will go much smoother - you will be better positioned to keep preclinical, clinical and manufacturing all together and moving down the same track.

"I do worry that as the field continues to grow and becomes more competitive and less open, these development pathways will put more and more strain on the regulatory agencies. I'm not sure how sustainable that will be in the longer term."

"...there are a lot of brilliant people in this field and I think if you really stop and brainstorm, there is always a way to figure out how to accomplish something."

Everyone always thought clinical was so important – and it probably still is for investors at least – but for me, CMC is absolutely critical. At the end of the day, understanding your product and process is what you're going to be inspected against. And those are the standards you are going to have to continue to meet for years to come.

I do think people are starting to get that, but I still find too many companies saying either 'we don't need to worry about it' or making excuses that they don't want to do something because it seems too hard.

But there are a lot of brilliant people in this field and I think if you really stop and brainstorm, there is always a way to figure out how to accomplish something. There really is no one defined path in cell and gene therapy - you have to approach every product separately and individually - so I think it's really important for people to be open to alternatives, and that goes for both industry and the regulatory agencies.

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CELL & GENE THERAPY INSIGHTS

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SPOTLIGHT

INTERVIEW

Brave new world - a new decade in allogeneic cellular immunotherapy

DAVID SOURDIVE is a co-founder of Cellectis and joined the Board of Directors in 2000. Dr. Sourdive combines a strong scientific expertise with experience in managing industrial programs bringing innovative technologies to industrial fruition. He served as Executive Vice President, Corporate Development, from 2008 to 2016 and as Executive Vice President, Technical Operations until July, 2019. In addition to his role at Cellectis, Dr. Sourdive has also served on the board of directors of the Mediterranean Institute for Life Sciences. David Sourdive graduated from École Polytechnique, received his Ph.D. in molecular virology at Institut Pasteur and completed a research fellowship in the Emory University Department of Microbiology and Immunology. His management training is from the HEC (Challenge +) and his decade-long experience in industrial program management was acquired at the French Department of Defense (DGA) prior to Cellectis' inception. .

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Q What are you working on right now?

DS: As the pioneers and most advanced developers in allogeneic CAR T-cell therapy, we are currently working at deploying our technology both in the clinic and through industrialisation in our manufacturing endeavours.

Part of our focus is progressing in our clinical investigations with our three wholly owned product candidates in the clinic: UCARTCS1 in

"In adoptive cell immunotherapies, we now see and better understand how genome engineering is the key to going after more challenging indications, such as solid tumors."

multiple myeloma, UCART123 in acute myeloid leukaemia, and UCART22 in B-cell malignancies and more specifically, acute lymphoblastic leukaemia. A key component in this clinical activity lies in its broadening the spectrum of the classical CAR-T approach, which has been very much associated with the CD19 paradigm in B-cell malignancies for some time – indeed, that is where our very first program, UCART19, actually started four years ago.

We are also building our own manufacturing facilities to be able to not only make critical raw and starting materials to engineer cells - to have the necessary RNA, vectors and so forth to implement genome engineering, - but also to commercially manufacture UCART final products. The raw and starting materials production will take place in our Paris "SMART" facility, which should go live in 2020, while the commercial manufacturing of UCART products will be done in our "IMPACT" facility in Raleigh, North Carolina - that will go live in 2021.

It is a moment of focus and excitement for us, but we are also preparing for longer-term. Since inception almost twenty years ago, when we became the first gene editing company, we have had the vision that gene editing is going to be transformative, especially in cell therapy. In adoptive cell immunotherapies, we now see and better understand how genome engineering is the key to going after more challenging indications, such as solid tumours.

Q Can you tell us more about the progress Cellectis is making in advancing its allogeneic cellular immunotherapy candidates through development?

DS: We are working out how our off-the-shelf allogeneic CAR T-cell technologies can be deployed in varying clinical situations. Multiple myeloma is a very different context from acute myeloid leukaemia, which is itself quite different from the B-cell malignancies. There are some similarities, previous teachings and understanding we can utilise but essentially, we are accelerating our progression on the learning curve. In particular, we are really starting to better understand how the UCART concept works in patients. For example, with the first UCART19 trials, we learned that the notion of redosing could apply as with classical off-the-shelf drugs and could be beneficial.

Bear in mind that an allogeneic CAR-T is not just the allogeneic version of an autologous therapy. It is an off-the-shelf pharmaceutical product and

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should be regarded as such. Although they do share some features with the autologous therapies, off-the-shelf allogeneic CAR-T products are much more than that, and we are now looking into ways of using off-the-shelf CAR-T that are quite different from the concepts used in the autologous space.

The other area where we are making real progress is in preparing for the next steps in clinical development. Right now, we are pursuing dose escalation Phase 1 clinical studies for our products, but the next step will be to go pivotal. Our preparations address the need to manufacture pivotal study materials that abide by tighter standards and are much closer to what will be required for commercial product manufacture.

Q Can you talk us through the challenges you have faced thus far, and that you anticipate meeting in the future as Cellectis' allogeneic cell therapies continue through the scale-up process?

DS: When we started the CAR-T programme a few years ago, we thought that making an autologous treatment would be challenging. We thought the logistics associated with that type of therapy would be quite a formidable enterprise for a small company like ours, and we wondered how it could possibly work from a business model perspective. It seemed at the time that this type of therapy was much more a business of the points of care – essentially a sophisticated graft – as opposed to the business of a pharmaceutical company. So from the very beginning of our program, we chose to go for an allogeneic approach and we identified key questions to be addressed - boxes we needed to check.

We thought that allogeneic CAR-T would have to be immediately available to patients who would not have to wait for a treatment to be manufactured for them. We thought that an allogeneic CAR-T would also not require a patient to be his or her own donor – in other words, that they would not have to be fit or large enough to be an effective donor. If you think of infants, for example, it's challenging to collect enough cells to make such a product. We also anticipated that the product could be made available in a much broader range of points of care, and not just those highly sophisticated hospitals that would be able to perform such complex manufacturing.

We were also very careful to consider affordability, expecting a lower cost of goods because hundreds of doses can be made from one single batch, which is what we are already able to do today. Also, as I mentioned earlier, we anticipated that as off-the-shelf products, UCARTs could be used with

"...we are pioneers in this field. We are breaking ground just about every week. Even when we talk to regulatory agencies, we have to define and invent new ways, innovative concepts, and be creative on all fronts. We are fitting a new concept into a pre-existing framework that may not necessarily have been conceived or designed to accommodate this type of product."

more flexibility, allowing redosing or combining with other drugs and so forth.

As we have moved forward over the years, we have seen each box checked, one by one. We verified the product's safety, which was something we were particularly careful about given the theoretical concerns about potential graft versus host disease. We verified that we could achieve a cost of goods that was in line with our expectations and that the product could be stored and made available to patients for immediate treatment with no requirement for materials donated by those patients.

There are challenges associated with this endeavour and they pertain essentially to the fact that we are pioneers in this field. We are breaking ground just about every week. Even when we talk to regulatory agencies, we have to define and invent new ways, innovative concepts, and be creative on all fronts. We are fitting a new concept into a pre-existing framework that may not necessarily have been conceived or designed to accommodate this type of product. Frankly, the way the cells work together and operate is still not fully described or understood. We believe that, at some point, we will use other concepts to better characterise these types of products.

Today, we describe cells from the immune system essentially by their ontogeny: i.e. where they come from. People will tell you 'these are lymphocytes' or 'these are CD8 T-cells'. They can tell you what the grandparent cells were, but they do not tell you what the cells actually do, how they interact with each other. It is a bit like assessing a soccer team by looking at the passport of each player, but not really looking at the role they perform in the team. We are missing some science that would better speak to the concepts, observables, even vocabulary, to describe what these cell populations are and do.

As we face this challenge, we are literally writing a piece of science where there is often no precedent, no tools or tests. Now that we have been able to manufacture these products, we can study them, develop the concepts and start filling the gaps.

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So, while there are some usual challenges that we are always confronted with in cell therapy, I would say the major ones pertain to our being pioneers in the field.

Q As the decade turns, where is technological innovation most badly needed in cell therapy manufacture, for you? What would do you expect to see the tool provider community working on to support industry further over the short-to-mid-term?

DS: I think there is a lot of focus today on trying to develop solutions for producing cells or streamlining manufacturing processes, but in my opinion, the primary pain point where we need help is on the analytical side.

We are using biological objects that are extremely complex. Cells are huge when compared to individual molecules that have been the century-long paradigm for pharmaceuticals. Moreover, these products are not pure but a mixture of cells, each of which has its own fate even though they may collectively act as one piece of tissue. Cells are living entities that change, multiply, adapt to their environment and interact with each other. They may start expressing new genes while downregulating others and modify their functionalities.

We currently lack some observational tools and metrics to be able to better describe what cell populations can do or become once infused into patients. A very simple and common way to illustrate this is the following: if you take a snapshot of the immune system of, say, 100 healthy individuals and you observe this with the current tools and metrics, using the current vocabulary, you will see 100 different immune systems each with its own repertoire of effectors and specificities. Moreover, if you take the same snapshot 6 months later, you will see 100 new, different immune systems that will have all changed and yet still be different from each other.

However, if these 100 people all catch the same 'flu or some other common virus, they will basically all respond in the same way: with some fever after roughly the same number of days, with some other clinical signs, and they will get rid of the virus, and about 5% of their cells will remain after a month and become part of their immune memory. The scenario will be more or less the same, so there are clearly

"We are using biological objects that are extremely complex. Cells are huge when compared to individual molecules that have been the century-long paradigm for pharmaceuticals."

commonalities between all of these healthy people that are simply not apparent when you look at their immune system with today's tools. In essence, what makes a mix of cell populations perform a particular scenario rather than another remains to be better understood and predicted. This example is just an illustration of what applies more broadly to cell therapy at large.

Our field would greatly benefit from players developing concepts, enabling technologies, markers and assays allowing us to measure what is important in cell populations and behaviour in a way that is both predictive and robust. This is absolutely essential for the cell therapy field moving forward although not often talked about… like an elephant in the room.

The second thing cell therapy could be helped by is access to technologies and solutions adapted to its own specificities, or even conceived of with those specificities in mind. This relates to the fact that cell therapy is only just recently being viewed as a market of its own. It is less and less this fledgling field where repurposing tools developed to manufacture antibodies or other proteins has been the status quo up until now.

Bear in mind that cell therapy grew in academia and hospitals and belonged to the world of grafts. It is now transitioning towards the world of industry, with off-the-shelf pharmaceutical products on the way. This concurs with cell therapy becoming a market per se. Some technologies or solutions need to be developed specifically for cell therapy, such as closed manufacturing systems that are commensurate with the size and number of cells we need to work with, that are easy to use for sampling, for assaying, for non-invasive monitoring, and so on. I am optimistic because we are starting to see evidence of that recognition for our field.

The third challenge lies in the potential global capacity crunch ahead of us for all sorts of critical raw and starting materials, many of which are custom-made for specific indications or even individual products - DNA or vectors, for example.

A first example is cellular starting material that is often collected under specific regulations that now need to also be regarded as useable in a pharmaceutical process. This requires handling pursuant to cGMP or GMP guidelines which implies investing in specific infrastructure and quality systems that are rarely found in collections sites.

Likewise, the global capacity to manufacture some critical materials is growing slowly because commissioning new facilities, training teams and setting up GMP capability takes time. Today, should you want to order a GMP AAV vector batch, you may be waiting for 2 years to obtain it - and this may only be a component of your final product, albeit an important one.

Because we could not wait for such a period of time, we decided to invest in our own SMART facility to make these critical raw and starting

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"The cell and gene therapy field at large would clearly benefit from the companies making these tools and materials for transducing, modifying or measuring cells to catch up."

materials, in addition to our investment in our final off-the-shelf allogeneic CAR-T cell product commercial manufacturing facility.

The cell and gene therapy field at large would clearly benefit from the companies making these tools and materials for transducing, modifying or measuring cells to catch up. The demand from industry is growing. Cell therapy is the next wave and acknowledging this fact means investing in the capacity that will support this growth.

As awareness of this gap is spreading, a lot of players are trying to move into the area and are advertising the fact they will soon be able to provide clinically useable vector, nucleic acids and so forth. Thus, there is reason to be hopeful and I expect in the not-too-distant future that making cell therapy opportunities available to patients will be a more rapid exercise.

Q What's your vision for the impact that gene editing platforms will have across the cell & gene therapy space over the coming decade, particularly in terms of driving progress in the allogeneic cell therapy field?

DS: We believe that gene editing and more broadly, genome engineering (that pertains to reprogramming genomes and not just edit individual genes) is going to be transformative for cell and gene therapy.

We're already seeing how this is enabling allogeneic products to become an industrial and clinical reality. But we think that genome engineering is essentially going to unleash the power of cell therapy and allow it to play a much broader role.

As an illustration, in adoptive T-cell immuno-therapy, a field we operate in, going after solid tumours will obviously not just rely on a chimeric antigen receptor (CAR), artificial TCR, or some other means of tumour recognition. Those advanced receptors are meant to make the tumour visible to immune effectors. However, most of these solid tumours are already visible to the immune system and have found ways to defeat it. In essence, for engineered cells to succeed where normal cells fail, they need attributes that are supra-physiological - i.e. more than normal. Cells need to be engineered to defeat the tumours and circumvent their defence mechanisms, e.g. by not being sensitive to checkpoint blockade, hypoxia, the microenvironment, etc.

"...by 2030, there will have been an element of standardisation, more and more centralised facilities will have come online, and the logistics will have been worked out a little better, although these therapies will probably remain expensive."

To repair a failing tissue or to have cells do more than just replace other, identical cells, they need to be engineered to perform something new that their sister cells fail to do. That is what will be really transformative. Allogenicity is one step that makes things more industrial and changes the game completely from that perspective, but it is just the beginning. The possibilities go way beyond that.

We believe that a central component of our field will lie in reprogramming different types of cells so that when combined and infused, they will execute a predetermined immunological scenario that we hope will make the patient better. It will be a bit like a battle plan, designed by combining infantry with artillery with air force because we think that is how we will defeat the enemy.

Genome engineering will enable executing this strategy, not just individual gene editing, but the engineering of multiple genes and pathways. We have already published results along this line of thinking. We believe that is the way to go after solid tumours in our own space, but it will also be a general approach for cell therapy at large.

Q What will the prevalent commercial manufacturing models for cell therapy look like in 2030?

DS: It is always complicated to speculate on what will happen in more than 10 years in our field. Whatever model is being used commercially in 2030 will have been largely finalised in terms of its development some 5 or 6 years before. So, to answer this question, we have to look at the models that will be developed over the coming 4 years or so, and that will succeed.

In the autologous space, the whole debate is between manufacturing at the point of care versus manufacturing at a central facility. There is a clear tension between the quest for consistency and better control which pushes towards things being centralised, and hospitals that would like to develop or retain manufacturing capabilities because it is a significant profit centre for them and a very high value added activity for these points of care that contributes to making them attractive in the eyes of patients.

I think there will always be this tension there but I believe that by 2030, there will have been an element of standardisation, more and more

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centralised facilities will have come online, and the logistics will have been worked out a little better, although these therapies will probably remain expensive. We are not directly involved in autologous cell therapy as of now, but that is my general impression.

Turning to the allogeneic field, as I have mentioned, we are already building a commercial manufacturing facility. It will be the first commercial cell therapy manufacturing facility to combine gene editing with cell therapy and we think that is a key milestone for the future in our field.

It has been designed to be very compact and multiproduct, because we believe that the coming years are going to be a time for combining versions and different attributes in these engineered products. We will need to be flexible and able to 'tweak' the cells rapidly, and we anticipate there is going to be huge synergy if we are able to centralise the manufacturing of these products.

While some countries may have restrictive import regulations pertaining to human cells, we believe the allogeneic cell therapy centralised manufacturing model will be the very model we are deploying at our future IMPACT facility in Raleigh, North Carolina. It will combine engineering, flexibility to grow, and capability to serve a very broad market in all major geographies.

The path to scale-up is clearly defined. There is also some scale-out at the same time because we can have multiple suites, and there is a strong element of disruption in the innovation when it comes to gene editing and genome engineering.

Q Finally, can you outline what the coming 12-24-month period holds in store for Cellectis?

DS: We will continue to move forward on three main fronts. The first one is clinical, of course. We are consolidating the UCART concept in the clinic in multiple indications, seeing how things develop and UCARTs operate, how to make best use of the product, dosing and redosing and so forth. All these elements are going to play out over this period, and we are very much looking forward to this clinical experience. It is very rewarding to see that the original concept that became a technology has now become a product that is helping patients.

The second front is manufacturing, as I have mentioned. We think there is huge value in being able to make these products ourselves, controlling the know-how at play with these cutting-edge technologies and products, and building a strong powerhouse to leverage the full potential of the approach as we broaden the UCART deployment in cancer.

Thirdly, we recognize that genome engineering can be applied beyond the field it has been used in so far. While we have started with haematological

malignancies, we are already exploring solid tumours and we are also looking into opportunities outside of the oncology field. For example, engineering the genome of blood cells could be a way to address many other indications and that is also an avenue we will explore over the coming year or two. We were pioneers in this ongoing revolution and we will continue to be at its forefront, bringing therapies to patients with unmet needs.

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CELL & GENE THERAPY INSIGHTS

2020 VISION
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SPOTLIGHT

INTERVIEW

Next steps for the iPSC and tissue engineering fields

IOANNIS PAPANTONIOU is a Chemical Engineer by training (graduated from the University of Patras, Greece) and is currently a Principal Investigator at the Institute of Chemical Engineering Sciences at the Foundation of Research&Technology-Hellas and a visiting Professor at Prometheus, KU Leuven. He obtained his PhD at Department of Biochemical Engineering, University College London funded by the National Scholarship Foundation of Greece. Subsequently he joined KU Leuven where he obtained funding to initiate an autonomous research track and is currently ATMP Research coordinator, within Prometheus – the KU Leuven division of skeletal tissue Engineering. He is active in addressing bioprocessing/translational challenges that would allow scalable and robust manufacturing of adult progenitor/stem cells, required for moving ATMPs for skeletal healing into the clinic. His task is to promote interfaces with industrial partners aiming at valorising research solutions generated within the platform along the entire ATMP bioprocess pipeline and has initiated several collaborations at the academia/industry interface. His research aims are to: Design and engineer progressively complex yet autonomous skeletal 3D living implants adopting breakthroughs in organoid technologies (Tissue engineered ATMPs); Develop a panel of metrics to enable quantitative definition of biologic events ensuring activation and maintenance of mechanism of action; Link therapeutic functionality upon implantation to the patient to *in vitro* quality attributes of skeletal ATMPs; Develop automated devices that can contribute to a cell therapy industry 4.0 equivalent. Integrate in silico derived tools for a self-regulated/adaptive manufacture of next generation living implants.

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Q What are you working on right now?

IP: In general, the purpose of my research is to develop manufacturing technologies for the scalable production of cell based ATMPs. In particular, processes that would allow the manufacturing of microtissue and organoid "building blocks" for the production of complex living implants and engineered tissues.

Q Let's begin by focusing on the induced pluripotent stem cell (iPSC) field. Firstly, can you reflect on recent breakthroughs in the space?

IP: There are two distinct areas here. One is the clinical use of these cells – major breakthroughs there mainly have to do with ongoing initiatives for the development of iPSC-derived products that cannot be detected by, or that are compatible with, a patient's immune system. So efforts there are focused on how to render this type of cellular product as clinically approved engineered tissues or cells.

The other exciting area is the development of organoids that faithfully mimic developmental events encountered in the body and result in the formation of patterned structures with functionalities reminiscent of the physiological counterparts.

These two aspects are for me the most exciting and most promising breakthroughs that have happened in the field in recent times, beyond the actual discovery of iPS cells.

Q Where do you expect to see the next advances in terms of clinical applications of iPSC-derived cells and tissues?

IP: Issue number one here, scalability, has been dealt with already to some extent – there are scalable platforms that can produce not only differentiated iPS cells but also quite defined populations of more differentiated progenitor cells. These may in turn be used as raw materials for the formation of clinically relevant cell types.

The second issue is how do you make the end product – the tissue product itself – and how do you build in the desired functionality? That's the next challenge to be addressed: how to develop functional tissues, and how to then compare them to the native tissues that you would like them to substitute for in terms of either presence or function? From the

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"That's the next challenge to be addressed: how to develop functional tissues, and how to then compare them to the native tissues that you would like them to substitute for in terms of either presence or function?"

manufacturing perspective, it will have to do with the combination with biofabrication technologies – on the one hand, how do you produce organoid modules, and on the other, how do you bring them together and merge them into a 3-dimensional end product that can be functional? In addition, a future challenge would be in the development of implants that possess not only the desired geometric features at the end of the process but also a space-related multi-functionality.

From my perspective, this is where this technology is really promising and where we should be heading in the next decade. But of course, as it evolves further, we will have to deal with the real world: we will have to make ATMPs that can be approved by regulatory bodies. They will have to reach the market in a cost-efficient way. So we will begin to face the same challenges that other cellular products have faced in the past and indeed, are still facing today.

Q Regarding iPSC-derived organoids and the non-clinical tools side, what's your view on likely future development directions?

IP: As I mentioned earlier, we are just starting to move beyond single organoid platforms – going from mimicking (to a certain extent) a single tissue to mimicking a whole system. Ideally, you would like to have a cheap platform where you could have multiple organoids that are all talking to each other which would allow you to replicate what's happening in the body much more faithfully and study it – moving from 'organ on a chip' to 'patient on a chip', if you will.

How to mimic the length scales you find in the body is a big challenge to be addressed in the future. Right now, we are more focused on the biology – how we can bring together certain organoids that can communicate and where the functionality of one can affect another. However, a really interesting next step would be how to also mimic the proportions of organs in these new chip technologies?

We do already have quite complex structures that can self-assemble and undergo developmental events – that is very exciting, but it has its limitations. I would like to see the field continue down the path of enabling these organoids to communicate with each, to fuse and form more complex structures.

"Ideally, you would like to have a cheap platform where you could have multiple organoids that are all talking to each other which would allow you to replicate what's happening in the body much more faithfully and study it - moving from 'organ on a chip' to 'patient on a chip', if you will."

Q What's your vision for how iPSCs could drive cell and gene therapy as a whole over the coming decade?

IP: I think they will be used everywhere, from screening of drugs to being used for clinically relevant products. I think they have a very interesting future as a cell type that can be used in gene therapy – engineered cell therapy with designer cells, basically.

So while iPSCs will be used for discovery, I personally really look forward to the clinical applications. That would be the major breakthrough, in my opinion, because it would move us towards making off-the-shelf products on demand. I hope for certain clinical indications that can become a reality within this time-window.

Q Zooming out again and looking ahead: in what specific area(s) do you expect to see cell therapy make its next major breakthrough(s)?

IP: I don't want to make huge predictions – I think small steps take many years in this field – but there are two areas I would pick out. Firstly, the continued development of CART will be important: establishing the viability of a CART business model and migrating it into the solid tumour space, and into more widespread diseases in general – not just orphan indications or small patient groups. Whether that can be achieved in the next decade, though, I am not sure.

Secondly, relating more closely to my own work, I would pick out cell-based functional implants for skeletal defects – making an industry out of that research field, basically. Obviously, you could predict that we can have livers and hearts coming out of a printer, but I don't think we're anywhere near getting there yet – that's very complex functionality. But I think with less complex systems you could potentially mass produce functional tissue that is iPSC-derived – I think that could be feasible within the next decade in the skeletal space. This would also contribute to a shift towards

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manufacture using CQAs that are linked to the potency of your product, which I think would be a very important advance for the cell therapy field.

Q What does the future of autologous cell therapy look like, for you? What form of manufacturing model will prevail?

IP: I think that a movement towards point of care manufacturing could be feasible. There's a lot of development from the technology side with bespoke systems that can handle multiple steps – not only cell expansion, say, but entire bioprocesses, upstream and downstream. You now have major industry players working with and actively supporting this sort of approach. So I do believe the technology side – 'GMP in a box', for instance – is feasible. You would certainly also need quite major improvements in hospital facilities, etc., although maybe not as big as some make out, because the equipment footprints could conceivably be relatively small.

You have companies starting to streamline raw materials supply – cells, media, etc. They are providing kits for developing specific products. I think this is part of a shift to a phase where there will be critical mass from all angles – hardware, software, materials – and also predictive cellular products. When all of this comes together, you could in theory be relatively certain of the consistency of products manufactured in this type of 'at the bedside' solution.

I actually think the logistics element will present the biggest challenge in that scenario, rather than production. You would have cell and gene therapy companies that wouldn't have huge networks, and that would struggle to supply, support and communicate with multiple hospitals. I think that would be the most difficult part. Blockchain technologies could support this endeavour, providing a framework for data/action traceability and security aspects that are key in a field where living products are manufactured, while in addition, patient-related information might be required and handled.

"I actually think the logistics element will present the biggest challenge in that scenario, rather than production. You would have cell and gene therapy companies that wouldn't have huge networks, and that would struggle to supply, support and communicate with multiple hospitals."

Economics will play the key role at the end of the day, of course. The most profitable approach that can be supported by the healthcare systems will win out.

Q Where in particular do you see challenges/opportunities for MSCs in the decade ahead?

IP: I think right now the trend is to use them for generating exosomes, extracellular vesicles, etc. That for me is not cell therapy anymore, it's becoming something else. Continuing down that pathway, you could potentially no longer require MSCs from patients – you could use immortalised cell lines and use them for mass production, essentially employing the traditional biopharma model for producing monoclonal antibodies and the like.

There is also the potential that iPSC-derived MSCs could substitute for autologous MSCs, should they be proven capable, so I do have doubts as to whether you will be able to make the case long-term for autologous MSCs as a platform for cell therapy or tissue engineering. I think they will probably play a diminishing role – again, especially if iPSC technologies manage to demonstrate clinical relevance.

I'm very much interested in MSCs – in understanding what exactly they are and identifying the true progenitor cell subpopulations within this diverse cell population, and maybe isolating them. But that is purely scientific endeavour at the moment and if it comes to fruition, then maybe your MSCs will not actually be MSCs anymore – perhaps they will be defined as a pure stem/progenitor cell population with defined properties.

I think this approach could maybe provide a viable alternative to what we now call "MSCs." The truth is, MSCs is a complex cell population within which there are certain progenitor cells that can have therapeutic potential. If we can understand this better, we can isolate this cell type to create products with far greater potency than is currently possible. That might also affect the way we produce or deliver these products.

MSC R&D is still a major pillar of the cell therapy field and I do think that will continue for the decade to come. I think its survival as a significant field beyond that timeframe will ultimately depend on if and how the iPSC field develops an ability to substitute or recreate the same cell types, and on whether MSC-based ATMPs will possess measurable mechanisms of action.

Q What needs to happen before the 2020s can be considered the decade of 'precision manufacturing' in cell and gene therapy?

IP: One important aspect is making devices that are self-adapting, self-managed – that can actually operate with minimum

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"For precision manufacturing, we need better characterisation tools. For example, how do you incorporate high throughput technologies such as RAMAN spectroscopy in bioreactors?"

human intervention. For example, how do we actually link sensor readouts with how the device is operating? A second key aspect will be to link the data we are obtaining from these sensors to our CQAs.

For precision manufacturing, we need better characterisation tools. For example, how do you incorporate high throughput technologies such as RAMAN spectroscopy in bioreactors? We need this type of QC tool that can be interconnected with a culture system. That is for producing cells; for making tissues, we would need to look at fabrication length scales – we would need technologies that can print the dimensions that match the *in vivo* ones, or at least dimensions that the cells or tissues that you are printing can relate to, so that they are not excessively foreign to the living entity you are using. This is another aspect I would call precision medicine, especially for the ATMP field.

So to summarise:

1. We need to better characterise our cell products – we still don't know them very well.
2. When we culture them in suspension (which is required, in my opinion) we need to be able to link the CQAs of our cells through sensors to the QC that we have measured with our precision technologies.
3. We need to make tissues in a very highly defined manner with length scales of tissues or cells (ideally below 500 μm, which right now is quite challenging to get down to).

Q Do you expect continuous manufacture to make a real splash in the cell & gene therapy arena? If so, where? If not, why?

IP: I think it's necessary, for sure – otherwise, you just have to embed additional complexity in what is already a very complex processing situation or landscape.

For allogeneic cell products, it's just a no-brainer in my opinion – that's a perfect fit. For autologous products, I think we could consider integrated systems such as 'GMP in a box' devices as continuous manufacturing solutions. It actually links to what I said before: it relates to the need to integrate our process units – our single technologies – into pipelines.

So yes, it will be needed for both allogeneic and autologous cell therapy products. The outlook for each will probably be very different. However, there will be common technologies that will be part of both. For example, the decision-making tools – data tracing, management and analysis software – will be similar. And these are very important tools we don't have right now – they are critical technologies that are needed by the entire field. We really need an ecosystem of companies or research groups to develop these types of tools – they will ultimately provide the umbrella beneath which all future integrated and streamlined cell therapy processes can run.

Q Finally, what do you hope to have achieved in your own work by the end of the next decade?

IP: I would really love to help deliver a platform that can produce organoid-based skeletal implants in a streamlined manner – an automated platform that can start from single cells, and can deliver large bioengineered tissues that are manufactured with high precision, high robustness, high reproducibility, and with embedded potency.

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SPOTLIGHT

INTERVIEW

Bioprocess decisional tools to enable cell and gene therapy commercialization

SUZANNE FARID is Professor of Bioprocess Systems Engineering at the Advanced Centre for Biochemical Engineering at University College London (UCL) and Deputy Head of Department (Education). She is Co-Director of the Future Targeted Healthcare Manufacturing Hub in collaboration with industrial and academic consortia to revolutionize the delivery of cost-effective stratified protein-based and personalized cell-based therapies to patients. She is also Director of the UCL-AstraZeneca Centre of Excellence. She leads research on 'Decisional Tools' to facilitate cost-effective bioprocess design, capacity planning, R&D portfolio management, root cause analysis and manufacturability assessments for biopharmaceuticals ranging from mAbs to cell and gene therapies. She sits on the ISCT Business Models and Investment Sub Committee, UK BioIndustry Association Manufacturing Advisory Committee and is a Fellow of the IChemE. She obtained her Bachelor's and PhD degrees in Biochemical Engineering from UCL.

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Q What are you working on right now?

SF: I'm a Professor of Bioprocess Systems Engineering at University College London, in the Department of Biochemical Engineering. There, I lead research on bioprocess decisional tools.

These are tools that integrate process and business models. We use them to evaluate the potential of novel or innovative manufacturing strategies, and to identify the best route to commercialization for the new modalities that are coming through. Our research spans biotherapeutics ranging from antibodies to cell therapies to viral vectors.

I'm also Co-Director of UCL's Future Targeted Healthcare Manufacturing Hub. This is funded by the UK Research Council (EPSRC) and industry, and it focuses on how personalized therapies can achieve success. We look at this from several perspectives: manufacturing, regulation, reimbursement, business and supply chain.

One of a number of things we're working on is creating these decisional tools to address the inherent complexities with personalized cell therapies such as autologous CAR T-cell therapies. We've been looking at what we believe are highly topical questions for those actively developing or considering CAR T-cell therapies, including:

- What is the current Cost of Goods (CoG) of these therapies?
- What are the key CoG drivers?
- How much do viral vectors contribute to CoG?
- Which manufacturing platforms are most cost-effective?
- How economically feasible are these therapies, ultimately?
- What are the risk–reward trade-offs between centralized and bedside/'GMP-in-a-box' manufacture?

Q Tell us more about the most significant challenges facing engineered cell therapy manufacturing business models, as you see them.

SF: Engineered T-cell therapies such as CAR T have been a real game-changer in the clinical oncology space, as evidenced by the recent approvals of Kymriah® and Yescarta®. However, if you look at the number of patients who have actually received these treatments to date, it's quite limited. Numerous challenges exist relating to the suitability of manufacturing processes as the supply of these products continues to increase: the complexity of the supply chain itself, Cost of Goods, regulatory frameworks, and the reimbursement models in the space.

On the manufacturing front we're dealing with a complex needle-to-needle supply chain. For one thing, we're looking at orchestrating three separate manufacturing pathways for CAR T therapies – the plasmid DNA,

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"We're trying to address all of these challenges through the Hub, looking at the manufacturing and business strategies we need to evolve around these therapies to allow them to potentially reach a wider patient population in future at a somewhat more affordable price."

the viral vector and the gene-modified cells. We then have the additional challenges of cold chain transportation, of logistics at the clinical site, and of quality control (QC) release of each individual patient's batch.

All of this obviously brings a lot of challenges on the Cost of Goods side and many now recognize the need to lower CoG sufficiently to enable feasible business models. We've been looking at how to achieve that by firstly seeking to understand what this threshold might be. Typically, in the biopharmaceutical space there's a preference for CoG to be below, say, 15% of the selling price – we're assessing whether that can also apply to CAR Ts.

On the reimbursement front, there's the initial challenge of how to evaluate the cost–effectiveness of potentially curative cell and gene therapies – if the traditional approaches used in the UK for appraising novel therapies were applied to CAR Ts, they probably would not gain approval for reimbursement. And of course, the other concern is that the price of these therapies may be unsustainable and presents an opportunity cost dilemma for healthcare providers such as the UK's NHS.

On the regulatory front, several of these therapies are coming to market in an expedited fashion, meaning drug development timelines are shortened. This potentially compresses the time available to conduct CMC development activities, which puts pressure on decision-making in terms of the work that may need to be done earlier and at risk.

We're trying to address all of these challenges through the Hub, looking at the manufacturing and business strategies we need to evolve around these therapies to allow them to potentially reach a wider patient population in future at a somewhat more affordable price.

Q In what particular areas do you see the greatest opportunity for the industry to positively impact CoG over the relative near-term?

SF: We see the key CoG drivers being related to materials, quality control (QC) and labor costs – they all represent key targets for CoG reduction.

One example of this could be reducing culture timeframes. This would also bring benefits from the patient angle: the faster these therapies can be manufactured, the greater the chance the patients will actually receive them.

In terms of reducing the cost of materials, there are numerous efforts to optimize viral vector production, which has traditionally relied on lab-based processes. There are clear opportunities there to reduce the contribution of viral vectors to gene-modified cell therapy CoG.

Reducing the dose of these therapies also has the potential to have an impact. This would require a better understanding of the mechanism of action, though. And QC is another angle where optimization can take place – exploring different models such as centralized QC release in order to reduce that burden.

All of this is in relation to autologous gene-modified cell therapies. Of course, the alternative being explored at the moment is allogeneic or off-the-shelf therapeutics, which have the potential to benefit from scale-up approaches to manufacturing and economies of scale that will translate to lower CoG. If some of the current challenges relating to immune response can be resolved, then that is obviously an attractive approach in terms of helping with the CoG dilemma.

Q Opinions differ within the cell & gene therapy space as to if, when and where continuous manufacturing will have a major impact on the field – what's your view?

SF: We've done quite a bit of work on continuous manufacturing in the antibody space, and I've witnessed the resurgence of interest in the field as co-chair of two of the ECI Integrated Continuous Biomanufacturing conferences in recent years, in Berkeley and in Portugal.

We see continuous biomanufacturing being actively investigated in the therapeutic protein space. The interest there is related to the inherent advantage of higher productivities, which can translate into smaller footprint facilities and potentially more agile manufacturing. However, while there have been a lot of companies putting their technology development efforts into evaluating the potential of continuous manufacturing in that field, not many have actually used it in clinical programs as yet.

That said, at the most recent conference, there were signs of a shift towards people starting to commit to using continuous manufacture for clinical programs, but gaps and challenges relating to that implementation remain – the analytics (the monitoring and control capabilities to facilitate implementation) and the availability of the GMP equipment operating in a continuous mode are two examples.

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"There is also the question of whether to adopt an automated, closed, all-in-one platform for manufacturing versus having a series of segregated unit operations."

Given that this is the current reality in the antibody space, which has been looking at continuous manufacture for quite a while, I think it will take some time to break through in the cell and gene therapy space. I know there are efforts ongoing, though: from the cell therapy perspective (perhaps more for allogeneic cell therapies than autologous) one can imagine the use of perfusion culture to improve productivity and lead to smaller facilities – you can envisage that linked to continuous volume reduction, washing and purification. And in viral vector manufacture, which is perhaps closer to protein manufacture, there are efforts looking at perfusion culture in fixed bed bioreactors, or even in stirred-tank bioreactors. It's easier to envisage continuous chromatography being used in viral vector downstream bioprocessing, too.

Q Can you dive deeper on the bioprocessing technology side of things – for instance, how and where specifically do you think automation will continue to develop and be implemented?

SF: On the cell therapy front, there is a clear need to shift from manual, open operations to more automated, closed operations, especially when you think about it from a commercialization perspective – that's definitely an overall drive in the space.

With this comes a number of considerations and decisions for developers. For example, there are automated units being borrowed from the biotech space as well as more customized offerings being created specifically for cell therapy. There is also the question of whether to adopt an automated, closed, all-in-one platform for manufacturing versus having a series of segregated unit operations – trading-off the simplicity of having an all-in-one box versus the flexibility that you gain when you have a more modular approach to manufacturing.

Another important consideration for automation relates to how best to deal with variability – for example, in the patient-sourced material for autologous cell therapies. I think that moving forward, we'll need to consider whether we can develop feed-forward and feedback control algorithms to cope with that variability – for instance, if your patient's source material cell concentration is lower than expected, can we make an informed decision

"Facilities of the future will be ideally digitally integrated … Industry 4.0 adoption … has the potential to be game-changing and a critical lever for future commercial success for the cell and gene therapy industry."

on how much to increase the cell culture time in order to produce the required final number of cells for treatment? That would obviously require cell characterization assays, the associated online analytics, and the process know-how to build the necessary modelling and control algorithms. Advances in this area will be particularly important in this space, given the life-threatening nature of the diseases and the correspondingly serious implications of having a failed patient treatment – you often can't just go back and make another batch if you have a batch failure, due to the fact the patients are generally significantly immunocompromised.

A further key area for future automation is in the QC space. Obviously, when we move to these more patient-specific therapies, there's an explosion in the amount of quality data, batch manufacturing records and release testing that is required. That's probably not sustainable in its current form, if we expect these therapies to treat more than a few hundred patients – if we want to be able to treat tens of thousands of patients, say, then QC will need to be more automated and we'll need to figure out how to move to continuous, real-time release methods.

Q You are standing in a typical commercial cell & gene therapy manufacturing facility in December 2029 – what does it look like?

SF: Facilities of the future will be ideally digitally integrated with fully automated manufacturing, real-time release testing, standardized procedures, real-time traceability and agile processes. This shift will align with efforts towards Industry 4.0 adoption that has the potential to be game-changing and a critical lever for future commercial success for the cell and gene therapy industry.

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CELL & GENE THERAPY INSIGHTS

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TOOLS AND TECHNOLOGIES OF TOMORROW

SPOTLIGHT

INTERVIEW

Analyzing key ATMP talking points through IP and regulatory lenses

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Q Can you firstly give us some background on your involvement and activities in the life sciences area as

a whole, and in cell and gene therapy/regenerative medicine more specifically?

BSM: Ever since I was a child, I've been fascinated by how organisms and cells achieved complex behavior. I studied Natural Sciences at Cambridge University, specializing in cell and molecular biology and genetics, but it was after an 8-week stint in '94 in Professor David Ish-Horowicz's lab at what was then the Institute of Cancer Research Fund (ICRF) Unit in Oxford that I became a huge fan of developmental biology.

Having obtained my PhD, and after a short Post Doc, I retrained to become a lawyer and intellectual property specialist. Life sciences has been a key focus and passion for me in my legal career too, and I've advised numerous pharma, biotech and medical device companies on a range of issues including regarding cell and gene therapy applications. I've also been a member of the UK Bioindustry Association Cell and Gene Therapy Advisory Committee for over 7 years.

JM: I've always had a passion for science, but I knew I wanted to be a lawyer, and I converted to law after completing my Natural Sciences degree. I joined Arnold and Porter in 2005, and completed my training here, specifically because of the life sciences practice. I'm now a Partner in that group, focusing on regulatory advice to the innovative pharmaceutical industry and indeed, to the broader life sciences sector.

As part of this work, we provide advice to companies on a range of first-in-class products including, in more recent times, Advanced Therapy Medicinal Products (ATMP). This advice focuses on navigating the rather complex regulatory framework, ensuring these products meet safety/efficacy requirements and helping them reach and stay on the market.

Q As you both look at cell and gene therapy as a whole with its various component technologies and modalities, what particular areas stand out for you in terms of current/ongoing IP- and regulatory-related concerns that need to be addressed?

BSM: Patentability is always an issue! However, there are a number of challenges that particularly concern cell and gene therapy products.

In the EU we have the Biotech Directive, which came into force in 1998. Its purpose was to harmonize what was and wasn't patentable in the biotech field. There's a broad exemption in that Directive for inventions that are considered to be contrary to public policy or morality, but it also excludes

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a number of inventions from being patentable for ethical reasons. Specific exclusions relevant to the cell and gene therapy field include processes for modifying the germ line identity of a human being, and the use of human embryos for industrial and commercial purposes.

It is this latter exclusion that caused much controversy in the cell therapy field, culminating in the Court of Justice of the European Union (CJEU) decision on the International Stem Cell Corporation ("ISC") case (Case: C-364/13, ECLI:EU:C:2014:2451, which was preceded by the Brüstle decision [Case: C-34/10, ECLI:EU:C:2011:669]). This landmark ruling related to the question of which human cells are deemed to constitute a human embryo such that their use in an invention is not patentable under the Biotech Directive.

The European Patent Office (EPO) had previously ruled in the WARF case (Enlarged EPO Board of Appeal decision G2/06) that inventions that required the destruction of human embryos in order to put them into practice were not patentable, and that the only exception to that would be those that involved the use of pluripotent embryonic stem cells that could be derived from cell lines which were publicly available at the filing date.

However, the CJEU went one step further and extended this exclusion significantly, saying that an invention is not patentable if that invention required the destruction of human embryos, no matter when this may have occurred. Additionally, they adopted quite a strict interpretation of what a human embryo is, covering all of the early stages of human development, and all other similar cells capable of commencing the process of development of a human being.

In short, as long as a cell cannot form a viable human being and the invention hasn't required the destruction of human embryos, then it is possible to obtain a patent for it – that is of course as long as it meets all of the other patentability criteria.

It's 5 years since the ISC decision and, in practice, the concerns over the ramifications of these decisions from the CJEU and the EPO, and the subsequent revised guidelines from the EPO, were probably exaggerated at the time. I think the concerns did not materially hinder the industry partly due to the fact the field was, and is still to an extent, in its infancy. There is currently not enough competition to drive companies to contest the validity of these patents, beyond opposing them before the EPO and we have yet to see any litigation in the UK courts concerning this type of technology. Furthermore, patent attorneys have (as ever) found ways around the limitations posed by the decision.

All this said, I could anticipate there being litigation in the future relating to the current EPO guidelines, because they now accept inventions using human embryonic stem cells filed after February 2008. This is on the basis that, at that point, those cells could be obtained without destroying

"One way around patentability issues is for companies to rely on confidential information and trade secrets, rather than trying to obtain protection through the patent system ... The UK courts are quite used to maintaining information confidential."

the embryos. However, I think that could ultimately be challenged before the courts because those embryos are frozen indefinitely, they are not re-implanted, and one would assume they would be ultimately destroyed.

Beyond these specific concerns on patentability that are particular to cell and gene therapy, there are also the usual general patentability hurdles. These include the fact that, under the European Patent Convention and the UK Patents Act, methods for the treatment of the human or animal body by surgery or therapy are excluded from patentability. Patent claims therefore have to be worded using a specific language – a legal fudge to avoid this exclusion. People refer to these types of claims as second (or further) medical use claims, and it's these that innovators are finding most challenging to obtain, enforce and successfully defend their validity. These challenges are relevant not just to the cell and gene therapy field but also, in particular, in relation to orphan drugs.

One way around patentability issues is for companies to rely on confidential information and trade secrets, rather than trying to obtain protection through the patent system. In the UK, we do have one of the most well-developed regimes for this, and the UK courts are quite used to maintaining information confidential. This is also something that is now possible across the EU thanks to the recent Trade Secrets Directive. And this could be an attractive option for certain technological aspects, because you could maintain exclusivity indefinitely, without having to invest in patent prosecution and maintenance fees.

But it is not without its own challenges. For example, if information can be reverse engineered, or is developed independently, you can't prevent the third party in question from doing so. The third party could then go ahead and patent similar or related technology that covers your confidential information, which would obviously make it difficult to expand your own commercial objectives. Moreover, if the confidential information becomes public through lawful means, there's no way you can protect it.

So, there are clearly costs associated with maintaining information confidential and it can be difficult to prove someone has misused that. As a result, companies do tend to use mixed strategies to balance the challenges in protecting these products and processes.

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JM: On the regulatory side, the areas that need to be addressed are more focused on the approval process, and then what happens when the product is on the market.

Everybody knows that the number of clinical trials involving ATMPs is booming – one statistic I saw recently is that there are currently over a thousand clinical trials ongoing around the world involving these products (ARM and BIA Report, Leading Innovation; The UK's ATMP Landscape, July 2019). This volume of activity clearly puts strain on the regulatory authorities. In addition, the EU framework for authorization of these products is now over 10 years old, having been implemented in 2007 (Regulation [EC] No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation [EC] No 726/2004), and while there have been amendments to try to update the legislation, the reality is that there are still relatively few products that are widely available on the market. And indeed, some of the products that have been approved have actually been withdrawn post-authorization due to difficulties with manufacture or reimbursement. I believe there are currently around 14 ATMPs on the market in the EU, which is not very many when you consider the number that are undergoing clinical investigation.

The reality is that most ATMPs have to go through the authorization process afresh each time, because of the unique aspects of the product, its manufacturing process, the starting materials, and the target disease. This all means there's pressure in terms of time and resources, both for the authorities and the companies. However, we have now reached a stage where a certain number of products have gone through this process; lessons and best practices have been learnt, difficult issues have been considered, and hopefully, there will be a more streamlined process going forward.

The other area that needs to be addressed from the regulatory side is that there are a lot of interlocking rules in this area that will impact the development and authorization of these products, not all of which relate to the medicine's authorization. For example, companies also have to consider rules on genetically modified organisms, and on blood, tissues and cells. These are areas of legislation that have been implemented differently across the EU, and member states take different views on these issues as part of the European Medicines Agency's (EMA) committees. For example, in relation to the interaction between the regulation on the collection and transfer of blood, tissues and cells, and the regulation on the authorization of ATMPs, we are aware that different EU countries take different approaches in terms of which regulatory regime applies at the various stages of the manufacturing process – at the beginning when you collect the starting material, and later, when you provide the final product to the patient. Despite the fact

these products are authorized at an EU level, there's a surprising amount of divergence at the national level, which leads to complications for both the regulators and the companies.

There are also additional requirements in relation to licensing of premises that handle tissues and cells, or receive imports of such products into the EU (for example, because part of the manufacturing process may be based in the USA), and for the quality testing that has to be carried out. All of these requirements are on top of the medicine authorization rules. This overlap and inconsistency has been identified as an area of concern, and discussions around how to streamline, or at least harmonize, these rules across the EU are underway in a bid to deliver some clarity in the future.

Q Beatriz, we are seeing – and will continue to see in the near future – the first examples of competition between marketed cell and gene therapy products. What are your expectations in terms of how robust patent protection will be in this space moving forward?

BSM: Before we even start to consider the robustness of patent protection, I would query the extent to which patent protection for specific applications may actually exclude competitors. This remark is due to the highly specific nature of the cell and gene therapy applications currently being developed.

For such specific applications, whilst robust patent protection is clearly preferable, it may well be that the scope of the protection – what is actually claimed in those patents – may not actually cover those competitor technologies. In other words, the patent system will not necessarily be relevant to provide exclusivity by asserting that a competitor product infringes an innovator's patent.

However, where I do think there will be more litigation, at least in the short-term, is with the platform technologies – that's where patent protection could certainly hinder competition.

The clearest current example is with the recently introduced genome editing techniques, such as CRISPR, which can and do have wide application. Valid patents in this arena are extremely powerful and lucrative. It's for this reason that we have seen hard-fought battles already over the first generation of CRISPR patents, particularly between the Broad Institute on the one hand and the University of California Berkeley (UCB) on the other in the US and Europe.

There is a crowded patent landscape developing around CRISPR technology. The Broad provided stats in July this year that the US Patent Office

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has apparently granted more than 80 patents with claims relating to CRISPR and/or Cas9 to more than 300 inventors from nearly 60 applicant organizations! In Europe, it's less: the EPO has issued more than 20 patents to 30 inventors from about 10 applicant institutions. But overall, globally, more than 1,500 patent applications have been filed but not yet granted.

Licensing activity in this arena has been fierce and well reported. The Broad Institute is very open about the approach that it's taking - its policy has been to make CRISPR tools readily available for academic and non-profit use, so no licenses are necessary for that work. Licenses are required, however, by research companies and companies wishing to sell tools and reagents – those licenses are non-exclusive – and then exclusive licenses may be obtained for human therapeutic use. Other organizations currently involved in licensing or sub-licensing deals in this space include UCB, the University of Vienna, Duke University, Massachusetts General Hospital, CRISPR Therapeutics, Editas, Caribou Biosciences… The list goes on.

So CRISPR is an example of where I do think that robust patents will be helpful. It will be interesting to see whether these first-generation patents are ultimately upheld and, if so, what their scope will be as well as what secondary patents will be the ones to stand the test of time.

Q Jackie, what are your expectations in terms of the impact of commercial competition upon future regulatory approval and market access environments for ATMPs?

JM: Market access continues to be very difficult for these products, given the high research and manufacturing costs – in particular, where the product is only used by a small patient population, and sometimes only a handful of patients across the entire world, leading to large costs per patient. It's no secret that the current economic environment across the EU means healthcare systems are struggling

"...companies also have to consider rules on genetically modified organisms, and on blood, tissues and cells. These are areas of legislation that have been implemented differently across the EU, and member states take different views on these issues..."

to fund these highly specialized medicines. When there are many patient groups with unmet needs, such high cost of treatment raises ethical and political issues; when should healthcare organizations divert finite resources to only a few patients, potentially leading to difficulties with being able to treat others? Finding ways to balance those difficult decisions is the reality for healthcare organizations, and for ATMP companies.

Authorities are also increasingly employing novel methods to procure cheaper access to products, and they are pushing the boundaries of the legislation in this regard. Again, companies have to contend with the resultant difficult market access conditions when trying to launch these products.

I think that new, tailored payment models will have to be developed to take into account the high upfront costs for ATMPs as compared to the often very dramatic long-term benefits of treatment. It may be necessary to spread the cost of treatment over time, or to agree some form of risk sharing payment scheme with healthcare organizations. However, that may require changes to healthcare accounting rules to properly account for the need to spread the cost of therapy over a number of years – that is not necessarily how these systems are currently set up. It will certainly also require collaboration between industry and the healthcare organizations to ensure that both sides are able to meet their obligations and patients are able to access these products.

The unfortunate reality is that if healthcare systems can't afford to pay for these products, and they're not commercially successful because reimbursement can't be obtained, then they may be withdrawn. Based on publicly available information, three ATMPs have been withdrawn for precisely this reason, one of which is reported to have only treated one patient while commercially available. After such long development and authorization processes, that's a real shame for patients.

Another issue is about the data necessary to support market access. These products often target rare diseases and/or may seek to be authorized through an accelerated review process, sometimes based on limited data sets, so these products can be placed on the market as quickly as possible. It's often difficult in such clinical trials to establish a proper control group, because it's not ethical to provide a placebo to half of the patients, meaning less robust or extensive data may be generated compared to, say, the data for more standard chemical products. This can lead to uncertainty of long-term outcomes. Apart from the difficulties this presents in terms of obtaining a marketing authorization, this uncertainty doesn't help in demonstrating to authorities that the product is cost-effective and should be reimbursed at a relatively high price.

All of these interlocking issues need to be addressed by companies moving forward if ATMPs are to flourish on the market.

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Q Given the increasing likelihood of commercial competition between multiple approved advanced therapies in some orphan disease indications, plus the recent ongoing discussion around orphan similarity involving regulators on both sides of the Atlantic, just how secure is Orphan Exclusivity (OE) moving forward, in your view?

JM: Some commentators believe that OE is currently too broad and covers too many products, which places undue strain on healthcare systems. The European Commission and the EU authorities are generally trying to reduce the scope of OE, narrowing which indications and products are covered. There have been a number of recent changes to the guidance (Commission notice on the application of Articles 3, 5 and 7 of Regulation [EC] No 141/2000 on orphan medicinal products, C/2016/7253) – and to a lesser extent, the legislation – that have impacted the interpretation of OE, such as when a follow-on product will be considered similar or clinically superior to a product that's already on the market. This has led to a number of disputes between industry and the EMA/European Commission about the interpretation of the legislation, some of which have resulted in cases before the European Court.

But actually, if you manage to retain orphan designation and receive OE when the product is authorized, it's relatively secure. A similar product for the same indication can only be placed on the market in quite limited circumstances, and that true monopoly right has been protected by the courts.

So, while there are legitimate concerns about the ability to obtain OE in the first place, once you have it, it should be relatively secure.

Q Can you each comment on the potential for the equivalent of biosimilars to impact cell and gene therapy in the near- to mid-terms?

BSM: I'm not a regulatory expert, but considering the length of time and the challenges that biosimilars have faced in reaching and becoming established on the market - and given that cell and gene therapy products are far more unique, patient-specific and technically complex than mAbs, for example - then I think it will be very difficult for the equivalent of biosimilars to be authorized any time soon or in the mid-term.

JM: I completely agree. It's very unclear to what extent manufacturers of follow-on products would be able to meet the requirements to enable

"...if healthcare systems can't afford to pay for these products, and they're not commercially successful because reimbursement can't be obtained, then they may be withdrawn ... Three ATMPs have been withdrawn for precisely this reason..."

them to prove comparability of their product with the reference product that is already on the market. And as Beatriz said, it took the authorities some time to get comfortable with the data necessary to approved biosimilars – it's likely there will be similar questions in terms of how the current guidelines should be applied to ATMPs. So, I don't think the launch of these products is at all imminent.

However, of greater concern currently is the issue of unlicensed follow-on products, and supply of unlicensed products under the various exemptions in the legislation continues to be controversial. There's an exemption in relation to advanced therapies in particular that allows for the supply of these products without a marketing authorization, providing certain conditions are met – for example, usage is within a hospital, within an individual member state, on a non-routine basis. We've seen in other circumstances that these exemptions are pushed to the limit by authorities, particularly where there may be perceived cost savings. These provisions have also been implemented quite differently in different member states – for instance, in terms of the definition of 'non-routine', and whether the exemption only applies if there is no authorized product on the market.

The potential for competition from such unlicensed products, which do not have to bear the heavy R&D costs and are not subject to the same stringent authorization regime, is seen as a real commercial risk by some companies.

Q Beatriz, what would be your advice for early-stage cell and gene therapy developers seeking to devise a patent protection strategy with likely future trends in mind?

BSM: I mentioned previously that companies in general, and certainly those that are early in development, need to consider a multi-pronged strategy covering patent protection, licensing deals, and the protection of confidential information or trade secrets. On top of that, besides protecting their own IP and portfolio, companies need

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to be aware of what their competitors and other third parties are doing to ensure that they have freedom to operate.

In the UK, over the past 2 years, there have been three Supreme Court decisions that impact this field in terms of what can and can't be patented. These decisions should be borne in mind by early-stage companies as they consider alternative or complementary strategies. They highlight three key aspects in particular:

1. Companies need to consider whether the product they are developing could be deemed to be equivalent to something that has been granted by a third party, and therefore be liable to patent infringement. Taking equivalence as an example: if part of your claim refers to DNA sequences, it may be that a nonidentical DNA sequence could fall within the scope of that claim and be deemed to be equivalent;

2. The amount of data required in patent applications is becoming more significant in this field – in particular, for inventions concerning second (or further) medical use. This presents the challenge for companies of whether they should seek patent protection early or wait until they have more data: if they file early, they could ultimately find their patent claims are invalid due to a lack of sufficient data; if they delay, they run the risk of a competitor filing an application first, or of a publication appearing that may make it difficult for them to have a subsequent application granted;

3. Some improvements made to a known product – to a cell line, for example – that are not necessarily obvious in advance of clinical testing may not be patentable. It is very important for early-stage companies to liaise with their legal counsel in order to find the best strategy for them in this regard. For example, companies might well seek patent protection over the actual product, the specific cell lines and the scaffolds used, but lab techniques and processes for making those cell lines may well be kept confidential.

Q Jackie, what would be your advice to early-stage cell and gene therapy developers seeking to devise a regulatory compliance and market access strategy – again, with likely future trends in mind?

JM: Naturally, we see many companies focusing on the data and expertise required to navigate the marketing authorization process. This is of course an important part of the company's strategy and it's not without its difficulties given the novel nature of these products. We are aware that opinions differ among competent authorities on the extent of data required for ATMP authorization and on the appropriate comparators to use in clinical trials, which are still being discussed and resolved.

It's important that companies seek appropriate advice from the regulatory authorities before submitting an application. In fact, there was a paper published just last month by some EMA regulators discussing a particular product and the advantages gained from the company seeking Scientific Advice during the development process and prior to submission (Schuessler-Lenz *et al.*, Regulators' Advice Can Make a Difference: European Medicines Agency Approval of Zynteglo for Beta Thalassemia, Clinical Pharmacology & Therapeutics, 08 November 2019). The product in question went through the approval process very quickly as a result.

Companies also need to focus on post-authorization issues. We discussed earlier that pricing and market access can be difficult, and it's important that these considerations are kept in mind early on in a product's development. As more and more ATMPs are launched, healthcare organizations are going to be seeking increasingly competitive prices, and companies need to make sure they collect the necessary data through their clinical trials to support these negotiations and avoid delays in launch of the product.

However, discussions with healthcare professionals and organizations before a product has obtained a marketing authorization are always difficult and raise compliance issues – this is also true for ATMPs, which raise particular questions in this area. For example, hospitals need to be trained on the collection and preparation procedure for the starting materials for the product, and on what information to give to patients as part of the consent process, but individual EU member states adopt very different views on how much of this can be done before marketing authorization. There may also be requirements relating to diagnostic testing, which again raise questions for reimbursement and how the company can support this type of testing as part of the product offering. Having a strategy in place as early as possible for how to approach these discussions, and when, will help ensure the early adoption of these products.

Finally, the scale-up of manufacture and batch-to-batch consistency are important, of course, and are things that some ATMP companies have struggled with following commercial launch. The practicalities of making these products commercially available when manufacturing sites are spread across the world have raised difficulties. This also links to broader compliance issues, such as how and when to provide information to healthcare professionals, and indeed, to patients, on any benefits that may be associated with the treatment. It may be necessary for a patient and their family to travel to a specific clinic and stay there during treatment – the degree to which companies are able to support such travel and associated costs is difficult to navigate. The Codes of Practice for the Pharmaceutical Industry (for example, the EFPIA Code of Practice, July 2019) don't deal with these issues – companies have to make decisions based more on first principles than any real guidance, and on a case-by-case basis with each product

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launch. Again, having a strategy in place relating to what the company will provide and on what basis is really important for getting these products to market as quickly as possible.

Q Standardization is a major talking point across the board for the cell and gene therapy field at present – in what particular areas would you like to see standardization initiatives pursued to the benefit of the sector?

BSM: I think minimum standards and standardization in general for tools, media, storage and transportation requirements in this sector would all be beneficial.

This does however come at a price from an IP perspective, as first mover companies gain patent protection for platform-related inventions. The aforementioned patent battles and extensive licensing deals in the CRISPR space are a good example of what can happen if the relevant standard is covered by patents.

In the future, we may well see developments in this arena to the extent that standards are formally adopted. Patents may be granted that are deemed essential for the application of these formal standards. In those circumstances, the owners of those standard-essential patents (SEPs) would need to disclose those patents during the development of the relevant standard, and they subsequently would be obliged to license those SEPs on fair, reasonable and non-discriminatory terms.

If standards were to be formally applied in this field, I would anticipate that patent disputes would follow – we've seen a significant number of disputes over SEPs in the telecoms field, for example. However, that said, I think there clearly is benefit and should be an objective to reasonably standardize, and also to continue to develop guidelines to assist in the commercialization of cell and gene therapy products.

JM: On the regulatory side, we've noted already that due to their unique attributes, at the moment, each ATMP has had to go through the authorization process afresh and more or less on a case-by-case basis. However, now that a measure of experience has been gained and there is increasing familiarity with these products, much-needed guidelines are beginning to emerge that will hopefully assist with the process moving forward.

For example, there was new manufacturing guidance published in 2017 (Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products, 22 November 2017) that adapted the general good

manufacturing practice requirements to the specific characteristics of ATMPs, such as decentralized manufacturing and complex supply chains – the aim being to ensure consistency of manufacturing despite variability in the starting materials and the complex global manufacturing chain. The guidelines very much take a risk-based approach, allowing manufacturers to be flexible as long as they have control systems in place, and proportionate to the level of risk.

Similarly, earlier this year, clinical guidance (Guidelines on Good Clinical Practice specific for Advanced Therapy Medicinal Products, 10 October 2019) was published that will greatly assist with the clinical development process and provide guidance for companies on how to structure their trials, including the number of patients necessary to demonstrate safety and efficacy, and appropriate comparators to use, which have been some of the major areas of concern.

These guidance documents have been drawn up based on the experience of the authorities in assessing the products that are already on the market. They provide much-needed detail for companies seeking to obtain an authorization and hopefully, over the coming years, we will see more guidance coming through covering a broad range of regulatory issues.

But it's also important that there are efforts to assist with standardization of the more practical aspects of treatment. At present, each product has its own collection protocol, its own storage process in a specific type of freezer (that the hospital has to procure), and its own booking and traceability protocol (each of which tends to involve different email- and web-based systems). While there are only a few products on the market, these differences are manageable for hospitals. But as the number of products increases, and if even a fraction of the thousand products that are currently in clinical trials reach the market, then these more practical aspects will also need to be standardized.

BSM: There's a balance to be found here. In some circumstances, guidelines will be sufficient – sometimes you require flexibility for new products and, if everything is standardized, you might actually limit innovation. But for other practical aspects, standardization would be a significant help.

JM: I agree. On the regulatory side, the authorities tend to develop guidance, which they can then adapt as necessary for specific products. This does cause difficulties as companies may not be able to understand or predict the authority's approach in a given situation. But more formal standards in relation to the practical aspects, such as refrigeration, traceability or booking, would help a great deal with the beginning and end of the supply chain.

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"...it's also important that there are efforts to assist with standardization of the more practical aspects of treatment. At present, each product has its own collection protocol, its own storage process in a specific type of freezer ... and its own booking and traceability protocol..."

Q Finally, on the topic of the current challenges around market access of cell and gene therapy products, what for you are the key considerations and next steps for the community as a whole to ensure patients can benefit from ground-breaking new therapies in the decade to come?

BSM: I think the challenges are more heavily weighted on the regulatory compliance side but, as ever, strong IP protection – whether by way of patents, confidential information, or a mixture of the two – will always be an important part of the puzzle. It is vital for ensuring innovators can access sufficient investment to enable them to bring a product to the market (assuming that product also clears all the regulatory and compliance hurdles, of course). Investors want reliable exclusivity of products and processes and if that cannot be secured, investment won't follow.

On a more general level, to promote the sector, I think there should be more reliable protection for novel cell and gene therapies through the patent and the regulatory systems – particularly for second (or further) medical use products. I consider this to be a key challenge both at present and moving forward.

JM: Ensuring patients benefit from these therapies will require cooperation between the healthcare systems, the health technology assessment bodies, and industry. All these stakeholders now have experiences to draw on from ATMPs that have been authorized and are on the market. Important lessons have been learnt and best practices can be defined. It's now time for stakeholders to work together to resolve some of the complications we've mentioned during our discussion, and to help streamline the process for future products.

Early engagement by companies with the authorities, patients and healthcare professionals will be a crucial component of this effort. This will help to ensure increased familiarity with the technology and the manufacturing

processes – and indeed, will increase the community's understanding of what these products can achieve.

Companies should also engage with industry bodies to provide their views as part of industry-wide discussions and responses to consultations to help drive harmonization in some of these areas.

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CELL & GENE THERAPY INSIGHTS

2020 VISION
TOOLS AND TECHNOLOGIES OF TOMORROW

SPOTLIGHT

INTERVIEW

Advancing iPSC-derived NK cell therapy

BAHRAM (BOB) VALAMEHR is the Chief Development Officer at Fate Therapeutics, overseeing the company's early development activities including 'off-the-shelf' cell therapy product candidates derived from the company's induced pluripotent stem cell platform. Previously, Dr Valamehr was the Vice President of Cancer Immunotherapy at Fate and prior to that played key scientific roles at Amgen, the Center for Cell Control (a NIH Nanomedicine Development Center) and the Broad Stem Cell Research Center developing novel methods to control pluripotency, to modulate stem cell fate including hematopoiesis and to better understand cellular signaling pathways associated with cancer. He has co-authored numerous studies and patents related to stem cell biology, oncology and materials science. Dr Valamehr received his PhD from the Department of Molecular and Medical Pharmacology at UCLA, his MBA from Pepperdine University and his BS from the Department of Chemistry and Biochemistry at UCLA.

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Q Tell us what you are working on right now.

BV: We are working on utilizing induced pluripotent stem cells (iPSC) to create off-the-shelf cell therapy. More specifically, we're

focused on hematopoietic development to derive effector cells that are multi-engineered to go after cancer.

Q Can you go into more depth on the Fate Therapeutics platform and approach, and what differentiates it in today's busy cellular immunotherapy field?

BV: Fate was brought together with the notion that stem cell biology is going to play a critical role in the future of medicine.

A little more than 10 years ago, we started working on iPSCs. We actually had multiple ideas brought in by the Scientific Founders, including Dr Rudolf Jaenisch and Dr Sheng Ding, focused on various different stem cell biology strategies, and iPSCs was one of these.

Initially, we focused on how to culture iPSCs in a way that's amenable to industrial platforms. For example, traditionally, pluripotent stem cells – whether iPSCs or human embryonic stem cells (ESCs) – were most often cultured as clumps on mouse embryonic fibroblast (MEF) cells. This was a very finicky process that required a lot of art and knowhow. For one thing, you have to culture the cells in the right size clump: they like to live in a community and when you dissociate them from this, they tend to die.

So we focused on how to make a culture system that was not so tricky to handle, that could be used routinely, and that could support single cell culture. This way we would spend much less time culturing iPSCs and more time learning the best way to derive desired cell types or to manipulate the iPSC's genetic makeup to modify them at the single cell level.

Following this early focus on industrializing the iPSC culture platform and genetic engineering at the single cell level, we learned how to differentiate the iPSCs into the lineage of choice. Initially, we worked on several strategies but ultimately ended up on hematopoietic differentiation. We've spent the past 5 years really fine-tuning hematopoietic differentiation towards $CD34^+$ hematopoietic progenitor cells and then towards T and NK cells, with a mindset of creating a pure product that is scalable, that can be made available off-the-shelf, and that uniformly contains the genetic engineered modalities that we introduced at the iPSC-stage, originally as a single cell.

In short, Fate's unique platform characteristic is the ability to take a single cell that has all the engineered modalities – all the preferred attributes, without the off-target effects one sees in most engineering strategies – and to create from that single cell a master cell bank that can be tapped into in a renewable manner to create off-the-shelf products at large-scale, and at a low cost per dose.

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Every vial we take from the master cell bank is uniform in composition when it comes to genetic engineering. We then differentiate that into a large number of T and NK cells, which bear the genetic modality – for example, of chimeric antigen receptor (CAR) – and now we have a true industrial platform that is very similar to that used for monoclonal antibody (mAb) production. With mAbs, you access a master cell bank of Chinese hamster ovary (CHO) cells, for example, that have been genetically edited to produce the antibody of choice, you manufacture that in a very reproducible and consistent manner, and you end up with your monoclonal antibody at large-scale. We're now doing that for cell therapy.

Q Why has Fate prioritized NK cells over other immune cell types in the pipeline, and what role do you see for NK cell therapies in the future immuno-oncology toolbox?

BV: We are actually focused on both T and NK cells. but I can certainly tell you why we love NK cells!

NK cells are the frontline immune cells that play a key role in keeping us healthy. They are multifaceted and have this unique ability to sense every single cell in your body and decide whether that cell is happy, or if it has been transformed or virally infected. Stress ligands that show up on transformed cells – i.e., cells that are now cancer or virally infected cells – differentiate them from healthy ones by giving the transformed cells a new surface profile. NK cells have the unique ability to sense those differences – they recognize that these cells have been stressed and then eliminate them.

They are very potent and in addition to being able to directly kill transformed or infected cells, they also secrete lots of pro-inflammatory cytokines such as TNF alpha and interferon gamma, which in turn recruit the second wave of adaptive immune cells – T and B cells – to join the fight against disease or infection.

NK cells also carry a unique receptor – the Fc receptor – which binds to monoclonal antibodies. Through targeting and attachment using this receptor, NK cells can be targeted to the antibody-coated cancer cells. For example, when the human body raises antibodies against certain cells that have been transformed or infected, NK cells will bind to the tail end of those antibodies which are bound to the transformed cells, which can then be eliminated by the NK cells. Monoclonal antibodies such as Herceptin were developed in part to target cancer cells in this way. In addition to blocking signaling pathway associated with cancer, Herceptin also mediates the antibody-dependent cellular cytotoxicity (ADCC) which is basically the recognition of the NK cell to the antibody to directly kill the cancer cell.

"Fate Therapeutics sees a huge opportunity in improving NK cells' ability to target and persist. If we can harness their power, then we can provide a whole new avenue of cell therapy that will be complementary to T-cell immunotherapy..."

So there are three different, unique ways in which NK cells attack transformed or infected cells. However, NK cells do have their drawbacks: they are not persistent, and they are hard to process and give back to the patient. Fate Therapeutics sees a huge opportunity in improving NK cells' ability to target and persist. If we can harness their power, then we can provide a whole new avenue of cell therapy that will be complementary to T-cell immunotherapy – the two are not in competition.

Q Many expect iPSC-derived therapies to become commonplace or even dominant in the cell therapy space over the coming decade and beyond – firstly, what are your own expectations in this regard and what timeframe would you put on this eventuating?

BV: I think it will be sooner rather than later, simply because of what's been happening at the forefront lately.

Firstly, CAR T cell therapy led by Michel Sadelain came into the clinical spotlight about 10 years ago and showed you could now really go after a cancer that has failed to be respond to other treatment strategies. That really opened the door for cell therapy. This was followed by the whole CRISPR phenomenon, with gene editing becoming a common practice where you can now routinely achieve 80–90% gene edited cell populations – not very long ago, we'd get less than a percent and be very excited about that!

This combination of facilitated gene editing with genuinely efficacious cell therapy has led to us now looking beyond that first wave of approaches, and asking how can we convert this technology into pharmaceutical drug process development strategy? And in that context, iPSC really becomes the true platform: now you can take that single cell, engineer it, create a master cell line, and from that master cell line you have a renewable process.

iPSCs are so crucial for the future because there's such a demand for this paradigm and in my opinion, no other platform can enable it. I think it's

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going to facilitate the rapid progress of iPSC to the clinic. In fact, we are already seeing this – there are so many new biotechs and also traditional pharmaceutical companies now considering iPSC as key portfolio strategy. I believe that within the next 5 years, we're going to see a lot of iPSC-derived products being assessed for safety and efficacy in the clinic.

Q Can you speak to some of the key challenges that you and others developing iPSC-based therapies can expect to encounter as these products continue to advance into/through the clinic and towards commercialization?

BV: First and foremost, there's a traditional thought that stem cells share common features with cancer cells – therefore, an induced pluripotent stem cell might look similar to a cancer cell purely in terms of its signaling cascade, although those signals are differently regulated. A lot of people carry the related misconception that just because some of these genes are expressed in an iPSC, you now have a starting material that is 'cancer-like'.

Getting over this taboo around iPSC-derived products causing a teratoma is something that I think we are well on the way to addressing – we're seeing this become less and less of a concern as differentiation processes improve and molecular tools become more powerful in their ability for high-resolution detection. At the end of the day, if you have a true platform that doesn't alter the genetic makeup of the cell in terms of creating genetic variations or chromosomal instability, and you have a final product that is completely free of or lacks the ability to de-differentiate into iPSCs, then you carry the same concerns as you would any other adoptive cell therapy. I think the scientific community will continue to get comfortable with the idea that it really doesn't matter where the NK cell came from – whether it's from cord blood, or a primary CD34-derived cell, for instance: an NK cell is an NK cell if you make and process it properly. I think having that understanding overcomes a major challenge.

Q Can you tell us more about the steps you are taking now to address these issues?

BV: We've created a cellular reprogramming process that is non-integrative, meaning we don't alter the genome and we don't insert transgenes into the starting material. When we reprogram the cell into an iPSC and then differentiate it into the cell product, there are no

"We've spent the past 5 years really fine-tuning hematopoietic differentiation towards CD34+ hematopoietic progenitor cells and then towards T and NK cells, with a mindset of creating a pure product that is scalable, that can be made available off-the-shelf..."

residual transgenes carrying the potential to de-differentiate that cell product back into an iPSC again. This addresses the first issue, which is the potential of the product to give rise to a teratoma. That ability simply does not exist.

The second part relates to residual iPSCs in the final product. What we've done here is create a differentiation process that gives us a completely pure product: we get 100% hematopoietic cells and of that 100%, 99% are NK cells, for example. We simply don't have any carryover iPSCs. We have confirmed this not only through phenotype profiling, but also through molecular analysis at a resolution that assures us the final product does not have any iPSC carryover.

Through our process, we both eliminate the potential for de-differentiation of the cells, and the presence of any carryover iPSCs in the final product.

Q Lastly, what are Fate Therapeutics key priorities and goals for the next 12–24 months?

BV: We have three iPSC-derived NK cell products cleared by the FDA for clinical investigation. First and foremost, the most important thing for Fate will be to execute on those clinical trials – to look for safety, and also to understand the kinetics behind a multi-dosing paradigm.

The second step for us is to continue to develop our pipeline with multi-edited products of T and NK cells, in order to go after certain diseases where we will need multi-edited strategies in order to completely eliminate the tumor.

So firstly, executing on the clinical trials, and secondly, boosting the pipeline through smart engineering strategies at the iPSC level to make final products that are highly efficacious and available off-the-shelf in a cost-effective and outpatient manner so that every patient has access to the treatment.

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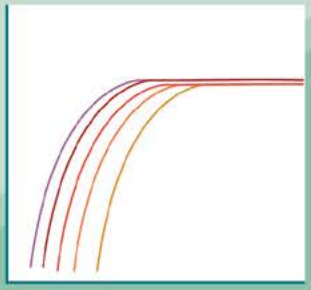
CELL & GENE THERAPY INSIGHTS

Vector Channel

ASSAYS AND TITERING EDITION



CELL & GENE THERAPY INSIGHTS

IN FOCUS:
Highlights from our Vector Channel

Assays and Titering

CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL:
ASSAYS & TITERING

EXPERT INSIGHT

Performance of in-house optimized orthogonal methods for titration of in-process and purified rAAV

Franz Schnetzinger, Kamila Pytel & Helena Pudilova

To be able to accurately characterize gene therapy products it is crucial to develop robust analytical strategies. For this we developed and optimized methods for quantification of rAAV genome (vg/mL) and capsid (vp/mL) titers, measured by qPCR/ddPCR and rAAV capsid ELISA assays, respectively. Our focus was on optimizing sample preparation steps and sample pre-dilution conditions into standardized protocols to improve on the assay precision and robustness. Using orthogonal methods, we confirmed the optimized conditions and found ddPCR to have superior performance over qPCR; particularly for in-process material. Overall our data emphasize the importance of thorough optimization and standardization of in-house developed analytical methods for advanced characterization of purified and in-process derived rAAV productions. Furthermore, we have established HPLC based protocols which correlate with viral titers observed by qPCR/ddPCR and ELISA and hence can significantly improve throughput and sample turnaround time.

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INTRODUCTION

Developing a manufacturing process for gene therapy products requires a panel of analytical methods for the characterization of the material throughout the production process and for the determination of the quality of the final material [1,2]. The focus at any stage of process development, but particularly in the early phase, is on the scale of vector generated by different process conditions. This requires robust titration methods of the viral particles and encapsidated vector genomes.

Over the last 2 years we have developed a suspension process with an animal-free upstream and two-step chromatography downstream purification for production of rAAV-based gene therapy vectors [3]. In parallel, qPCR and ddPCR based methods for genomic titer determination have been set up, a capsid ELISA has been implemented for titration of virus particles, gel-based methods for purity evaluation, and liquid chromatography-based assays for the assessment of viral titer and product quality (in terms of presence of aggregates and empty particles) have been developed.

Although applications of rAAV titer methods have been described in the literature their implementation is not as simple as copy and paste of a published protocol. Assay development requires careful consideration of the intended purpose of the method and an appreciation of those method attributes which can affect method capability. Minor variations in the sample preparation or handling can have a significant impact on assay performance. In this article we share some examples on how optimization of such steps can improve method robustness. Additionally, we describe rapid alternatives to PCR or ELISA based rAAV titration based on liquid chromatography.

The assays discussed in this manuscript are being executed in our in-house R&D labs to support product and process development. Hence, the methods are not yet formally validated. Nevertheless, the examples on method optimization are equally applicable to rAAV titration methods developed for product release.

EXAMPLE 1: OPTIMIZED SAMPLE PREPARATION FOR GENOMIC TITRATION

The ability to accurately define rAAV concentration is critical both for process development and QC release of purified material as dosage of rAAV vector products is most commonly based on the genomic titer (vg/mL) [2]. qPCR has for some time been the gold standard for this purpose. With the adoption of digital PCR for rAAV titration this approach is rapidly becoming state of the art. It offers increased robustness and accuracy due to absolute quantification being an inherent property of the methodology.

However, despite the improvements achieved in recent years in both qPCR and ddPCR equipment and chemistry, the development of a genomic titer assay for rAAV products is still a challenging task. Particularly analyst to analyst variability and the robustness of sample treatment steps when executing digestion steps are areas prone to introduce errors. While the former can be addressed by a combination of analyst training and automation of key liquid handling steps, the

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BOX 1

Implementation of appropriate control samples.

Independent of the analytical method the implementation of control samples to monitor the performance of the method is of utmost importance. The selection and implementation of an appropriate control sample should be one of the first steps in any assay development activity, as it allows to gain an understanding of the method's capability. This control sample, or samples if the method is used at multiple in-process stages, is in addition to any assay controls required to monitor the correct execution and robustness of the method.

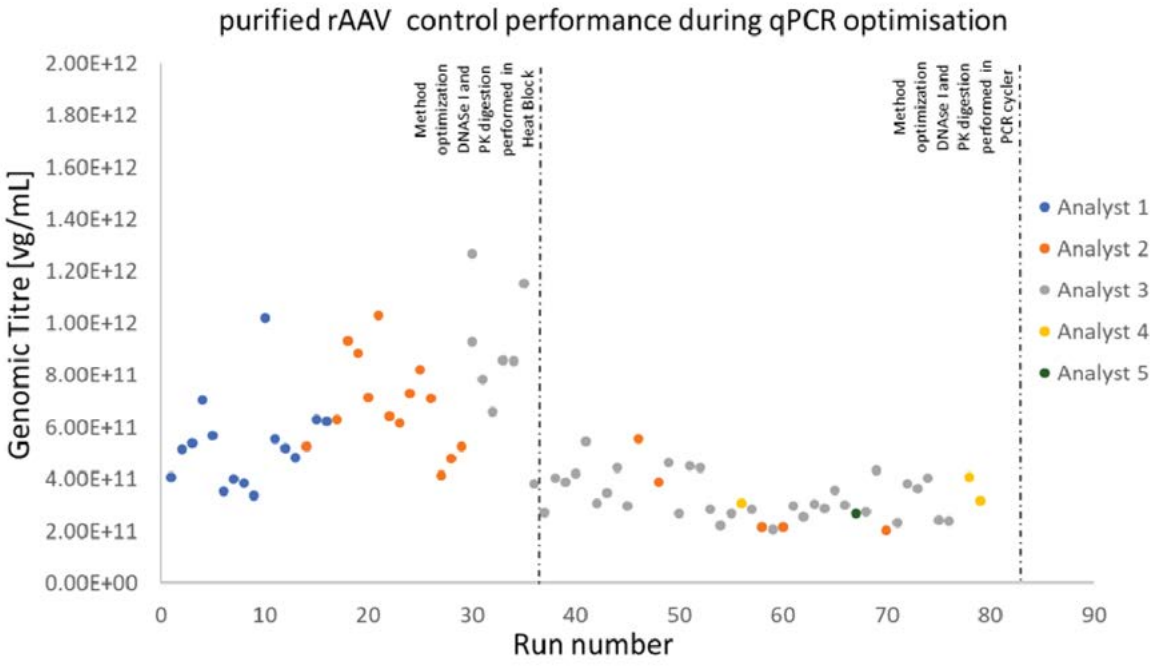
For our development work, single-use aliquots of crude vector from end of upstream processing and final, purified vector were prepared. Appropriate dilutions of these materials are run in each titer assay. These long-term data are trended and provide valuable insight in the robustness of the assay. In addition, the historic results can function as a baseline for further optimization of the method through Design of Experiment (DoE) activities.

robustness of sample treatment is much more challenging to control. Especially in the presence of abundant and diverse protein and nucleic acid process and product related impurities in crude samples [4].

With regards to digestion steps, it is important to consider both concentration of DNase I and Proteinase K [5] and timing required for selected reagents to reduce levels of residuals and other process related contaminates to release the capsid contained DNA. Thus, the focus should be on in-process samples when optimizing digestion steps to release encapsidated DNA. The more contaminated with cell debris, host cell DNA and residuals material, the more difficult to clear it and open up the capsid particles. Furthermore, process residuals can interfere with the assay and

FIGURE 1

Evaluation of purified rAAV control performance during qPCR optimisation when digestion steps executed in Heat Block or PCR thermal cycler.



Data are expressed as average genomic titre [vg/mL] of internal controls per run and color-coded as per analyst performing assay. Data contained between dash dots is significantly different (t-test, $p<0.05$).

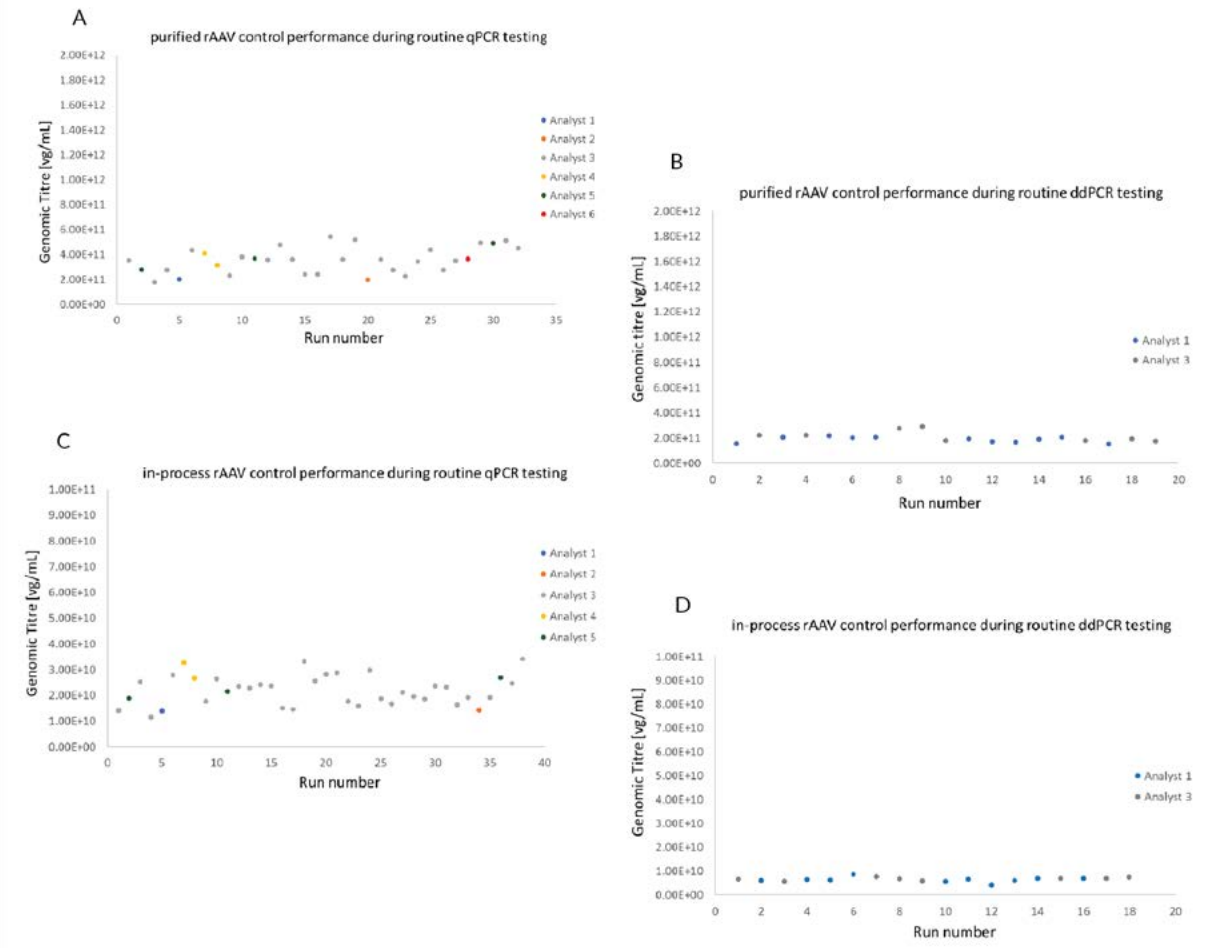
lead to an incorrect determination of genomic titer.

To ensure accurate titration in presence of free residual DNA, we have adopted the digestion protocol from Ai *et al.* 2017 [6]. In the procedure optimized for rAAV material, samples – including test material and controls – are incubated with DNase I for 15h–16h at 37 °C. This is followed by EDTA addition to stop Dnase I from potentially digesting released rAAV DNA. When cleared from all contaminants, material is treated with Proteinase K and subsequently used in the qPCR/ddPCR assay. Mastermixes and amplification conditions have been optimized for the used reaction chemistry and oligonucleotides.

Another important factor which we decided to investigate Was equipment used for overnight DNase I digestion. This is because we observed variability in titer results of our rAAV control. Upon comparison of both standard laboratory thermoblock and Bio-Rad PCR C1000 thermal

FIGURE 2

Evaluation of internal controls performance in qPCR and ddPCR assay.



(A & B) Purified rAAV control tested during routine qPCR (n=32) and ddPCR (n=19) runs, respectively. **(C & D)** In-process material control tested during routine qPCR (n=38) and ddPCR (n=18) runs, respectively. Purified control and in-process control were digested using the same protocol and reagents. Data are expressed as average genomic titre [vg/mL] of internal controls per run number and color-coded as per analyst performing the assay.

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BOX 2

Analytical HPLC methodologies.

IEX-HPLC methodology: salt concentration gradient, NaCl 0 – 250mM at high pH with additional salts at constant concentration.
AFF-HPLC methodology: neutral buffered capture step, low pH buffer rAAV elution step, low pH acid post-elution wash=CIP, neutral buffer re-equilibration.

cycler (Figure 1) we noticed a significant reduction in titer variability (statistical significance was confirmed by two-sided t-test assuming unequal variance and $\alpha = 0.05$) when purified rAAV control digested in the PCR thermal cycler overnight. This was, we assume, due to an enclosed environment which heats the vial from all sides. Which in turn creates reduced sample evaporation and condensation on the lid and results in a more homogeneous reaction environment.

While the optimization of the digest led to reduced qPCR variation, inter-assay CV was still substantial both for purified material (CV 29.2% across 32 runs) and in-process material (CV 26.5% across 38 runs). Using ddPCR method developed in parallel to the qPCR assay as an orthogonal method allowed us to more precisely define genomic titers of tested material (Figure 2 A, B, C & D). For this purpose, we directly transferred qPCR method onto ddPCR using EvaGreen chemistry instead of SYBRGreen. As a result of which we observed less variability of coefficients of variation when assessing vector's titer by ddPCR for purified material (CV 17.8% across 19 runs) and in-process material (CV 14.3% across 18 runs) indicating additional advantage of the method over qPCR and confirming the superior performance of ddPCR reported recently [5].

To conclude, ddPCR quantifies using endpoint instead of real-time amplification and it is less affected by inhibitors of amplification that may be present in the sample [7]. Moreover, ddPCR is also not affected by batch to batch variability of plasmid DNA used for preparation of the standard curve, inconsistent standard curve performance, or bias in plasmid DNA quantitation. The latter being the most likely root cause for the difference in qPCR and ddPCR genomic titers shown in Figure 2.

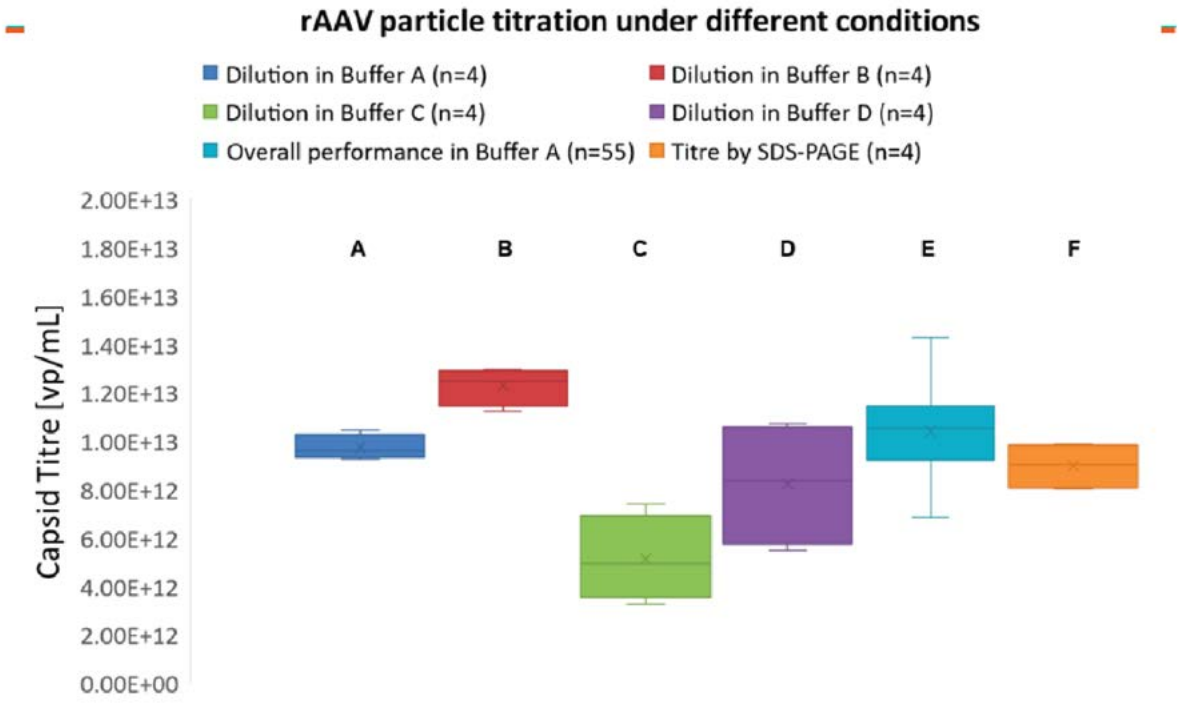
EXAMPLE 2: STANDARDIZED PRE-DILUTION OF CAPSID ELISA SAMPLES

While genomic titer provides the yield of rAAV particles containing the qPCR/ddPCR target sequence only, the capsid titration returns the total number of rAAV particles; including capsids containing the target sequence, empty particles, and particles encapsidating non-specific nucleic acids. As this capsid titer can vary significantly from the genomic titer, understanding the relationship between genomic and capsid titer is an important step in characterizing rAAV preparations. Furthermore, it informs process development on lot to lot variation of the vector material.

Typically, the sandwich ELISA is the format of choice for determination of capsid titer of rAAV preparations. Albeit the principles of implementing an ELISA assay are well established, the specific sample conditions of rAAV vectors require

FIGURE 3

Comparison of capsid titres using different diluents for rAAV sample dilution is shown as box plots.



Boxes represent the values between Q1 and Q3, whereas whiskers represent 1.5 interquartile range, horizontal lines represent medians, crosses represent average values. Box plots A, B, C, and D show the capsid titre when using different buffers for sample dilution (n=4 for each buffer) performed by same analyst across 4 ELISA plates. Box plot E shows the overall assay variability after selection of buffer A. Box plot F shows the capsid titre based on SDS-PAGE confirming buffer A as the most suitable diluent for ELISA samples. Mean titres in vector particles per millilitre (vp/mL) and coefficient of variation (%CV) for each condition are: A) 9.73E12, 5.5%; B) 1.23E13, 6.6%; C) 5.12E12, 34.6%; D) 8.23E12, 31.2%; E) 1.03E12, 14.9%; F) 8.98E12, 11.0%.

additional considerations during method development. Specifically, the formulation of the purified vector often contains excipients in order to reduce aggregation and loss of vector due to binding on charged container surfaces. Potential matrix effects were an unknown variable when we started capsid titration. In order to elucidate whether the surfactant could interfere with the assay, leading to higher variability and/or a negative or positive bias in the assay, we investigated the repeatability of the method using different commonly used ELISA buffers. Furthermore, we evaluated which dilution buffer would be most suitable to bring purified vector samples within the range of the ELISA standard curve.

Figure 3 shows the data gathered during the buffer evaluation of an rAAV capsid titration method. Four commonly used buffers for sample dilutions in ELISA assays were compared by preparing independent dilutions of a well characterized, purified vector material. The four different preparations (A, B, C, and D) were tested on one ELISA plate and quantified against the kit standard per kit instructions. The plate was repeated three times for a total of four determinations of each buffer. All four runs were performed by the same operator using the same lot of ELISA kit.

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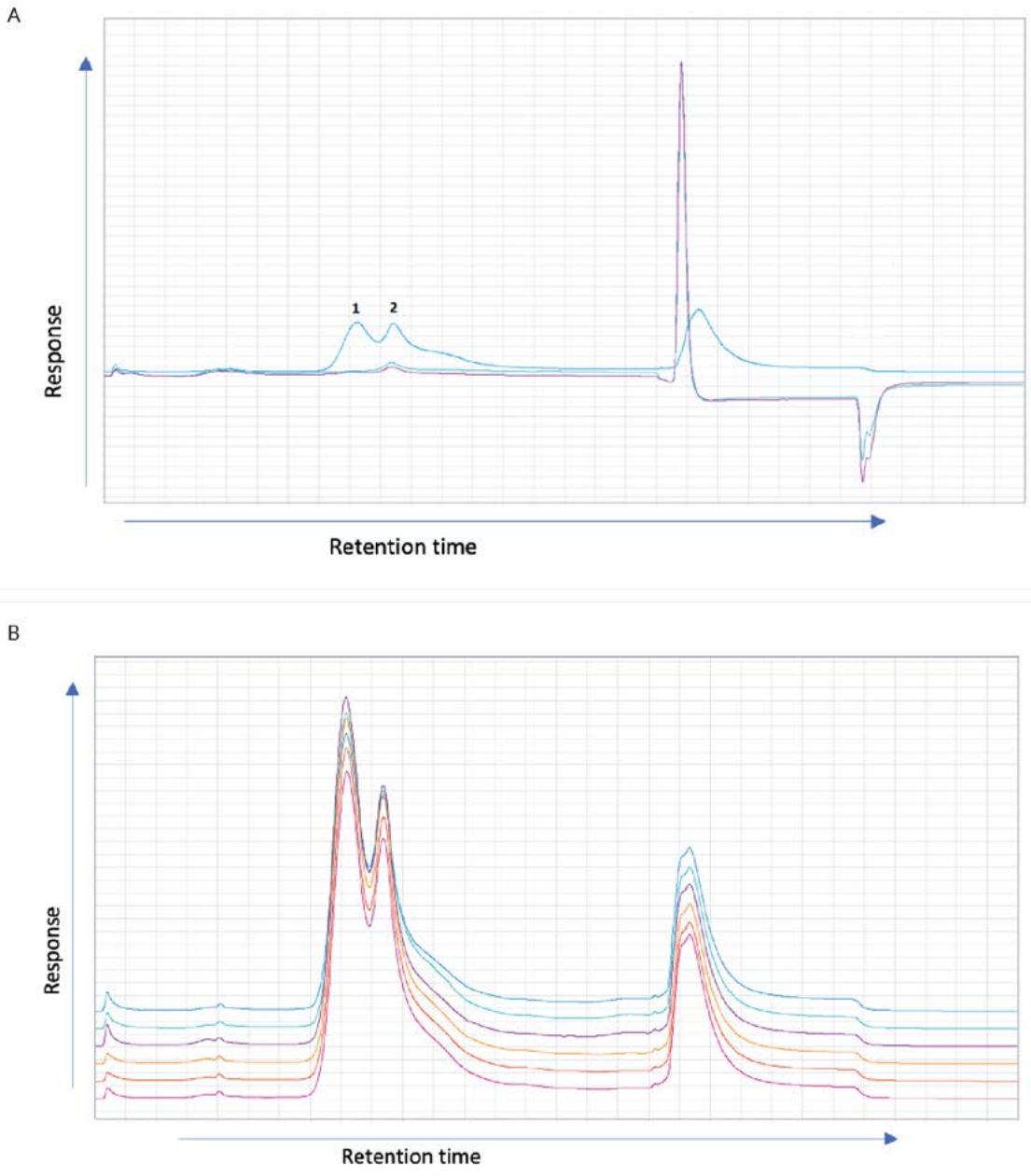
FIGURE 4

Representative chromatograms of AAV samples.

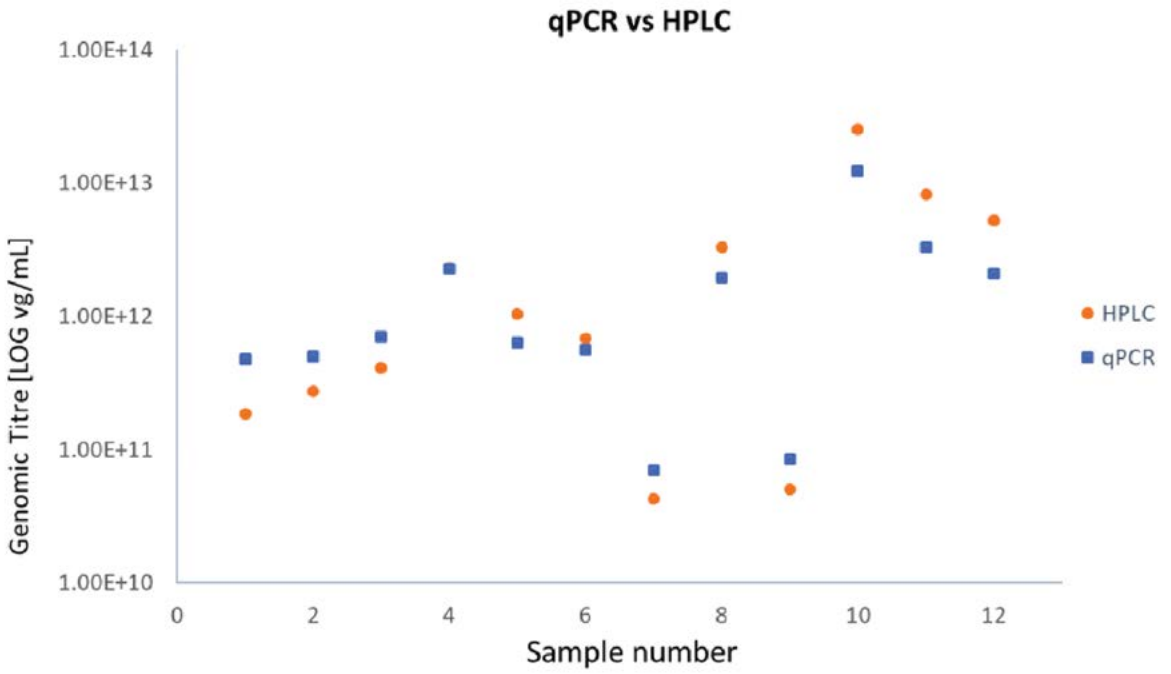


(A) Chromatogram of the UV response at 260 nm (green), 280 nm (lilac) and fluorescence (blue) obtained by IEX-HPLC. Peaks: 1. Empty rAAV capsid, 2. Full rAAV capsid. Fluorescence peak intensity is greater than for the A280/A260 signals and increases the lower level of detection. **(B)** Stacked chromatogram showing the fluorescence response of 6 injections from IEX performed at preparative scale.

The buffers C and D resulted in undesirable variation of the assay with a CV >30%. Buffers A and B performed similarly in terms of sample variability with a CV of 5.5 and 6.6%, respectively. Yet the average titers were significantly different from each other. To confirm the range of the expected capsid titer a quantitative SDS-PAGE was performed which was adapted from a published protocol [8]. These

FIGURE 5

Observed correlation between genomic titre obtained via qPCR (blue squares) and IEX-HPLC (orange dots); correlation coefficient = 0.9914 (calculated using formula correl (X,Y) = $(\sum(x-\bar{x})(y-\bar{y})/\sqrt{(\sum(x-\bar{x})^2 \sum(y-\bar{y})^2})$ where $\bar{x}$ and $\bar{y}$ are the average of x and y values, respectively).



data confirmed buffer A as the most suitable diluent for rAAV samples as SDS-PAGE results were not significantly different1 from ELISA results when using buffer A as diluent. Since finalization of the ELISA protocol, with Buffer A as the selected diluent, the method has been run by four operators using multiple lots of assay reagents. Across 55 runs, performed by 4 analysts using 3 different ELISA kit lots over a period of greater than 12 months, the method variance is <15% based on the variance of the assay control.

EXAMPLE 3: RAPID IN-PROCESS SAMPLE TESTING

Titration of genomic and capsid titer of rAAV preparations are indispensable analytical tools for development of rAAV manufacturing processes and the characterization of the generated material. These methods have one significant drawback, however – throughput. Digest, incubations, and PCR reactions require time. Furthermore, the number of samples that can robustly be handled within an assay is limited. A potential solution for this issue is moving to a different platform altogether. (Ultra-) high performance liquid chromatography (HPLC) applications based on affinity and ion-exchange separation have previously been described [9–11].

We developed HPLC titration assays based on the purification steps used in our downstream processing, affinity chromatography (AFF) to capture rAAV particles followed by ion-exchange (IEX)

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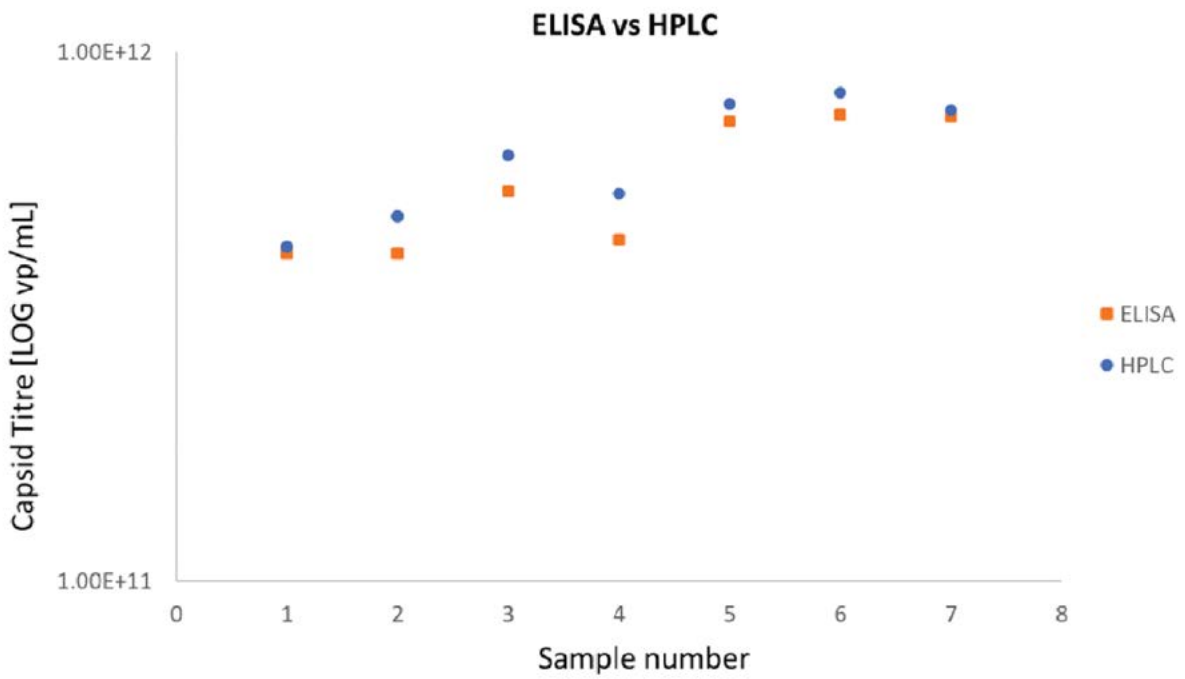
FIGURE 6

Observed correlation between capsid titre obtained via ELISA (orange squares) and AFF-HPLC (blue dots); correlation coefficient = 0.9798 (calculated using formula correl (X,Y) = $(\sum(x-\bar{x})(y-\bar{y})/\sqrt{(\sum(x-\bar{x})^2 \sum(y-\bar{y})^2)}$ where $\bar{x}$ and $\bar{y}$ are the average of x and y values, respectively)



chromatography for full/empty separation (Figure 4). This allowed us to feed the method development data back into the manufacturing process. And, vice versa, data from process development informed analytical method development on important topics such as stability of the samples and importance of sample preparation. Understanding of variability of the sample impurities introduced in upstream and downstream processing steps can help with choosing the best preparation method for analytical samples (e.g., filtration, centrifugation, dilutions or pH adjustment prior to the IEX).

In both HPLC applications an rAAV control sample is used for the quantification of test samples (see Box 2 for chromatographic methodologies). For this, a serial dilution of the control sample is injected and the fluorescence signal recorded. The linear relationship of the nominal concentration of each dilution with the fluorescence signal is then used to quantify the test samples. Nominal genomic and capsid titer of the control material are based on the qPCR and capsid ELISA methods described above.

IEX-HPLC AS A RAPID ALTERNATIVE FOR QPCR

During downstream process development the large-scale preparative process was transferred onto an analytical column in order to optimize the separation without the need for large volume of crude vector. Albeit the move to the analytical column was initially made to reduce the scale of the preparative work we

soon found this to be an excellent tool for quantifying rAAV vector rapidly and reproducibly in about 15 minutes.

Whilst developing both the preparative and analytical scale of the IEX chromatography we were gathering information on the stability of the vector material using both qPCR and IEX-HPLC for titration. Sample stability studies were also performed to determine the appropriate sample preparation for IEX-HPLC testing. In addition, understanding the impurities in the in-process samples was crucial for the method development and again preparation of the samples for analysis.

Comparing results from IEX-HPLC with qPCR data shows a good correlation between the two methods (Figure 5). Based on these data the IEX-HPLC method was applied as an orthogonal method for our in-process samples.

AFF-HPLC AS RAPID ALTERNATIVE FOR CAPSID ELISA

Similarly to IEX-HPLC, the AFF-HPLC is derived from the preparative protocol. To establish the analytical method, we packed the same resin of the downstream purification process into an analytical column and tested it under similar conditions to our preparative method. As affinity chromatography captures both full and empty rAAV vector particles we could confirm that the fluorescence peak area of the AFF-HPLC method correlates closely with the capsid titer determined by ELISA (Figure 6).

Due to the greater automation and sample throughput of HPLC analysis compared to ELISA testing, we have used this AFF-HPLC to screen upstream development samples prior to ELISA testing to rapidly identify the impact of changes to upstream conditions. Furthermore, the AFF-HPLC not only works as an orthogonal technique for capsid ELISA quantification but provides a tool for purification of upstream samples on small scale for additional characterization.

TRANSLATION INSIGHT

Accurate determination of vector titers is crucial for the development of safe and efficacious gene therapy products. The examples from our in-house method development show only a small piece of the work that needs to go into the optimization of each assay. Yet they highlight the challenges the industry is facing in developing robust and reliable rAAV titration methods. Seemingly small changes in equipment or reagents can have a significant impact on the assay performance.

Despite decades of rAAV research the industry is far from defining standardized assays for vector titration; particularly for genomic titer assays numerous protocols have been published. While primer/probe systems need to be specific to the material tested, differences in the available protocols go beyond that; set up of the digest (both DNase I and Proteinase K digest or DNase I digest only; buffers used, concentration of enzymes, and conditions of digest), type of dilution buffers used, and the used PCR chemistry vary significantly in available protocols. On top of that the different equipment platforms with bespoke software solutions add another layer

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of complexity and make a comparison of product titers across different protocols practically impossible.

Hence, in the absence of a more unified approach for vector titration care must be taken to appropriately evaluate the impact each step in an assay can have on the final result. As highlighted by our examples it is important that this optimization considers all operations, reagents, and equipment concerning sample handling and preparation. This, however, requires time and resources. And analytical resources for method development are often in direct competition with the need for sample analysis from process development.

We found IEX-HPLC and AFF-HPLC to be suitable orthogonal methods for genomic and capsid titration, respectively. Particularly the qPCR/ddPCR bottleneck can be avoided by using IEX-HPLC for screening in-process samples as there is no need for digesting samples. Similarly, AFF-HPLC can reduce the need for more resource intensive ELISA assays. Combining the high-throughput of HPLC methods for screening and using qPCR/ddPCR and ELISA to follow up on interesting observations of the HPLC methods allowed us to streamline the sample analysis. With further optimization these HPLC methods have the potential to become valuable process analytical technology (PAT) tools in the rAAV manufacturing process.

Last but not least, the analytical chromatography can be used as a suitable small-scale model of our downstream purification process. This reduces the burden of generating high volume crude vector material and thereby increasing the throughput of different processing conditions which can be screened in parallel.

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CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL:
ASSAYS & TITERING

INTERVIEW

Emerging tools and needs for AAV vector characterisation

FABIEN DORANGE holds a PhD in virology and worked as a post-doctoral researcher in the gene therapy field . Before joining Genethon, Fabien was Head of R&D viral safety in a CRO company (Texcell, France). He joined Genethon in 2016 and currently heads the analytical development department. He is responsible for developing analytical assays for characterization and release testing of gene therapy AAV vectors.

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Q What are you working on right now?

FD: We are working on the development of analytical assays for our main gene therapy projects here at Genethon, which focused on rare genetic diseases including Crigler-Najjar Syndrome and Duchenne Muscular Dystrophy. We started a clinical trial last year for Crigler-Najjar Syndrome.

We are also working on establishing potency assays, which is quite challenging in this field in general, but particularly so when you're dealing with a disease that impacts structural function, such as a dystrophy.

The analytical team is also working on the evaluation of new technologies for increasing the throughput and improving the accuracy and precision of analytical methods.

Q The need for innovation in viral vector bioprocess analytics has become an extremely timely and pressing topic of late for the whole gene therapy field – can you frame for us why this is, from your perspective?

FD: From my point of view, in order for gene therapies to benefit fully from accelerated or expedited development pathways such as fast-track designation, we need to generate applicable analytical tools to support the development of both upstream and downstream bioprocesses. We are really dependent upon this characterisation knowledge, which is why we need to implement robust, precise and high-throughput methods.

We also require sufficient materials for the development and clarification of specific assays for these analytical tools – for example, we sometimes need to characterise reference materials that will be used for transferring methods to a CMO or CRO.

Q Can you pick out any emerging technologies or techniques which particularly interest you in this area at the moment?

FD: For the determination of AAV vector genome titres, which define the dose of product, we are currently working with Droplet Digital PCR (ddPCR) technology. ddPCR has shown very promising results so far, offering better precision than the current gold standard, qPCR. It is my belief this technology should drive greater efficiency in AAV vector process development. This is quite important for calculating recoveries, etc.

Droplet digital PCR (ddPCR) can be used in the vector genome quantification and also in DNA impurities (plasmid or host cell DNA) quantification. Two-dimensional ddPCR has also recently been used to evaluate AAV genome integrity using targeted sequences to both ends of the viral genome.

So we feel this is a complete method. However, it is important

"in order for gene therapies to benefit fully from accelerated or expedited development pathways such as fast-track designation, we need to generate applicable analytical tools to support the development of both upstream and downstream bioprocesses."

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"In addition to ddPCR, I think next-generation sequencing is an important method moving forward. It could be very useful for the characterisation of all encapsidated DNA sequences, including the vector genome but also the cell DNA sequence and AAV plasmid DNA - it can give you an overview of the contaminating DNA in your samples."

to take care regarding the dilution series for the sample preparation, as we need to dilute the vectors quite a lot in order to be in the right range of quantification. You also need to consider the aggregation level, as vector aggregates may lead to inaccurate VG titers with ddPCR.

In addition to ddPCR, I think next-generation sequencing is an important method moving forward. It could be very useful for the characterisation of all encapsidated DNA sequences, including the vector genome but also the cell DNA sequence and AAV plasmid DNA - it can give you an overview of the contaminating DNA in your samples.

Q What's your take on analytical tools being repurposed from the mAbs world – in particular, what are the challenges with adapting them to viral vector production, and how do you seek to address them?

FD: There are many methods used for conventional biologics such as mAbs that are similar for the characterisation of AAV vectors, including all the impurity testing methods such those for DNA and other protein impurities.

However, while there are some common methods that we can use, we also need specific reagents due to the fact we use production cells that are different from those in the mAbs world. For example, when it comes to the quantification of the residue of cell proteins, we have access to only a limited number of commercial kits. There's a clear need to generate more specific reagents such as platform- or process-specific reagents for AAV vectors.

AAV vectors also have additional features to mAbs: they comprise both protein and nucleic acid components, and so we need methods to characterise viral capsid and encapsidated vector genome. We also need to verify the identity of the vector genome, the capsid serotype, and the infectivity of the viral vector. And we need to establish the potency of the product, which requires a specific cell-based assay.

One final important consideration is the fact we have access to a much lower volume per batch with AAV vectors used for muscular diseases than we would if we were working with mAbs, for example.

Q Where do you see progress in bringing analytics inline, specifically – and where do the key gaps of deficiencies remain in this regard?

FD: In my view, there are no in process analytical tools available today that can easily control inline AAV production - that can readily generate process efficiency information. We clearly need an in-process method to characterise the main quality attributes of AAV vectors with a short time to result.

Q Finally, can you pick out 2-3 future tools which would be on your wish-list as technological breakthroughs that could make the greatest difference to gene therapy manufacture?

FD: Today, we are missing tools for the deeper characterisation of AAV vectors. We need to develop assays that deliver a greater knowledge of AAV products in order to improve product quality and patient safety.

We also need high throughput technologies, automation, and shorter time to results – both for controlling the manufacture of AAV vectors and for QC testing to decrease time to release.

And as we discussed earlier regarding the relatively small batch volumes we have with AAV, we also need analytical tools that require reduced quantities of product.

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CELL & GENE THERAPY INSIGHTS

Supply Chain Channel

MATERIALS COLLECTION & LOGISTICS AT THE CLINICAL POINT OF CARE

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Materials Collection & Logistics at the Clinical Point of Care

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SUPPLY CHAIN FOCUS: MATERIALS COLLECTION & LOGISTICS AT THE CLINICAL POINT OF CARE

CHANNEL CONTENT

FOREWORD

Managing starting materials collection and logistics at the clinical point of care

JOHN DM CAMPBELL is Associate Director of Tissues, Cells and Advanced Therapeutics at the Scottish National Blood Transfusion Service (SNBTS) in Edinburgh. He completed his PhD in Pathology at Edinburgh in 1995, and has worked in the cellular therapy field for 30 years in the academic, industrial and healthcare sectors.

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The cell and gene therapy field has undergone enormous expansion in the past decade in terms of the numbers of products being developed and their use in clinical trials. Unprecedented levels of investment are driving cell and gene therapy to marketing authorizations and these living medicines are having major impacts in previously untreatable diseases. While a multiplicity of products are now in development, these products share at least one thing in common – procurement of an appropriate starting material. Procurement of properly consented and tested starting materials is the foundation of the entire cell and gene therapy industry – in this supply chain quarterly spotlight we examine three distinct but inter-related parts of this dynamic field.

Sharon Zahra and colleagues discuss the first steps in the supply chain, setting out the frameworks for collection and testing of starting materials. They highlight the common areas of good practice in starting material procurement which pertain, whether this is a relatively simple donation such as leukapheresis or a more challenging material such as a tissue biopsy. The authors are experts in understanding the partnership between the medical, nursing and laboratory staff involved in collection of starting materials and the manufacturer and discuss vital aspects of training and validation. Manufacturing of cellular therapies is now commonly taking place across different

jurisdictions and the authors discuss how good practice is supporting this extended supply chain, but also highlight areas where further harmonization will be required.

Neil McGowan and colleagues examine the next step in the chain – the characterization, testing and storage of the starting materials after collection, ensuring that they meet critical quality attributes. They discuss in detail the initial packaging and storage strategies for different starting materials, and highlight good practice that is vital in ensuring consistency in the subsequent manufacturing steps, whether these involve fresh or cryopreserved material. Cryopreservation is commonly used for starting materials, but there is still variation in cryopreservation methods, and the type and grade of clean rooms used – here the authors discuss these issues with reference to the latest regulatory guidance. The authors also discuss at length the analysis of starting materials - the different methods used, the specific requirements unique to tissues versus liquid samples as starting materials, and how this analysis must go hand in hand with the manufacturing and final product critical quality attributes. Often the starting material is the key comparator in a number of assays regarding phenotypic changes and genetic stability and is thus key in ensuring safety of the final manufactured product.

In the third part of this spotlight, an interview with Peter Olagunju provides a thorough insight into the intricacies of starting materials procurement, which present challenges even for a company with a highly focused portfolio such as Bluebird Bio. Many of the areas discussed in the two articles are well illustrated in Peter's interview as he discusses Bluebird's strategic approach to deal with issues such as autologous (mobilized) versus allogeneic (steady state) products; a supply chain with no cryopreservation steps and integrating hospitals into the supply chain for collection of starting material and delivery of finished product. Peter also looks to the future, with the potential for hundreds of candidate therapies to progress to late stage clinical trial and marketing authorization within the next few years. He highlights areas of regulation/audit, product labelling and integration of different IT portals which require joined-up thinking support the trials of the future.

A recurrent theme from all three articles therefore is the need for standardization, and to build industry-wide consensus in order to support the massive expansion of the cell and gene therapy industry.

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CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: MATERIALS COLLECTION & LOGISTICS AT THE CLINICAL POINT OF CARE

EXPERT INSIGHT

Challenges in the procurement of starting materials for ATMP manufacture

Sharon Zahra, Lynn Manson, Lisa Jarvis & Marc Turner

Somatic cell, genetically modified and tissue engineered advanced therapies share a common generic supply chain with other substances of human origin such as blood components, tissues, minimally manipulated cells and solid organs. The quality, safety and efficacy of the medicinal product at the point of administration to the patient is contingent on the entirety of its provenance including the selection and screening of the donor, procurement and distribution of the starting material, manufacturing, and return to, and management within, the healthcare environment. Information, chain of identity and regulatory compliance must flow bi-directionally, often across several different organizational boundaries. Moreover, the heterogeneity of advanced therapies is accompanied by considerable diversity in supply chains and complexity and disruption to existing modes of operation which act as a barrier to adoption. Overcoming these challenges and realizing the benefits of this new generation of therapies requires a new philosophy of long-term collaboration, mutual understanding and partnership between commercial and healthcare organizations.

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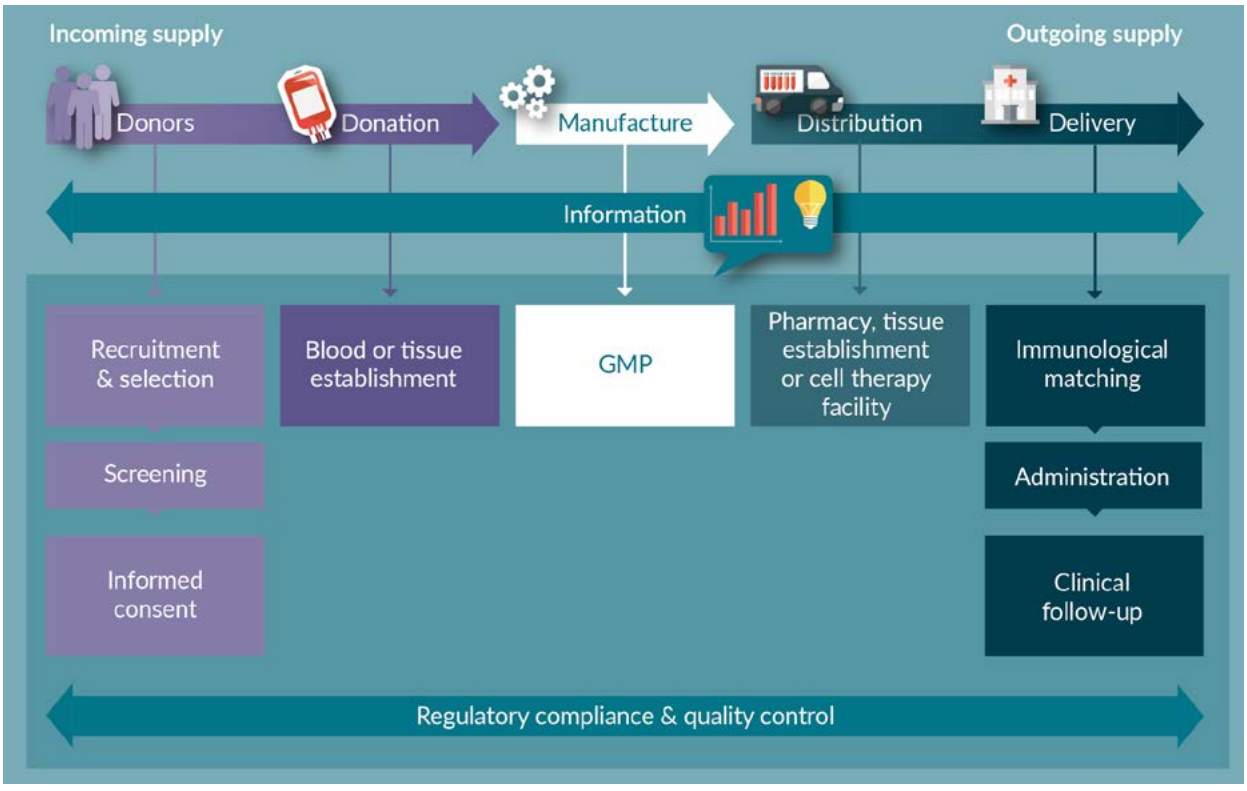
INTRODUCTION

The quality, safety and efficacy of Advanced Therapy Medicinal Products (ATMPs) are highly contingent on the integrity of their supply chain. In the case of somatic-cell therapy medicines (including those subject to genetic modification), tissue-engineered medicines and those consisting of cells combined with medical devices (combined ATMPs), the generic supply chain is similar to that of other substances of human origin such as blood components, unmodified cells and tissues and solid organs for transplantation (Figure 1). In this context the provenance of the starting material is of critical import to the quality and safety of the medicinal product and therefore issues such as information flow, chain of custody and quality and regulatory compliance need to be considered bi-directionally across the whole of the supply chain [1,2].

Starting materials are heterogeneous and may consist of peripheral blood cells such as hematopoietic stem / progenitor cells, T cells, NK cells or monocytes, tissue-derived cells such as mesenchymal stromal cells from bone marrow or adipose tissue, or solid tissue such as skin or tumor samples. The starting material may be donated by the patient her/himself (autologous) or another person (allogeneic) and the quality thereof may be impacted by biological variability between and within donors particularly, in the case of autologous starting materials, the patient's underlying diagnosis, past and current treatment and current health.

FIGURE 1

The supply chain for substances of human origin.



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The donor selection, screening and consent processes may vary dependent on the nature of the donor and starting material. Procurement may be carried out by apheresis in a dedicated unit, by aspiration in a clinical environment or by biopsy or excision in a surgical theatre, again dependent on the starting material. Donor selection and screening, procurement, labelling and traceability requirements may vary across different regulatory jurisdictions leading to particular problems importing or exporting starting materials for ATMP manufacture across international borders. Finally, of course, even a specific defined starting material may be used by different organizations in the manufacture of different ATMPs in different contexts. Clinical trials are highly controlled with relatively small numbers of patients being managed in accredited centers. Procurement of starting material from much larger numbers of donors/patients in a general clinical environment can challenge both competencies and resources.

In most regulatory jurisdictions, donation of starting material for ATMP manufacture is regulated under the frameworks designed for procurement of tissues and cells for direct human use – for example, in the European Union under the Tissues and Cells Directives (2004/23/EC) as transposed into Member State law and in the US under the Code of Federal Regulations (21 CFR 1271) and the Public Health Service Act (Sections 351 and 361). Other jurisdictions may not have specific regulation pertaining to blood, tissue and cell procurement and manage this under medicinal product legislation. In addition, professional accreditation bodies provide guidelines for procurement of some cells and tissues (such as the FACT-JACIE standards applied to clinical apheresis units) [3].

Although the general purpose and themes of regulatory legislation are similar across international jurisdictions, differences in detailed practices with respect to donor selection and screening criteria, procurement, packaging and labelling present challenges where starting materials and/or medicinal products are crossing international boundaries.

In the face of such multifaceted complexity there are therefore significant challenges in developing standardized approaches to the procurement of starting materials. A degree of standardization is, however, essential to ensure safety of products and allow simplification of processes, scale out and reduction in costs and risk of error, and will require partnership amongst healthcare organizations, manufacturers and regulatory authorities if ATMPs are going to realize their potential for widespread global adoption. This article outlines the main challenges and potential solutions in this regard.

DONOR SELECTION

Donor selection criteria include determinants of both suitability and eligibility. Suitability encompasses ensuring that the donor's health and wellbeing are not compromised by undergoing the procurement process whilst eligibility ensures that the risk of infection or other disease transmission through procurement, storage, manufacture and administration is minimized. These criteria

may vary significantly dependent on whether the donation is for autologous or allogeneic purposes:

Autologous donation

For autologous ATMPs the donor of the starting material is also the recipient of the medicinal product and as such donor eligibility (i.e., to minimize transmission of infectious, autoimmune or neoplastic disease) does not apply. However, it is still important to ensure that the patient is suitable to undergo the procurement procedure without being exposed to undue risk and that the starting material will not be compromised with respect to its quality and safety (e.g., due to contamination from an ongoing bacterial infection). Care also needs to be taken to protect the staff and facilities involved in procurement and manufacturing, and to ensure that the risk of cross-contamination of any other products manufactured in the same facility is minimized.

In some cases, such as the manufacture of autologous CAR-T products [4], there may be a limited window of opportunity to procure suitable starting material when the patient's performance status is adequate following previous treatments and before progressive disease takes over. Careful benefit–risk assessment of the suitability of the patient is therefore required on a case by case basis.

Allogeneic donation

When procuring starting material for manufacture of allogeneic ATMPs, donor selection needs to be performed carefully to ensure not just the suitability of the donor but also their eligibility to donate starting material as aspects of their health may impact on the quality and safety of a manufactured medicinal product that could be used to treat numerous patients over several years [5]. Such assessments need to include a full medical, travel, social and behavioral history and, in some jurisdictions, physical examination of donors.

A donor medical history needs to be taken to assess the potential impact of any previous or current medical condition on the quality of the donated material and the risk of disease transmission. The impact of any medications the potential donor may be taking also needs to be considered, both in terms of their potential to impact negatively on the quality or safety of the medicinal product during manufacture and in terms of potential direct toxicity to the recipient(s). The donor's past medical history must also be considered when determining donor suitability, especially if donation will require a general anesthetic or surgery.

Donor travel (both recent and in the distant past) also needs to be considered when assessing the risk of travel-related infections that may be transmitted through the donated material. Most travel-related risks e.g. potential exposure to West Nile Virus or Zika virus are associated with recent travel (in the previous 12 months) as most such infections are normally cleared by the potential donor within a number of months; however travel in the more distant past, e.g., childhood residency, also needs to be taken into account when considering the risk of infections that may persist in a subclinical form (e.g., malaria).

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Similar to donors of other substances of human origin, an assessment of the potential donor's social and behavioral risks must be carried out due to the risk of infectious window period donations. Evidence of high-risk behavior in the donor e.g., tattoos in a non-regulated environment or intravenous drug use, should lead to donor deferral even in the face of negative results for markers of currently identified infections.

If a potential donor does not meet screening criteria but there is an urgent clinical need such that deferral of the donor would result in greater risk to the intended recipient than use of the ineligible donor, a documented protocol exception can be considered subject to a risk assessment on a case-by-case basis.

DONOR MICROBIOLOGY SCREENING

For autologous donors, some countries do not mandate transfusion/transplant-transmitted infection testing and this is captured on the product label. However, to support standardization of practice, reduce the risk of exposure of staff and facilities and of cross-contamination of other products, it is usual to carry out the same testing regimen in both autologous and allogeneic donors.

Donation screening can be broadly divided into two main categories: mandatory and discretionary testing. Mandatory tests are an absolute requirement prior to release of substances of human origin and are defined in the relevant legislation. Most countries will, as a minimum, mandate serological testing for hepatitis B and C viruses and human immunodeficiency virus I/II although molecular testing may also be mandated to increase the safety of the product by reducing the possibility of infectious window period donations [6].

Additional or discretionary testing may be performed because of a specific additional or identifiable donor or recipient risk: for example testing for West Nile Virus RNA in donors who have travelled to a West Nile affected region in the 28 days prior to donation or serological testing for *Cytomegalovirus* (CMV) for immunosuppressed CMV-negative recipients.

Due to the higher sensitivity of screening tests, as compared with diagnostic tests, screening algorithms are used. Donations with initially reactive results for infectious disease markers are initially repeat tested in duplicate with the same screening assay. Repeat negative donations can be released for use. On the other hand if repeat testing identifies a repeatedly reactive sample further testing is required by a designated reference laboratory. Based on the results of the confirmatory testing, donations may or may not be considered suitable for use.

All microbiological tests or reagents for the testing of blood, tissue or cell donors must comply with regulatory requirements on medical devices for *in vitro* diagnostics which can and do vary across different jurisdictions. Microbiological tests require high quality samples to ensure accurate results. Samples should be of sufficient volume to allow initial screening, any subsequent confirmatory testing and archiving. A quality management system should be in place to monitor the performance of tests, ensuring

that sensitivity and specificity meet state of the art standards.

PROCUREMENT

Cellular apheresis

Cellular apheresis can be used to procure a wide variety of different starting cell types used in the manufacture of different types of ATMPs including hematopoietic stem and progenitor cells, alpha-beta T cells, regulatory T cells, gamma-delta T cells, NK cells or monocytes [7–9].

Collection is generally carried out using continuous-flow apheresis machines, though some units still use intermittent flow systems. In either case the apheresis machine settings need to be set to optimize mononuclear cell collection, as indicated by the product yield. The apheresis procedure will generally take 3 to 6 hours to complete depending upon the size of the donor, the flow rates through the venous access devices being used, the pre-collection peripheral blood count and the target yield. Consideration should be given to the need for a temporary wide bore double lumen central venous access device to ensure that vascular flows are optimized and uninterrupted during the procedure for interface optimization.

Whilst allogeneic donors will have normal peripheral blood counts this may not be the case for autologous donors where underlying disease and the treatment thereof may result in failure of the donor to reach the required pre-collection peripheral blood target cell threshold above which collection can proceed. The target cell and threshold will vary depending upon the manufacturing process and ATMP. The decision to collect may be based around a peripheral blood absolute target-cell count or total mononuclear cell count. Close liaison with the referring clinical or study team is critical to ensure that autologous procurement is carried out in an appropriate clinical window of opportunity.

Once the target cell threshold has been achieved, mononuclear cell collection commences, often with manual adjustments to the inlet flow rate and collection hematocrit being made by the specialist apheresis operator to optimize target cell yield. The total blood volume to be processed will be impacted upon by the pre-collection peripheral blood count, the donor's size and the target yield, and so a relative (e.g., 2.5 times total blood volume) rather than an absolute (e.g., 10 liters) processing blood volume is preferable. The total blood volume to be processed can be calculated using a formula based on the target cell yield and pre-collection peripheral blood count. It is advisable to use a relatively low machine collection efficiency (approximately 40%) in the calculation for autologous donors to allow for relative or absolute cytopenia due to disease or treatment. The actual machine collection efficiency can be calculated once the product yield is known. Concurrent plasma may also be collected and ACD-A anticoagulant added to large volume collections, if requested by the ATMP manufacturer. An absolute (or per kg recipient body weight) target yield may be advised by the manufacturer. If so, a post-collection mononuclear or target cell count will facilitate yield calculation and inform the need for a second collection. Generally, if the target

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cell threshold is exceeded the calculated total blood volume processed and the inlet flow rates are stable, the target yield will be exceeded and it may be possible for excess cells to be stored for future use. The platelet and red cell content of the collection can also be quantified on a sample of the starting material.

Tissues

Standardization of solid tissue procurement as a starting material for ATMP manufacture is usually more challenging than that of mononuclear cells by apheresis [10].

Procurement of tissues as starting material for autologous use may require surgical biopsy or excision and will be collected from patients who are usually, by definition, unwell. Such patients are likely to have a range of underlying medical conditions and may be treated in many different medical facilities that may be geographically distant from the processing center and may be carried out by many different clinicians, most of whom will be specialists in the treatment of the underlying condition of the patient rather than in procurement of starting material for ATMP manufacture. This means that each individual clinician carrying out ATMP starting material procurement may do so only on a handful of occasions. Standardizing such retrievals is therefore challenging, particularly where sending the starting material to the processing facility is time sensitive to ensure the viability of the cells in the donated material. Good communication and engagement with all areas who may be involved is key to ensure that the material retrieved is suitable, is handled in a manner that ensures the survival of the relevant cells and is not contaminated during retrieval and handling prior to reaching the manufacturing facility.

Procurement of starting material for allogeneic ATMPs is more straightforward to standardize as the activity can be concentrated in a small number of centers so that the staff involved develop the requisite experience. Procurement of allogeneic starting material is normally from otherwise healthy individuals who may be donating the starting material as part of a different procedure e.g., skin donation during reconstructive surgery with the donated skin then being used as a source of cells that can be expanded into ATMPs.

A close working relationship between the procurement team and the manufacturing facility is important to ensure that the tissue is handled as required both to minimize the risk of introduction of contamination during the retrieval process and to ensure the quality of the donation by maintaining the tissue under the required temperature conditions during transport to the processing facility. Clearly written standardized operating procedures for donation handling are essential.

LABELLING & CHAIN OF CUSTODY

It is essential that the starting material is labelled in such a way as to both comply with the legal and accreditation requirements for tissue and cell procurement and provide the information required by the manufacturer as part of its quality management system and licensure. In the case of autologous starting

material, patient-specific identifiers such as name, gender, date of birth and hospital number may also be required in order to ensure that the resultant medicinal product is administered to the same person when it returns to the clinical environment.

Whilst tissue/cell labelling has become more standardized in some jurisdictions e.g., through the application of the Single European Code in the EU, interpretation can vary across different countries and Tissue Establishments and in-house labelling may persist in parallel. Some jurisdictions do not prescribe labelling requirements. The situation is compounded where different manufacturers require different types of labels containing different information to be applied to the starting material. In addition, labels must be checked to ensure that adhesives do not compromise the integrity of the tissue container and are suitable for the conditions under which they will be stored.

Use of a standardized labelling system to ensure consistent terminology and coding of cellular products is required by collection facility accreditation bodies and underpins traceability, tracking and surveillance of cells and tissues across countries. Several standardized labelling systems are available, including the Eurocode and ISBT 128 labelling systems, the latter being overseen by ICCBBA [11]. The starting material must be packaged in containers that have been validated to ensure that its quality and safety are not compromised during distribution to the manufacturing site. The transfer of the starting material from the control of the collection facility to the control of the manufacturer must be fully documented and a copy of the relevant documents confirming chain of custody filed in the donor's records. Before transfer can occur, the criteria required to allow the material to be released from the control of the collection facility must be verified as having been met, accompanying documentation completed and checked, and testing and warning labels applied. Sign off that these steps have been completed satisfactorily must be provided by the collection facility and accepted by the receiving manufacturer to provide clarity around responsibility for the product as it passes along the chain of custody.

CONCLUSIONS

The challenges in procurement of starting material for ATMP manufacture are often underestimated. Considerations such as donor selection and screening criteria, procurement procedures, packaging, labelling and storage may vary between different cell and tissue procurement organizations, between procurement organizations and manufacturers, and between different manufacturers. The situation is exacerbated where the procurement and manufacturing organizations are in different countries and/or regulatory jurisdictions. Addressing these challenges requires close partnership between the procuring organization, the manufacturer and the hospital providing the clinical treatment. Development of a set of common international standards would reduce the complexity and costs of manufacture and delivery of ATMPs and facilitate the development of a global industry that would benefit patients around the world.

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CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: MATERIALS COLLECTION & LOGISTICS AT THE CLINICAL POINT OF CARE

EXPERT INSIGHT

Cell and tissue starting materials for ATMPs

Neil W McGowan, John DM Campbell & Joanne C Mountford

The recent, rapid expansion in the use of cellular therapeutics has vastly increased the need to collect and process suitable starting materials from cell or tissue samples. These materials vary from relatively simple peripheral blood cell populations to the more complex tissue materials or established cell lines or isolates. In an evolving regulatory environment, adhering to good practice for acquisition, transport and processing is increasingly complex. Also, to understand the outcome of variations introduced during the manufacturing process and to permit more efficient process development, extensive analysis and retention of samples of the starting material is essential. However, these critical early steps are often overlooked and not optimized or standardized. In this article, we address some of the main challenges for the supply of high quality, consistent starting materials including: transport and hold time, cryopreservation, initial processing and the analysis of tissue and cellular materials. Variation in current practise highlights opportunities to standardize handling and testing of some common starting materials in order to increase consistency and quality control during the manufacture of these technically challenging advanced therapy medicinal products.

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INTRODUCTION

With the rapid increase in Advanced Therapy Medicinal Product (ATMP) manufacturing seen over the last 3 years, the pressure and demands on supplies of starting materials have also risen dramatically. Alongside the proliferation in therapies, regulators are increasingly looking for additional, complex characterization of products at all stages including the starting material(s). Starting materials generally fall into three categories: 1) Cell preparations such as apheresis collection of peripheral blood mononuclear cells; 2) Tissue samples commonly including skin and adipose tissue or umbilical cord; 3) Established cell lines such as pluripotent stem cells (PSC), or expanded primary cell isolates including mesenchymal stromal cells (MSC), that have been previously manufactured. The challenges presented by these sources are divergent, particularly with regard to the risk of contamination during collection, which is a major concern for tissues but less so for cells collected in a closed process or those that have been previously manufactured and undergone Quality Control (QC) and sterility testing before banking.

The evolving regulatory environment, and the development of more complex identity and functional testing, highlights the need for extensive analysis and the retention of samples at all stages of the process from starting materials and throughout final manufacturing. This is of particular importance for those products that undergo extensive population doublings, and/or lengthy culture periods, as these are at particular risk of genetic variation introduced, or selected for, by the process. Hence, retention of starting material is essential for comparative analysis to ensure stability during manufacturing, for consistency, comparability, look back and to account for any additional testing requirements.

This article is partnered with that of Zahra *et al.* in the same issue, covering the requirements for donor selection and sample collection, so it is assumed that these steps have been undertaken prior to the steps discussed herein. Also, as the particular role of regulators has been covered extensively elsewhere [1] and vary between international agencies we have concentrated on the practical, and sometimes overlooked, challenges of the procurement and processing of starting materials for ATMP manufacturing.

Figure 1 illustrates common routes for the acquisition and processing of stating materials, with the particular stages discussed below, also whilst it is highly recommended that samples be retained at all stages, those points where analysis and sampling is essential are highlighted.

PACKAGING, TRANSPORT & HOLD TIMES

Within the EU the procurement of tissues and cells is regulated under the terms of the Tissues and Cells Directive (2004/23/EC) [2]. However, immediately following this event the regulatory position transitions to the appropriate ATMP regulatory and guidance documents (1394/2007 and Eudralex Vol 4, Part IV respectively [3,4,]), at which point tissues or cells become part of the supply chain for the manufacture of medicinal products. Therefore, controls required for the subsequent transport and any hold

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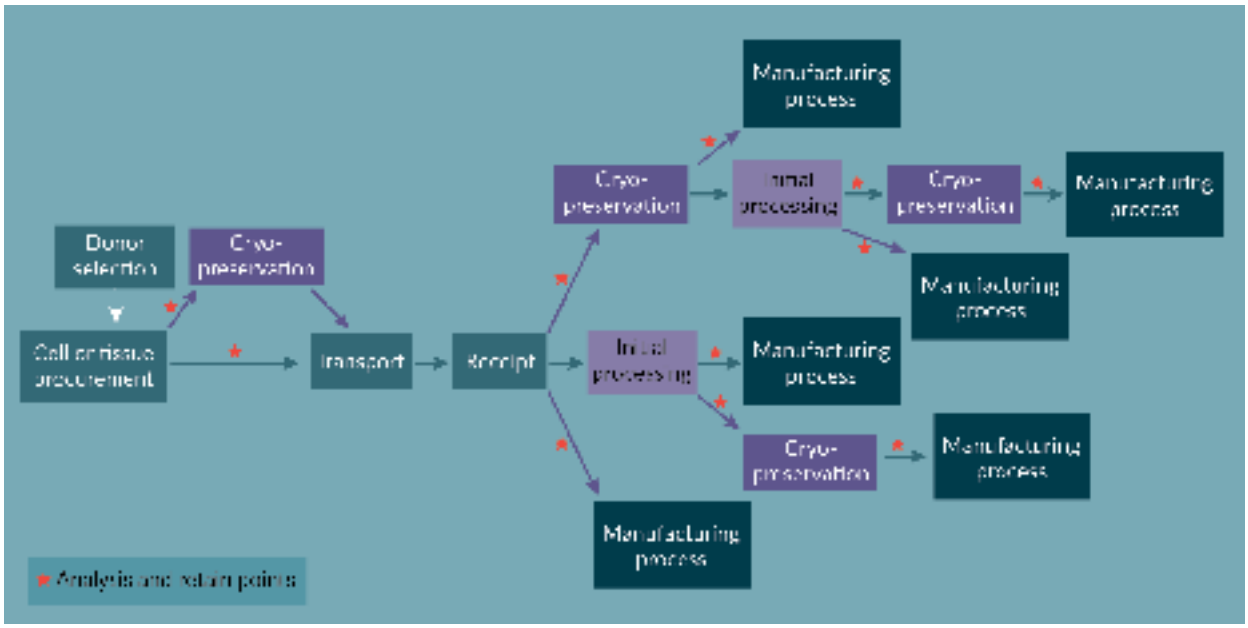
FIGURE 1

Common pathways for starting materials entering an ATMP manufacturing facility.



The route may vary depending on source of material, for example apheresis collection of PBMC that will be used in a closed manufacturing process would follow a direct route – shipped at controlled temperature, received and directly entered into the manufacturing process. Tissue based starting materials are more likely to require initial processing before cryopreservation or manufacturing, following a more complex route. Cell lines or banked primary isolates e.g.,: PSC or MSC, will be received as cryopreserved stocks and usually require initial processing or conditioning, and potentially additional cryopreservation as intermediate banks, before final manufacturing and/or clinical application.

times are of paramount importance to ensure the safety, integrity, efficacy and ultimately the compliance of manufacturing procedures employed in the generation of downstream products.

There are many aspects that require careful consideration prior to transport and for the purpose of this article it is assumed that donor informed consent, testing and procurement have all occurred in accordance with the appropriate regulations for the country of origin, bearing in mind the requirements for autologous vs allogeneic donations remain quite different, commensurate to risk.

Immediately following procurement, the tissues or cells require careful packaging. Procedures will obviously be quite different depending on the nature of the procurement procedure and indeed the tissue or cell. For tissues requiring surgical excision the immediate concern should be to minimize the risk of contamination by packaging within a sterile container (usually within a secondary or tertiary container) with an appropriate transport solution, ideally a licensed medicinal infusion solution (e.g., crystalloids such as saline or Plasmalyte) or organ preservation solutions of the appropriate grade (e.g., UW solution). Tamper-evident seals should also be applied to the containers before packaging to ensure product integrity upon receipt. In the case of mononuclear cell apheresis donations, it is obviously easier to protect the product by sealing the lines attached to the cannula. However,

care must be taken to ensure that seals are performed correctly and repeated to provide a further level of security. At this stage it is common for samples to be taken for both cell enumeration/identity and sterility. Whilst this can often be performed closed by utilizing the existing bulbs on the apheresis bags it is essential to ensure that products are mixed thoroughly prior to sampling to obtain a representative sample.

Once the starting material has been contained the next requirement is labelling to maintain the audit trail, which applies to both the material itself and any samples derived from it. Within the EU the coding directive applies to the procurement of starting materials for ATMPs. In reality it is often difficult to apply Single European Code (SEC)-compliant labels to starting material containers directly and therefore this can be also be achieved via accompanying documentation which identifies the Donation Identification Sequence (DIS): composed of the ISO Country code, the EU Tissue Establishment Code and the Unique Donation Number (usually ISBT-128 barcode, which should also be applied to all product containers). Accompanying documentation should be controlled and contain the required information to enable the onward distribution of products in an GMP-compliant manner i.e. containing any required information on the donation, timings, staff involved and where appropriate donor/patient identifiers (particularly in the case of autologous products or directed donations). It may also be appropriate to include other Critical Quality Attributes (CQA) on the documentation at this stage that could be relevant to downstream manufacture (e.g., sample weight and/or volume, clinical observations etc.). This documentation can also be utilized as a checklist to ensure that the correct steps are followed and verified by two members of staff to ensure the highest standards of GMP are in place from the outset.

It may be necessary to transport the starting material to the manufacturing facility directly, or alternatively retain locally in a secure location depending on timings. In our experience, the former is the usual scenario given that hospitals often lack the secure, controlled and monitored areas required as part of the ATMP starting material supply chain. In this regard, thought inevitably turns to transport and in particular storage temperatures. For tissues surgically excised it is usual to immediately cool in order to maintain tissue viability and minimize the potential for bacterial growth in the event of contamination. This can be achieved via the use of transport containers validated to maintain a temperature range between 2-8oC, either through the use of pre-cooled packs within insulated containers or alternatively more complex and expensive devices with integrated active cooling and heating functions. The latter is often more appropriate when longer distances are required although consideration needs to be given to battery life and in some cases continuous electrical supply during transport, which necessitates the use of specialist, experienced couriers.

Depending on the nature of the starting material, the transport solution, the manufacturing process and ultimately the final ATMP that will be manufactured it may be more appropriate to transfer at controlled ambient (usually 18-24oC) although this is actually more difficult to achieve in practice and requires

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the more expensive transport devices. This is particularly relevant for some mononuclear starting materials collected by apheresis and consideration must be given to interim storage under these conditions upon receipt at the manufacturing facility. It is often easier to store a product at 2-8oC in a secure, monitored fridge than it is to find a controlled ambient incubator or monitored room to serve this purpose. Alternatively, it may be more appropriate to consider immediate cryopreservation of the starting material (as described below) when longer hold times and/or distances have to be negotiated prior to manufacture, transport would then be via validated dry shipper with security measures and labelling procedures applied as above.

Hold times prior to the initiation of manufacture are often necessary to avoid onerous out of hours service provision given that most, if not all surgical and/or apheresis procedures will be conducted during working hours within a hospital environment. Therefore, unless immediate manufacture is essential an overnight hold should be incorporated into the process to prevent these issues. Ideally this should be in continuity with the storage temperature utilized for the initial transport. As with other aspects of the supply chain for starting materials, the details of overnight hold are not standardized, the length of time and temperature for the same material may vary between centers. This variation in handling procedures at the earliest stages of a process can induce differences in the material that will persist throughout the entire manufacturing process, leading to variation even within processes that are well defined in the later stages. Therefore, conditions for overnight hold should be validated for specific tissue or cells and ideally standardized between centers.

CRYOPRESERVATION OF STARTING MATERIALS

For many ATMP starting materials, particularly mononuclear cell donations from apheresis collections an interim step in the supply chain can include cryopreservation. This has several obvious benefits; allowing an extended hold time prior to manufacture, which in turn facilitates scheduling whilst de-risking the process as it allows time for additional testing prior to subsequent manufacturing steps. In addition, this simplifies logistics given the ability to transport material worldwide in secure vessels at cryogenic temperatures. The only aspect that needs careful consideration before implementation of such a step is the potential impact of cell loss observed upon thawing. This has become the method of choice in the CAR-T cell field particularly in the autologous setting whereby patients attend local apheresis units for collection before manufacture at a centralized facility as part of a hub and spoke type model. Importantly, in the EU the cryopreservation of ATMP starting materials is not considered 'substantial manipulation' [3] and therefore it is important that these procedures are performed in compliance with the EU Tissues and Cells Directive [2] and the Competent Authority for the country of origin informed as appropriate.

Liquid nitrogen-based controlled rate cryopreservation remains the method of choice, although the particular cryoprotectant mix utilized and the 'profiles' employed

for different starting materials remain variable. In our experience the use of penetrating (e.g. DMSO), non-penetrating cryoprotectants (e.g., HAS) and support solutions (e.g., Plasmalyte) or other commercially available products (containing all three components), together with subtle differences (rate of temperature drop, final temperature and modifications related to eutectic points/latent heat release) in the specific profiles vary between collection centers and indeed between product to product. The choice of a particular method often lacks a robust evidence base to support its use and tends to represent 'custom and practice' in most instances. Similar issues are encountered when using established cell lines e.g., pluripotent stem cells (PSC) or primary isolates such as mesenchymal stromal cells (MSC), as starting materials. Both of these materials will have been previously manufactured and stored as master or working cell banks (MCB, WCB), but again variation in custom and practice results in a wide range of cryopreservation agents, protocols, storage format and temperature options as seen with PBMC preparations.

It is beyond the scope of this article to compare and contrast different cryopreservation methodologies for different cell and tissue types but this is an area that would benefit hugely from internationally agreed criteria for common starting materials. This need is exemplified by the increasing number of centers that are now involved in the cryopreservation of mononuclear cells as part of the CAR-T supply chain. Consideration should also be given to the use of non-liquid nitrogen based controlled rate cryopreservation devices that can reduce the time period between the addition of cryoprotectant and initiating of cooling. Moreover, the use of ultra-low freezers (-80oC to -150oC) for long-term storage has obvious benefits in terms of cost, infrastructure and reduced potential for cross-contamination when compared to conventional liquid nitrogen.

INITIAL GMP PROCESSING & BIOBURDEN REDUCTION

Initial processing of starting materials for ATMPs, whether autologous or allogeneic, usually takes two forms, in the case of mononuclear cell apheresis donations this will usually involve thawing if cryopreserved, cell selection/depletion (where appropriate), followed by immediate culture in the presence of GMP-grade media supplemented with growth factors and/or cytokines. Where the process involves tissues, or in some cases organs the first step usually involves a combination of surgical techniques and frequently a decontamination/bioburden reduction procedure (incubation/rinse in a decontamination or antibiotic solution). This is particularly relevant when you consider that a typical operating theatre environment is usually equivalent to EU Grade C/D standards, whereas open aseptic manufacture is employed within EU Grade A/B environments. It is therefore prudent to assume the starting material is contaminated and take steps accordingly to mitigate the impact of a contaminated starting material within an aseptic manufacturing facility.

Initial processing of tissues or organs for ATMPs should be conducted within controlled areas ideally

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dedicated for that purpose. Certainly, if cells derived from tissues or organs are subsequently required to be manipulated as part of open manufacturing procedures (EU Grade A/B conditions) the initial process should be conducted in an alternative room (in our experience usually EU Grade A/C) and incorporate a decontamination step or at the very least a bioburden reduction measure as a prerequisite. One other measure to mitigate the risk of contamination is to include antibiotics in culture media although this should be restricted to the early stages of culture. This should also be avoided in EU Grade A/B rooms given concerns around the validity of subsequent sterility testing and environmental monitoring due to the potential for a bacteriostatic, rather than bactericidal effect. All of these risks should be comprehensively described and evaluated within a Contamination Control Strategy. This document should be prepared to document the requirements for segregation of products and processes, which in turn will help define the requirements for rooms, grade and AHUs, etc. This has become a regulatory expectation (Eudralex Vol IV, Annex 1) [4] and is essential for a multi-product facility.

For prototcols using established cell lines or primary isolates as a starting material, the route to manufacturing can be more straightforward, with the thaw step post-transport/storage forming the first step of the final manufacturing process.

ANALYSIS OF STARTING MATERIALS

Analysis of the starting materials used for production of ATMPs is often most critical in the development phase, informing the routine data that will be collected once manufacturing is established and in process control (IPC) parameters. Critical analysis of the key properties of the starting material is vital to understand the outcome of variations introduced to the developing manufacturing process. This development and analysis process can be challenging, particularly in the autologous therapy field, where a limited amount of unique material is available for validation and manufacturing. Scarcity may also be an issue with allogeneic therapies where small amounts of stromal or stem cells require to be expanded and kept in the desired state through many rounds of cell culture. Broadly there are three starting material scenarios requiring analysis – Blood, or other cellular sample, manufactured into a cellular product; Tissue manufactured into a cellular product; Tissue manufactured into a 'solid' tissue composite graft.

Cellular starting materials

Initial analysis of the fitness of any starting material will involve cell counts, viability and phenotyping. A variety of specialist counting devices are used for cell counts and viability (usually based on exclusion of specific dyes), and can produce differing results, and should therefore be standardized between centers. The use of flow cytometry is becoming more common for viability assessment but, as with automated cell counters and manual methods, generally depends on dye exclusion to discriminate live/dead cells. It is essential that an appropriate dye is chosen for the condition of the sample, for example the use of trypan blue on cells

directly after recovery from cryopreservation results in a very large under-estimation of viability as the membranes of viable cells are temporarily permeable due to DMSO exposure and may therefore permit ingress of dye despite being fully viable. Care must also be chosen to use preparation techniques suitable to the cellular material – e.g., single cell counting for PBMCs, but counting of healthy small colonies of pluripotent cells used as a starting material. As discussed below for thawed samples, the analysis method should accommodate the format of the starting material as far as possible, and not require extensive processing to suit the analytical method, as this will reduce the accuracy, particularly of cell counts.

Flow cytometry remains the mainstay for analysis of blood or apheresis starting materials for ATMP [5]. This may be a relatively simple screening of the donor or the donation e.g. CAR-T cell manufacture requires a minimum number of $CD3^+$ cells to be successful, but the decision to collect the starting material may be based on the CD3 count of the patient rather than the CD3 count in the collected starting material [6]. However, the timing and degree of testing is not standardized across the CAR industry. In collection of leukapheresis for e.g., collection of $CD34^+$ stem cells, the usual approach is to assess the $CD34^+$ cell count in the blood of the mobilized patient or donor as a reliable marker for the $CD34^+$ content of the collected graft.

Caution should be exercised in testing donor samples when the assay is for a surrogate of cellular function. As an example, donors are pre-screened for anti-EBV antibodies to indicate that a collection should contain EBV-specific T cells. These collections are used to manufacture EBV-specific T cells to treat EBV-driven lymphoma [7]. In our laboratory, manufacturing success has now been increased by screening donor samples in a functional assay of interferon production in response to EBV-derived peptides [8]. This ensures that the EBV antibody-positive peripheral blood collection contains sufficient EBV-specific memory T cells to then be expanded and manufactured into a product. When apheresis is used for selection of a specific cell type at the initiation of manufacture, e.g., selection of $CD14^+$ cells, flow cytometry analysis is essential to ensure that the starting material is suitable. A collection containing too many cells presents as great a challenge as too few. A new first in human trial of macrophages for therapy of cirrhosis found greatly elevated levels of $CD14^+$ monocytes in patient collections compared to healthy donors which would have overwhelmed the CliniMACS prodigy CD14 selection system without adjustment of the cellular concentration of the starting material [9,10]. Clear such variations would change the approach to the development of the process and for example, necessitate the use of patient starting material at an early stage of the development rather than using, more easily available, healthy donor material.

Tissue starting materials

When tissues are used as starting materials 'fitness' to produce a cellular product will commonly be determined by physical properties assessed during the translation of the process (testing, procurement and informed consent are addressed by

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Zahra *et al.* in this journal). Testing the suitability of starting material for use in a manufacturing process will commonly focus on the age of the donor (relevant for autologous and allogeneic products); the environment where the tissue is procured; storage solution and containers; shelf life of explanted tissue; decontamination required and microbial control strategy; specialist dissection and initial culture of the dissected tissue. All of this work will determine the key quality attributes of the starting material to manufacture a cellular product. It is often not possible to prospectively isolate and characterize the desired cell type from a tissue sample before the start of manufacturing e.g., MSC from umbilical cord Wharton's jelly, therefore if these general criteria are critically evaluated, manufacturing success should be as high as possible. Quality control will often be improved by interim analysis of the cells growing from the explant, usually for cell count, viability and phenotype. These results at defined time points may be used to devise stop/go points in the initial cell culture which can be used to avoid the expense of full manufacturing costs in a process likely to fail.

When a tissue is used to produce a tissue/cells composite product, there may not be an opportunity to analyze the growing or final cellular product. An example of this would be corneal limbal epithelial stem cells grown and transplanted on amniotic membrane used to treat severe ocular disease [11]. These grafts are transplanted onto the surface of the eye, and present a unique challenge in understanding the starting material, which is a small explant taken from a donated cornea. In these cases, non-destructive sampling of the product is challenging, although small remnant samples of the product may be available after surgery. Best practice to control for starting material fitness and subsequent manufacturing success is make duplicate GMP cultures from the same material, one for transplantation, and one to be destructively tested, usually by fixation and immunohistology [11], although it may also be possible to harvest cells from the destroyed graft for flow cytometric analysis. While time-consuming, and less accurate than liquid sample analysis, this analysis of fixed start and end material does have the advantage of generating retention samples which may be re-visited for further analysis at a later date.

Tissue processing cell isolation can be lengthy, often requiring prolonged culture for the emergence of the desired cell type from an explants and extensive expansion over many population doublings to achieve sufficient cell numbers for banking and/or further manufacturing. This prolonged culture period, and the stresses associated with *ex vivo* culture, added to the potential biological bottlenecks introduced by freezing and/or cell selection, present significant potential for genetic damage and therefore genetic stability and the retention of a normal karyotype is of particular concern. Analysis of cell lines and primary isolates is generally less practically challenging as such starting materials have previously been processed, banked and undergo extensive QC as part of the banking process. However, as such starting materials will be more extensively manufactured, often undergoing two or more expansion, cryopreservation and banking cycles before manufacturing into a final product, it is

even more essential that identity and genetic stability/integrity of the material is assessed and ensured. As all individuals carry a unique combination of polymorphisms, copy number variations and mutations in their genome, and testing of a final product without reference to the specific starting material may lead to the assumption that an uncommon DNA sequence or mutation may have arisen during culture and pose a risk of tumor formation. For example, it is not uncommon for individuals to carry cancer associated mutations with no apparent detrimental effect, however if such a mutation were to be detected in a final product the product may be rejected. If it can be demonstrated by comparison to the starting material that this sequence or mutation is normal for that individual, then concerns may be allayed, particularly in the case of autologous products. The use of genetic testing methods such as genome sequencing, single nucleotide polymorphism and oncogene detection arrays are not currently required for ATMP as there is little evidence upon which to risk assess the large volumes of data generated by these techniques. However many organizations, including our own, are choosing to retain suitable samples or to perform these analyses For Information Only as a method to accrue data that might form the basis for future assessment of the effect of manufacturing processes on the genetic stability and integrity different starting materials.

Analysis of retained reference starting material

All aspects discussed above concentrate on ensuring that a starting material is suitable for manufacturing, and will produce a viable product. A different aspect of analysis of the starting material is to provide a reference material for the success and safety of the manufacturing process. This can prove to be technically challenging, and care must be taken in ensuring that the analysis does not introduce undesired artefacts.

In a relatively simple ATMP, such as the macrophages used to treat cirrhosis in the MATCH trial [9,10], the starting material of CD14-selected monocytes is used to define the success of the macrophage manufacturing process. $CD14^+$ cells do not express the macrophage markers 25F9 and CD206, and the success of the manufacturing is demonstrated by the *de novo* expression of the markers by the final macrophage product, measured by the delta in fluorescence intensity compared to the starting material. Thus the phenotype of the starting material is essential to determining the success of manufacturing, but also controls for donor to donor variability in the initial expression of the macrophage markers. In this case the starting material is not physically retained, rather the analysis data is used. This is particularly applicable when cells are used with no cryopreservation step in the manufacturing – freezing starting material cells for later analysis would not be representative, and freeze/thaw would risk materially altering the properties of the cells when analyzed.

The majority of cellular product manufacturing strategies will involve cryopreservation steps, often at multiple time points. The starting material may also be frozen to allow manufacturing from the same initial collection at multiple time points. This provides an opportunity to

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retain meaningful QC samples of the starting material from each stage of manufacture. Alternatively, QC samples may be paraformaldehyde fixed, as discussed for the limbal stem cell products. Analysis of these (usually cryo-) preserved samples e.g., by PCR for gene expression is relatively straightforward for ensuring expression of desired genes for cellular identity, and that undesired genes are not expressed which could be a safety concern. The use of thawed samples for determination of viability, cell count and phenotype can present challenges. Cell losses and reduced viability are commonly found in thawed samples. Great rigor must be applied in devising thawing strategies to minimize alterations in the sample due to processing.

CONCLUSION

In summary, as with most aspects of cellular therapy, the requirements for procurement and processing of starting materials are rapidly evolving. However if must be emphasized that there is no 'one-size-fits-all' solution at any point in the procedures and that product specific development and validation of steps such as transport, cryopreservation and bio-burden reduction are essential to optimize the potential for successful manufacturing. However, there are opportunities to standardize handling and testing of some common starting materials, for example PBMCs obtained by leukapheresis. The international industry could benefit from standardization of these essential steps as it may help reduce variation and increase robustness of manufacturing. Also, with the introduction of more complex and detailed testing technologies, whilst it is still essential to define and fix suitable CQA for product or material release, the importance of analyzing and retaining samples throughout, either For Information Only or as in process controls, cannot be overstated.

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CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: MATERIALS COLLECTION & LOGISTICS AT THE CLINICAL POINT OF CARE

CHANNEL CONTENT

INTERVIEW

Evolving the supply chain to counter key challenges in apheresis and leukapheresis collection

PETER OLAGUNJU is the Vice President of Patient Operations with bluebird bio. In this role, Peter has responsibility for managing the CMC related Centers of Excellence (COE) interfaces including the collection of patient cells, manufacture of drug product, and operational execution of patient treatments. Prior to bluebird bio, Peter most recently served as Senior Director, Global Technical Operations at Valeant (through the acquisition of Dendreon). He was at Dendreon for roughly 5 years where he managed the US and EU manufacturing operations and supply chain, including contract manufacturers, external testing sites, and the apheresis network for those regions. Prior to Dendreon, Peter had leadership positions within the quality function at a monoclonal antibody organization (ZymoGenetics) that was acquired by BMS in 2011. Peter did his undergraduate studies at the University of Illinois and completed an MBA program at the Foster school of Business (University of Washington).

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Q What are you working on right now?

PO: Here at bluebird bio we have two therapeutic franchises, one focused on oncology and a second that is focused on severe genetic disorders – mostly blood disorders – that are correlated to

a defect in a single gene. These two franchises are how we organize our business. Between them, we have four core products either nearing or at the commercialization stage.

One product was recently approved in Europe by the EMA – ZYNTEGLO, which is a therapy for transfusion-dependent β-thalassemia. We're obviously super excited about that – our first commercial product.

> "...we have four core products either nearing or at the commercialization stage."

We then have three additional products nearing commercialization – one in oncology, one for sickle cell anemia, and a third (Lenti-D) for a severe genetic disorder called cerebral adrenoleukodystrophy (CALD).

Q Can you frame for us the key challenges relating to apheresis/leukapheresis facing cellular immunotherapy companies today?

PO: Firstly, a key point of clarification that relates to the two franchises I've described: on the oncology side, the starting material is a leukapheresis, which is non-mobilized. However, on the severe genetic disorders side, it's a mobilized apheresis. The significance there is that for a mobilized apheresis, there is a requirement for pre-collection activities. You are mobilizing targeted cells that are different to the target cells in oncology. These (CD34-positive) cells come from the bone marrow and you mobilize them 5 days ahead of collection using two small molecules – this is to ensure that the targeted cells to be collected ($CD34^+$) are in the patient's bloodstream and available for collection at the appropriate time.

There is more coordination needed and there are additional steps required for a mobilized CD34-positive cell collection versus a leukapheresis or apheresis where the target collected cells are T cells for oncology therapeutics.

One of the key challenges on the severe genetic blood disorders side is collecting blood in the mobilized setting. Collecting targeted cells in the blood of patients whose blood is already compromised introduces additional technical challenges just from the collection perspective.

The other key challenge that's seen across both oncology and severe genetic disorder areas is patient-to-patient variability. Until you have a large enough data set to enable multi-variable analysis and allow the correlation of certain trends you may see, it's difficult to build manufacturing and downstream process tolerances to account for all of the patient-to-patient variability we can see in the starting material. That becomes a really key hurdle with apheresis.

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Q How has bluebird bio's front-end supply chain evolved to meet these challenges?

PO: We've taken a strategic approach where we've created a group that's acutely focused on collecting cells that are suitable for the manufacturing process – our Apheresis Operations Group.

This is a team of nurses who have worked in apheresis collection centers previously, and who are now tasked with helping train their counterparts – the nurses actually doing the collections in our partnered hospitals. They are engaged in training them on our collection-specific parameters based on disease indication.

We feel this is a real point of strategic differentiation – to have nurses on staff who have the rapport, the relationship and the understanding to build trusted links relating to supporting those nurses who are actually doing the collection. That is a key way in which we have evolved our thinking and taken a different strategic approach.

Another is in how we organize from a CMC perspective. With autologous products, where the hospital becomes part of your supply chain, you are required to think and organize yourself in a different way. At bluebird bio, this has manifested in the creation of a group called Patient Operations, which is the team that I lead. It's a fully integrated team focused on optimizing the experience for the patient with that Apheresis Operations Team, beginning with collection and moving through to drug product manufacturing.

Our collections are fresh, meaning we have a 48-hour shelf life, and so the Drug Product Manufacturing Team is effectively a customer of Apheresis Operations - there's a tight relationship or connection there.

The third group in Patient Operations is what we call Therapy Services. This is a team working with our contract manufacturers and with the hospitals to align manufacturing capacity or slots with availability from an apheresis scheduling perspective. All the underlying business processes related to being able to confirm those schedules in real-time is driven by Therapy Services.

We've also implemented IT solutions to enable us to meet the challenge of evolving efficient business processes that scale over time, and that possess the logic to assign slots to patients who enroll for our therapy.

These are some of the ways in which we have evolved our thinking and developed a fit-for-purpose model for our CMC interactions with hospitals and institutions.

Q There are increasing calls for cell & gene therapy companies to collaborate and standardize in order

to alleviate growing pressure on apheresis centers – where in particular do you see the requirement/ opportunities for such initiatives?

PO: This is really a critically important point. We do get a lot of feedback from treatment centers about this issue. There are different numbers bandied about in terms of cell and gene therapies in clinical trials today – some say more than 600 in clinical development, others say more than a thousand. But whatever the truth is, it's a very big number! Even if a relatively small percentage of these product candidates become commercial, that's potentially a big burden for these treatment centers, especially if each product has its own bespoke processes and requirements.

Three key areas have risen to the forefront in terms of identifying where we can harmonies certain 'non-value' differences in the process.

One is around the audit approach. Is there an opportunity to have a unified auditing system whereby we're able to share audits? This could involve a third-party auditor – it could involve leveraging FACT (Foundation for the Accreditation of Cellular Therapy) or JACIE (Joint Accreditation Committee ISCT-Europe & EBMT) audits to meet that requirement, for instance.

The second area is around labelling. It could be highly beneficial to come to a common, aligned convention around what the data fields should be and what is the general approach – is it on demand labelling, or is it sending labels to the site? As with the audit approach, this could potentially reduce the burden at the point of care.

The third one is around portals or IT systems to access and control the enrolment, ordering and scheduling of a product. If each company has a different portal, you can envisage a future where that becomes cost prohibitive and disruptive to the point of care – they would be investing time in non-value-added activities instead of spending time in clinical care and managing patients.

Those are three important areas that have been identified where there are opportunities for standardization.

Q Can you distil for us the key components of an optimal commercial apheresis supply chain as you see them?

PO: One of the key components is having a process where you have the option of cryopreserved apheresis - building that into your process development, generating data on it, and having the option to be able to freeze cells at the point of collection. It allows

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for greater latitude, it decouples the collection from the manufacturing, and it allows for more flexibility in the whole treatment process.

> "One of the key challenges on the severe genetic blood disorders side is collecting blood in the mobilized setting."

The second key element is ensuring you have enough volume going through the apheresis center. It's important to have a right-sized network where there's enough repetition, enough volume going through the center to avoid memory loss of how to do the procedure in-between patients. That's a key consideration in your network design.

The third is from an oversight perspective: really understanding what the needs of those key stakeholders at the center are – the apheresis nurse, the staff in the cell therapy lab. Understanding both their needs so you can execute your process correctly, but also how to engage with them so that there's no confusion. This boils down to having an approach that provides them with adequate support, whilst not asking them to carry out redundant activities that don't create value.

Those are the considerations for ensuring you have an optimal apheresis supply chain, from my perspective.

Q Finally, what will be your/your team's key priorities and goals over the coming 12–24 months?

PO: Our key focus is related to the core programs I described at the start – we call them the 'core four': ZYNTEGLO for transfusion-dependent β-thalassemia, which is launching in Europe –and we are in the midst of pursuing US filing activities; IDE-CEL, which is the treatment for multiple myeloma, partnered with Celgene/BMS; LentiGlobin for sickle cell disease; and Lenti-D for the treatment of CALD.

We're primarily concentrating on making sure we can execute in supporting those four core products, and then also on optimizing the process – in other words, on being able to get to a place where we can reduce COGs and increase capacity on both clinical and commercial sides within our supply chain. Those are some of our key initiatives as we move forward.

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CELL & GENE THERAPY INSIGHTS

COMMERCIAL INSIGHT: NOV 2019

Commercial insight: cell and gene therapy

Providing a critical overview of the sector's commercial developments – M&As, licensing agreements & collaborations, financial results, IPOs and clinical/regulatory updates, with commentary from our Expert Contributors.

CELL THERAPY

Mark Curtis. Financial Portfolio Manager, Emerging Technologies, Lonza AG, Switzerland

eGenesis, a Boston Biotech founded by George Church, closed its second round of financing in November, a Series B of $100M, to advance its xenotransplantation program into the clinic. Innovation in the Biotech world is happening incredibly fast, but it is rare to find a company with a vision as bold as eGenesis, which is to take a pig, engineer its genome to be porcine virus free, and then use its organs for human transplant. North of the border, in Canada, Notch Therapeutics came out of stealth mode with an equally impressive technology – a novel platform for allogeneic T cell manufacturing. Notch struck a deal with Allogene out of the gate, which will give Allogene access to clonal stem cell lines to build its future pipeline. Until now, Allogene has focused on donor derived T cells. Notch has plans to develop its own therapies as well, using its Engineered Thymic Niche platform, which provides an *in vitro* environment that mimics the human thymus to support T cell development.

GENE THERAPY

Richard Philipson. Chief Medical Officer, Trizell Ltd, UK

This month illustrates well the ups and downs of drug development, with Alnylam building its portfolio of approved RNAi therapeutics following the approval of Givlaari™ (givosiran) for the treatment of acute hepatic porphyria, whereas Solid Biosciences continues to experience problems with its

microdystrophin-based gene therapy for Duchenne muscular dystrophy, which could prove terminal for the product. Elsewhere, fascinating data are emerging from Harvard researchers and Rejuvenate Bio, which provide early evidence that a combination gene therapy, using three genes linked to health and lifestyle benefits (FGF21, sTGFβR2, and αKlotho), seems to reverse multiple age-related diseases in mice. Of course it's a very long journey from a mouse to a human, but nevertheless an important first step in proving the concept of gene therapy for diseases of aging.

CLINICAL/REGULATORY

KIADIS PHARMA SHIFTS FOCUS TO NK CELL THERAPY

Amsterdam-based biopharmaceutical company Kiadis Pharma has announced its plan to discontinue development of ATIR101 and to focus all resources and investments on the company's NK-cell therapy platform and product candidates. ATIR101 was the company's lead product candidate and latest decision will also see the company stopping its ongoing Phase 3 trial.

Kiadis is focused on developing cell-based immunotherapy products for treating blood cancers and inherited blood disorders. ATIR101 was developed as an adjunctive immunotherapeutic on top of allogeneic hematopoietic stem cell transplantation (HSCT). This is intended to provide the patient with a functional and mature immune system that can fight infections while not eliciting severe graft-versus-host disease (GvHD), thereby bridging the time until the immune system has fully re-grown from stem cells in the transplanted graft.

The trial was designed to show superiority of ATIR101 over the existing post-transplant cyclophosphamide (PTCy) protocol. However, the Phase 3 trial designed to be conducted in 250 patients was not enough to demonstrate superiority over PTCy and the team believed discontinuing the study would be the best strategy.

The company will focus instead on its NK-cell program which consists of off-the-shelf and haplo donor cell therapy products for the treatment of liquid and solid tumors. Kiadis' proprietary off-the-shelf NK-cell platform is based on NK-cells from unique universal donors, expanded and activated *ex vivo* using our PM21 particle technology. The platform has the potential to make NK-cell therapy products rapidly and economically available for a broad patient population across a potentially wide range of indications.

The company's pipeline includes; 1) A Phase 1/2 study to evaluate K-NK002 as an adjunctive treatment to the current standard-of-care haploidentical HSCT with PTCy, 2) A phase 1/2A study to

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evaluate K-NK003 as a treatment for patients with relapse and refractory acute myeloid leukemia and 3) Multiple pre-clinical programs to evaluate NK-cell therapies for the treatment of solid tumors.

In addition, Kiadis is implementing a restructuring program to refocus the organization on its NK-cell therapy platform, which will result in a reduction of approximately half of its workforce, a reduction in external clinical trial costs associated with the Phase 3 study, and a reduced company cash burn. The company ended the third quarter of 2019 with approximately €47 million of cash.

CRISPR GENE EDITING SHOWS PROMISE IN TREATING BLOOD DISORDERS

Interim data from two ongoing Phase 1/2 studies of CRISPR Therapeutics - Vertex research collaboration has yielded encouraging results for CTX001, an investigational *ex vivo* CRISPR gene-edited therapy developed for patients suffering from β-thalassemia and sickle cell disease.

CTX001 is an autologous stem cell therapy which leverages CRISPR editing to engineer a patient's hematopoietic stem cells to produce high levels of oxygen carrying fetal hemoglobin (HbF). The therapy is being tested and is expected to treat sickle cell and β-thalassemia patients who are currently dependent on regular blood transfusions.

CTX001 is currently being tested in two Phase 1/2 studies, for treating patients with 1) transfusion-dependent β-thalassemia (TDT) and 2) sickle cell disease (SCD). Preliminary data obtained from one patient each from these trials has shown that the treatment is safe and effective.

In the 2 years ahead of starting the trial, the TDT patient needed an average of 16.5 blood transfusions per year and the SCD patient experienced seven vaso-occlusive crises per year – a common complication of SCD where blood cells stick together and block blood vessels, damaging tissues and organs.

The TDT patient received CTX001 in the first quarter of 2019 and 9 months after receiving the treatment, the patient no longer needed transfusions and had near-normal hemoglobin levels. The SCD patient received CTX001 in mid-2019 and 4 months after administering the therapy, the patient was free of vaso-occlusive crises and had similar hemoglobin levels. Both patients also had high levels of red blood cells expressing fetal hemoglobin – more than 99% for the former and more than 94% for the latter – a sign that the CRISPR-edited treatment did what it was designed to do.

The treatment was safe and although serious adverse events occurred in both patients, none of them were related to CTX001.

Both trials (CLIMB-Thal-111 in TDT patients and CLIMB-SCD-121 for severe SCD patients) will enroll up to 45 patients and follow patients for approximately 2 years after infusion. Enrollment is ongoing at several clinical trial sites in the USA, Canada and Europe.

CTX001 is being developed under a co-development and co-commercialization agreement between CRISPR Therapeutics and Vertex which was signed in 2015. Following the news, Vertex's stock rose up 2.3% and that of CRISPR Therapeutics jumped 17%.

Dr Samarth Kulkarni, CEO of CRISPR Therapeutics commented:

"We are very encouraged by these preliminary data, the first such data to be reported for patients with beta thalassemia and sickle cell disease treated with our CRISPR/Cas9 edited autologous hematopoietic stem cell candidate, CTX001. These data support our belief in the potential of our therapies to have meaningful benefit for patients following a one-time intervention. We continue to enroll these studies as we drive forward to develop CRISPR/Cas9 therapies as a new class of transformative medicines to treat serious diseases."

CELGENE'S CAR-T THERAPY OFFERS HOPE FOR LYMPHOMA PATIENTS

Long-term follow-up data from Celgene's CAR-T therapy trial presented at the 61st American Society of Hematology meeting has provided promising results for Lisocabtagene Maraleucel (liso-cel) in patients with relapsed/refractory (r/r) large B cell lymphomas.

The trial was conducted in 342 patients; 268 pts received liso-cel at 3 different doses and 24 patients received nonconforming product. Primary endpoints were treatment-emergent adverse events (TEAEs) and overall response rate (ORR).

Safety analysis showed favorable safety profile in the patients. 79% of patients had grade ≥3 TEAEs, primarily cytopenias. CRS or NE occurred in 47% of pts. Any grade CRS occurred in 42% of pts at a median onset of 5 days; only 2% had grade ≥3 CRS. NEs occurred in 30% of pts (grade ≥3, 10%) at a median onset of 9 days (Table). 19% of pts received tocilizumab and 21% received corticosteroids for CRS and/or NEs.

The study demonstrated durable clinical activity and met all primary and secondary efficacy endpoints. Among patients evaluable for efficacy (n=255), ORR was 73% and the CR rate was 53%. Progression-free survival after liso-cel infusion was substantially longer than PFS from the immediate prior therapy.

FDA APPROVES ALNYLAM'S GIVOSIRAN FOR TREATING ACUTE HEPATIC PORPHYRIA

The FDA has approved Alnylam's GIVLAARI™ (givosiran) injection for treating patients with acute hepatic porphyria (AHP). The approval was based on the results from a Phase 3 trial (ENVISION) which showed significant reduction in the rate of porphyria attacks in AHP patients.

AHP is a family of ultra-rare metabolic disorder characterized by

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debilitating, potentially life-threatening attacks and, for some patients, chronic manifestations that negatively impact daily functioning and quality of life. It is caused by a defect in the heme biosynthetic pathway in the liver resulting in the accumulation of heme precursors called porphyrins. Long-term complications of AHP includes chronic neuropathic pain, hypertension, chronic kidney disease and liver disease.

Givosiran is a subcutaneously administered RNAi therapeutic targeting aminolevulinic acid synthase 1 (ALAS1). Monthly administration of givosiran was shown to significantly lower induced liver ALAS1 levels in a sustained manner and thereby decrease neurotoxic heme intermediates, aminolevulinic acid (ALA) and porphobilinogen (PBG), towards normal levels. By reducing accumulation of these intermediates, givosiran has the potential to prevent or reduce the occurrence of severe and life-threatening attacks, control chronic symptoms, and decrease the burden of the disease.

The approval news has come less than four months after acceptance of the New Drug Application. The ENVISION Phase 3 study was conducted in 94 patients with AHP, at 36 study sites in 18 countries. AHP patients treated with Givosiran experienced 70% fewer porphyria attacks compared to placebo. It also resulted in a similar reduction in intravenous hemin use, as well as reductions in urinary aminolevulinic acid (ALA), and urinary porphobilinogen (PBG).

Givosiran also previously received Breakthrough Therapy Designation from the FDA and Orphan Drug Designation in the US, as well as Priority Medicines (PRIME) Designation from the EMA and Orphan Drug Designation in the EU.

The approval of Alnylam's Givlaari™ (givosiran) for the treatment of acute hepatic porphyria (AHP) marks the company's second RNAi therapeutic to be approved in the last 16 months. Givlaari joins Onpattro® (patisiran) – indicated for the treatment of the polyneuropathy of hereditary transthyretin-mediated amyloidosis – in its stable of approved RNAi medicines. Givlaari is administered once a month by subcutaneous injection, and acts through RNA interference to reduce circulating levels of neurotoxic intermediates aminolevulinic acid and porphobilinogen, factors associated with attacks and other disease manifestations of AHP. The treatment requires careful patient monitoring; significant elevations in transaminases were seen in 15% of patients treated with Givlaari, and another 15% experienced renal toxicity. Nevertheless, these adverse effects can be monitored, and the approval offers an exciting development for the AHP patient community.- Richard Philipson

EXCELLTHERA'S DRUG BOOSTS UMBILICAL CORD BLOOD CELL DIFFERENTIATION

The number of patients undergoing a blood stem cell transplant is increasing each year. About half of the transplants fail because the

disease either returns or result in graft versus host disease (GvHD), or because the patient dies due to the chemotherapy and radiation treatments that accompany the transplant.

Stem cells used for these transplants are mainly harvested from the blood itself or from bone marrow. Only around 7% of transplants use stem cells harvested from umbilical cord blood. The occurrence of GvHD is low when using umbilical cord blood stem cells, however these cells are rarely used because the cords are small and do not contain enough quantity of cells to treat an adult.

In an attempt to overcome this issue, researchers at the Institute for Research in Immunology and Cancer (IRIC) of the Université de Montréal identified a small molecule drug that could boost stem cell counts in cord blood and the team has published early clinical data showing a quick expansion of blood cells as well as a low complication rate.

The drug has been licensed to Montreal-based biotech ExCellThera, which was founded by the study's co-senior author Dr Guy Sauvageau. The therapy received FDA's Regenerative medicine advanced therapy designation and the company is launching new clinical trials in the US and Canada in patients with high-risk leukemia.

In the recent article published in *The Lancet Haematology*, Dr Sauvageau and colleagues used UM171 to culture cord blood and infused it to 22 patients with blood cancers, some of whom had already failed a previous stem-cell transplant. When transplanted, the UM171-expanded blood cells established themselves quickly. None of the patients developed serious chronic GvHD during a median follow-up period of 18 months. And despite the patients being identified as high-risk, only one died due to treatment-related hemorrhage.

Data showed that UM171 prompted the stem cells to multiply by an average of 30-fold in just seven days. It also significantly increased the number of key immune cells in the graft blood, such as antigen-presenting dendritic cells and mastocytes.

Researchers are hopeful that the procedure could increase the percentage of genetically compatible transplants significantly.

Dr Sandra Cohen, co-author of the article commented:

"The most impressive result is the low mortality rate associated with UM171 transplantation compared to conventional cord transplantation. Not a single patient needed immunosuppression treatment after 13 months, whereas with normal transplants, 50% of patients require such treatment at that point. No other biotechnology procedure has produced these kinds of results."

CRISPR-EDITED T CELLS PROVE SAFE IN SMALL CANCER STUDY

Researchers at the Abramson Cancer Center of the University of Pennsylvania (UPenn) have provided early data from the first-ever clinical trial that uses CRISPR/Cas9 technology to edit patient's T cells. UPenn is

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conducting the ongoing study together with the Parker Institute for Cancer Immunotherapy and Tmunity Therapeutics.

The team first used CRISPR/Cas9 editing to remove three genes. The first two edits removed a T cell's natural receptors to make sure the immune cells bind to the right part of the cancer cells. The third edit removed PD-1, a natural checkpoint that sometimes blocks T cells. Following this, a lentivirus was used to insert an affinity-enhanced T cell receptor (TCR), to direct the edited T cells to target an antigen called NY-ESO-1 on cancers.

The edited T cells were infused back into the patients, so far, three participants - two with multiple myeloma and one with sarcoma. Data revealed that the CRISPR treatment was well tolerated and the edited T cells expanded and bound to their tumor target well.

The three patients who received the CRISPR-edited T cells had failed all previous treatments and nine months following the current therapy, are still though it's too early to assess their overall response.

The trial will now expand to 18 patients with multiple myeloma, synovial sarcoma or myxoid/round cell liposarcoma and is expected to run through 2022.

Dr Edward A Stadtmauer, the study's principal investigator at UPenn, commented:

"This trial is primarily concerned with three questions: can we edit T cells in this specific way? Are the resulting T cells functional? And are these cells safe to infuse into a patient? This early data suggests that the answer to all three questions may be yes."

SOLID BIOSCIENCES HALTS DMD GENE THERAPY TRIAL

The FDA has placed clinical hold on Solid Biosciences's Phase 1/2 gene therapy trial (IGNITE DMD) for Duchenne muscular dystrophy (DMD) after a patient suffered serious kidney and blood-related injuries.

This is the third time that the company is halting the trial and all the events have been related to safety concerns about the gene therapy under investigation, SGT-001. After each of the past two clinical holds, FDA had allowed the trial to continue.

DMD is a progressive, X-linked degenerative disorder caused by the absence of dystrophin and is the most commonly inherited neuromuscular disease. Dystrophin protein levels are affected due to out-of-frame mutations in the dystrophin gene.

SGT-001 is Solid's lead gene therapy candidate and it uses an AAV9 vector to deliver micro-dystrophin, a shorter but functional form of the dystrophin protein to the muscle cells of DMD patients. The therapy was proven to restore micro-dystrophin in muscle cells and had the potential to slow or stop the progression of DMD in preclinical trials.

Six patients were dosed with SGT-001, including three patients in the first cohort at a 5E13 vg/kg dose and three patients in the

second cohort at a 2E14 vg/kg dose. Patients in the first cohort and two patients in the second cohort were reported to be doing well and are being followed per the study protocol. The third patient in the higher dose cohort experienced a treatment-related serious adverse event (SAE). It was characterized by complement activation, thrombocytopenia, a decrease in red blood cell count, acute kidney injury, and cardio-pulmonary insufficiency. Currently the patient is being closely followed by his care team and is showing signs of recovery.

Solid will work with the FDA to resolve the hold and determine next steps for IGNITE DMD.

SGT-001 was developed based on the work by Dr Jeffrey Chamberlain (University of Washington) and Dr Dongsheng Duan (University of Missouri). It was granted Rare Pediatric Disease Designation and Fast Track Designation in the US and Orphan Drug Designations in both the EU and US.

The news emerging from Solid Biosciences on its microdystrophin-based gene therapy treatment for Duchenne muscular dystrophy looks ominous, with another patient experiencing serious safety issues following treatment, and the FDA placing the program on clinical hold. The company has previously reported that the low dose of SGT-001 failed to show signs of activity, but there have now been three patients in the higher dose cohort that have experienced safety issues. It looks like the company and its Duchenne treatment are increasingly being boxed into a corner, unable to find a dose that has therapeutic activity, but which doesn't produce serious adverse effects. The competition is also fierce, with Sarepta Therapeutics and Pfizer both developing gene therapies for DMD, while collaborators CRISPR Therapeutics and Vertex Pharmaceuticals are exploring the possibility of developing a treatment for multiple types of muscular dystrophy based on gene editing. - Richard Philipson

GENE THERAPY COMBO TREATS AGE-RELATED DISEASES IN MICE

Harvard researchers together with the biotech firm Rejuvenate Bio have used a combination gene therapy approach to reverse multiple age-related diseases in mice. If proven successful in larger animal models and humans, the strategy could be a major step in treating multiple age-related diseases and aging itself.

Aging is associated with several diseases including heart failure, kidney failure, diabetes and obesity, and the presence of one lead to another. There is no single treatment that could treat all these diseases and therefore patients are required to take multiple drugs, increasing the risk of negative side effects and affecting their lifespan.

A latest study by Prof. George Church and team at Harvard University's Wyss Institute for Biologically Inspired Engineering tested whether a combination of gene therapy using longevity-associated genes could improve age-related diseases in mice. To test this, they used three genes that had

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previously been shown to render increased health and lifespan benefits in mice: FGF21, sTGFβR2, and αKlotho. The team hypothesized that providing extra copies of these genes to diseased mice through gene therapy could similarly improve age-related diseases and confer health benefits.

The team first created separate gene therapy constructs for each gene using AAV8 vector and injected them either individually or in combination with the other genes into mice models of obesity, type 2 diabetes, heart failure, and renal failure.

Results showed that a one-time administration of gene therapy with FGF21 alone could reverse weight gain and type 2 diabetes in obese, diabetic mice. Combining FGF21 with sTGFβR2 reduced kidney atrophy by 75% in mice with renal fibrosis. Heart function in mice with heart failure improved by 58% when they were given sTGFβR2 alone or in combination with FGF21 or αKlotho. This shows that a combined therapeutic treatment of FGF21 and sTGFβR2 could successfully treat all four age-related conditions, therefore improving health and survival. Interestingly, combining all three genes together resulted in slightly worse outcomes.

The study also noted that the injected genes remained separate from the animals' native genomes and did not modify their natural DNA and could not be passed to future generations.

Findings from this study published in *PNAS* demonstrate the potential of gene therapy for treating diverse age-related ailments and the efficacy of combination gene therapy in improving health and lifespan by addressing multiple diseases at once.

Prof. Church commented:

"Achieving these results in non-transgenic mice is a major step toward being able to develop this treatment into a therapy, and co-administering multiple disease-addressing genes could help alleviate the immune issues that could arise from the alternative of delivering multiple, separate gene therapies for each disease. This research marks a milestone in being able to effectively treat the many diseases associated with aging, and perhaps could lead to a means of addressing aging itself."

Prof. Church is developing the therapy in collaboration with Rejuvenate Bio, a biotechnology company that he co-founded in 2017. The company is pursuing gene therapy treatments for dogs.

LICENSING AGREEMENTS & COLLABORATIONS

NOVOHEART TEAMS UP WITH ASTRAZENECA FOR 'HEART-IN-A-JAR'

Novoheart, a global stem cell biotechnology company developing engineered miniature living human hearts using stem cells and

bioengineering approaches, has announced a collaboration with global biopharmaceutical company AstraZeneca.

In collaboration with the Cardiovascular, Renal and Metabolism therapy area of AstraZeneca, Novoheart will work together to develop the world's first human-specific *in vitro*, functional model of heart failure with preserved ejection fraction (HFpEF), a common condition among the elderly and in women, with the reported prevalence approaching 10% in women over the age of 80 years.

The models will help drug developers for accurate preclinical testing of the effectiveness and safety of new drugs, maximizing the successes in drug discovery whilst minimizing costs and harm caused to patients.

The initial phase of the project aims to establish a new *in vitro* model, leveraging Novoheart's proprietary 3-D human ventricular cardiac organoid chamber (hvCOC) technology, that reproduces key phenotypic characteristics of HFpEF. Also known as 'human heart-in-a-jar', the hvCOC is the only human engineered heart tissue available on the market to date that enables clinically informative assessment of human cardiac pump performance including ejection fraction and developed pressure.

Regina Fritsche Danielson, SVP, Head of Research and Early Development, Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, commented:

"There are significant unmet treatment needs in patients with heart failure with preserved ejection fraction. By combining Novoheart's proprietary hvCOC model with our expertise in heart failure, we aim to create the first in vitro *model reproducing phenotypic characteristics of heart failure with preserved ejection fraction. This could bridge the gap between* in vivo *animal models and clinical trials to help accelerate the drug discovery process by providing human-specific preclinical data."*

BLUEBIRD BIO TO COLLABORATE WITH FORTY SEVEN FOR ANTIBODY-BASED CONDITIONING REGIMEN

bluebird bio has entered into a research collaboration with Forty Seven to pursue clinical proof-of-concept to study an all antibody conditioning regimen for use in combination with bluebird's autologous lentiviral vector hematopoietic stem cell gene (LVV HSC) therapy.

The study will use Forty Seven's antibody-based conditioning regimen, FSI-174 (anti-cKIT antibody) plus magrolimab (anti-CD47 antibody) in the gene therapy platform.

Conditioning is a pre-requisite for hematopoietic stem cell transplantations (HSCT) and most *ex vivo* LVV HSC gene therapies whereby a patient's own stem cells are depleted first from the bone marrow to facilitate the engraftment of the new HSCs through a process. However, the risks associated with using traditional conditioning agents like chemotherapy and radiation limits the types of patients who are eligible for gene therapy.

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To overcome this, bluebird bio has partnered with Forty Seven, a biopharmaceutical company developing antibody-based approaches to cure cancer, to test novel antibody-based conditioning regimens in its stem cell gene therapy. The collaboration will focus on a conditioning approach aimed to deliver reduced toxicity and will initially target diseases that have the potential to be corrected with transplantation of autologous gene-modified blood-forming stem cells. If successful, the new conditioning regimen could allow for more patients to undergo gene therapy. Under the terms of the agreement, bluebird bio will provide its *ex vivo* LVV HSC gene therapy platform and Forty Seven will contribute its innovative antibody-based conditioning regimen for the collaboration.

Dr Jens Peter Volkmer, Founder and VP of R&D at Forty Seven commented:

"Forty Seven is advancing the pioneering work on CD47 and cKIT from our scientific founder, Irv Weissman's lab. We have shown that antibody blockade of CD47 can synergize with other antibodies targeting cancer to promote tumor engulfment. Based on this experience, coupled with the results of preclinical studies, we are eager to explore this dual-antibody approach for the potential treatment of non-malignant diseases".

ALLOGENE PARTNERS WITH NOTCH TO CREATE STEM CELL-BASED ALLOGENIC CELL THERAPIES

Allogeneic CAR-T therapy developer Allogene Therapeutics has entered into a license agreement with Notch Therapeutics to develop induced pluripotent stem cell (iPSC)-based allogeneic cell therapies. In addition to the agreement, Allogene has acquired a 25% equity position in Notch.

Allogene Therapeutics, founded by former Kite Pharma executives, is taking another major initiative in the CAR-T space by entering into a collaboration with Notch to develop iPSC-based allogeneic CAR (AlloCAR) therapies.

The initial focus of the collaboration would be to develop iPSC-based AlloCAR therapy products for non-Hodgkin lymphoma, leukemia and multiple myeloma. Under the partnership, Allogene and Notch will create allogeneic cell therapy candidates from T cells or natural killer cells using Notch's Engineered Thymic Niche (ETN) platform.

Launched in 2018 by iPSC pioneers Juan Carlos Zúñiga-Pflücker and Peter Zandstra, Notch Therapeutics is developing advanced approaches to differentiate mature immune cells from iPSCs. The Notch ETN technology platform offers potential flexibility and scalability to produce stem cell-derived immune cell therapies. iPSCs could prove to be a better starting material for AlloCAR T therapies because they are renewable and could have improved efficiency of gene editing, greater scalability of supply, product homogeneity and more streamlined manufacturing.

Under the terms of the agreement, Allogene will make an upfront payment of $10 million to Notch. Notch will be responsible for the preclinical research of next-generation iPSC AlloCAR T cells. Allogene will clinically develop the product candidates and will have the exclusive worldwide rights to commercialize the resulting products.

Notch is also eligible to receive up to $7.25 million upon achieving certain agreed research milestones, up to $4.0 million per exclusive target upon achieving certain pre-clinical development milestones, and up to $283 million per exclusive target and cell type upon achieving certain clinical, regulatory and commercial milestones.

The companies are hopeful that the iPSC-based AlloCAR therapies, if successful in cancers, could be extended to treat other immunity-related diseases such as infectious diseases, autoimmune diseases and aging.

Dr David Chang, President, CEO and Co-Founder of Allogene Therapeutics commented:

"This collaboration exemplifies Allogene's long-term commitment to advancing the field of cancer treatment as we continue to expand and progress our innovative pipeline of off-the-shelf AlloCAR candidates. Though treatments made from iPSCs may take longer to get into the clinic than Allogene's current pipeline – which is based on donor T cells – they will be the "next chapter" in cell therapy."

NOTCH THERAPEUTICS

Notch Therapeutics was founded by two Toronto prominent Toronto scientists, Peter Zandstra and Juan-Carlos Zuniga-Pflucker, who have developed a platform that allows for differentiation of induced pluripotent stem cells into a variety of immune cell types. While most companies developing allogeneic T cell therapies today have taken a donor approach, where T cells are derived from a single donor and then expanded, Notch will use a clonal, pluripotent stem cell source. The result is the ability to generate much larger numbers of doses with essentially no genetic or phenotypic variability between doses. Notch will drive efficiency in manufacturing by implementing a variety of tools to engineer cells in its upstream process, such that differentiated cell therapies carry modifications, such as CARs, gene edits, cloaking, and other synthetic enhancements. The companies platform is based on the the thymic niche, the region of the body where T cells mature, and is an absolute game changing innovation for cell-based immunotherapy for oncology and other applications.-Mark Curtis

FERRING SIGNS DEAL WITH BLACKSTONE FOR BLADDER CANCER GENE THERAPY

Switzerland-based Ferring Pharmaceuticals has joined hands with Cambridge, MA-based investor Blackstone Life Sciences to pump $570 million into a bladder cancer gene therapy project.

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The deal will see the creation of a new company called FerGene, focused on the global development and marketing of nadofaragene firadenovec, a gene therapy currently in Phase 3 trial for bladder cancer. Blackstone will invest $400 million and Ferring is to invest $170 in the new company. Under the terms of the agreement, FerGene will hold the US marketing rights, while Ferring holds the rights outside of the USA.

Nadofaragene firadenovec is an AAV-based gene therapy containing the gene interferon alfa-2b intended for patients with high-grade non-muscle invasive bladder cancer that is unresponsive to Bacillus Calmette-Guerin (BCG) therapy, the current standard treatment.

The treatment is being tested in a Phase 3 trial where the therapy is administered by catheter into the bladder of the patient every three months.

The therapy works by releasing interferon alfa-2b and turning the patient's own bladder wall cells into multiple interferon microfactories, enhancing the body's natural defences against the cancer.

Nadofaragene firadenovec was developed by Dr Colin Dinney at the University of Texas MD Anderson Cancer Center. The therapy received FDA's Breakthrough Therapy designation.

TAKEDA TO COLLABORATE WITH MD ANDERSON FOR OFF-THE-SHELF CAR NK CELL THERAPY

The University of Texas MD Anderson Cancer Center and Takeda Pharmaceuticals have signed an exclusive license and research agreement to develop cord blood-derived chimeric antigen receptor-directed natural killer (CAR-NK)-cell therapies, 'armored' with IL-15, for the treatment of B-cell malignancies and other cancers.

Under the terms of the agreement, Takeda will receive access to MD Anderson's CAR-NK platform and the exclusive rights to develop and commercialize up to four programs, including a CD19-targeted CAR NK-cell therapy and a B-cell maturation antigen (BCMA)-targeted CAR NK-cell therapy. Takeda and MD Anderson will also conduct a research collaboration to further develop these CAR-NK programs.

MD Anderson's allogeneic CAR NK platform, developed by Dr Katy Rezvani, professor of Stem Cell Transplantation and Cellular Therapy, isolates NK cells from umbilical cord blood and engineers them to express CARs against specified cancer targets. CAR NK cells are modified with a retroviral vector to deliver genes and enhance their effectiveness to attack specific tumors. A CD19 CAR increases the cells' specificity for B-cell malignancies while the immunocytokine IL-15 enhances the proliferation and survival of the CAR NK cells in the body.

In contrast to current CAR T-cell therapies that utilize a patient's own genetically modified T-cells and require a multi-week manufacturing process, CAR NK cells are intended to be manufactured from a

non-related donor source and stored for off-the-shelf use, allowing treatment to be delivered more rapidly.

It is anticipated that the CD19 CAR NK-cell therapy could be administered in an outpatient setting. In an ongoing phase 1/2a clinical study treating patients with relapsed and refractory B-cell malignances, the CD19 CAR NK-cell therapy has not been associated with the severe cytokine release syndrome (CRS) or neurotoxicity observed with existing CAR-T therapies.

Dr Rezvani commented:

"Our vision is to improve upon existing treatments by developing armored CAR NKs that could be administered off-the-shelf in an outpatient setting—enabling more patients to be treated effectively, quickly and with minimal toxicities. With their expertise in hematologic malignancies and commitment to developing next-generation cell therapies, Takeda is the ideal collaborator to help our team advance CAR NK-cell therapies to patients in need of treatments."

FINANCE

EGENESIS RAISES $100 MILLION IN SERIES B FINANCING

eGenesis, a biotechnology company utilizing gene editing technologies for developing safe and effective human-compatible organs to address the global organ shortage, has announced the successful completion of a $100 million in Series B financing.

By pioneering an alternative source of human-compatible organs, eGenesis is committed to help solve the global organ shortage. Over 110,000 patients are on the organ transplant waitlist in the USA alone, with approximately 20 patients dying per day because they are unable to find a suitable organ donor.

The funds will let the company accelerate its kidney xenotransplant program into the clinic, as well as support advancement of a range of other xenotransplant programs across islet cell, liver, heart, and lung. The focus of the company is to rapidly advance an entirely new set of options across the transplantation field.

"With this new round of financing from industry leaders, eGenesis is well positioned to continue to advance the development of human-compatible organs to address the dire shortage in the U.S. and around the world," said Paul Sekhri, president and chief executive officer of eGenesis. "The concept of cross-species organ replacement, known as xenotransplantation, has re-emerged due to recent advancements in gene editing led by eGenesis, and will become a safe and effective solution for the hundreds of thousands of patients currently on the organ transplant waitlist globally."

Xenotransplantation is the transplantation of organs, tissue and cells

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from one species into another. Pig has been considered the most suitable donor for humans. However, obstacles in virus transmission and the body's immune response have prevented porcine organ xenotransplantation from entering clinical development. eGenesis uses gene editing tools such as CRISPR to overcome incompatibilities related to virology and immunology.

eGenesis hopes these methods can be wielded against the endogenous retroviruses found in pig organs, as well as against the body's rejection of foreign material. It's currently developing a kidney transplant as its lead candidate and hopes to expand its future work in xenotransplantation to areas such as cell therapy.

The financing was led by Fresenius Medical Care Ventures (FMCV), with participation from new investors including Leaps by Bayer, and Wellington Partners. Existing investors including, but not limited to, ARCH Venture Partners, Biomatics Capital, Alta Partners, and Khosla Ventures all participated.

eGenesis was founded on the premise of using xenotransplantation to address the gap in organs required for transplant in humans. Through the application of gene editing technology eGenesis is engineering the pig to become a source of human compatible organs for transplantation, with a lead program in kidney transplant. The company has successfully utilized CRISPR/Cas9 technology to clear the pig genome of porcine endogenous retroviruses and other elements that could lead to immunogenicity in humans. If the platform is broadly successful, eGenesis would become a direct competitor to regenerative medicine companies developing iPSC-derived therapies for transplant applications, including a number of companies developing therapies for type 1 diabetes. eGenesis recently closed a $100M Series B financing to progress its kidney transplant program into the clinic.-Mark Curtis

VIGENERON RAISES FUNDS IN SERIES A FINANCING

The German biotech ViGeneron has raised an undisclosed sum in a Series A financing round to advance an ophthalmic gene therapy that could deliver bigger DNA sequences into a wider range of cells than current technology.

The round was led by two Chinese investors, the VC firm Sequoia Capital China and the pharma and medtech giant WuXi AppTec. ViGeneron will use the funds to advance its preclinical gene therapy technology for treating two undisclosed blindness conditions that currently have no approved treatments.

ViGeneron, based in Munich, develops AAV-based gene therapies to target cells in the retina for retina disorders.

A major limitation of AAV vectors is that they can't carry long DNA sequences and ViGeneron is working towards developing vectors that are designed to carry different sections of the gene into a cell. These DNA sequences are separately transcribed into mRNA molecules that

are then assembled together into a single mRNA molecule carrying the instructions to create a therapeutic protein.

TOKYO'S HEARTSEED RAISES $26 MILLION TO DEVELOP STEM CELL INJECTIONS FOR HEART FAILURE

Heartseed, a Tokyo-based biotechnology company developing iPSC-derived cardiomyocytes for treating heart failure (HF), has announced that it has raised 2.8 Billion-yen (approx. $26 million) at Series B round, bringing its total financial backing to 3.8 Billion yen (approx. $35 million) since it was founded.

Proceeds from the funding will be used to support two clinical trials in its pipeline. The company expects to initiate Phase 1/2 clinical trial for its lead pipeline HS-001 for HF with reduced Ejection Fraction (HFrEF) in late 2020. The company is also supporting an investigator-initiated clinical trial for Dilated Cardiomyopathy led by Keio University, which is expected to be initiated in the first half of 2020.

The company was founded in 2015 by Prof. Keiichi Fukuda and his group at the Department of Cardiology, Keio University, Tokyo, to develop and commercialize cardiac regenerative medicine.

Participants in the Series B included new investors, SBI Investment, JMDC, Gene Techno Science, Nissay Capital, SMBC Capital, and an existing investor, Astellas Venture Management LLC., which has supported Heartseed from Series A.

Prof. Fukuda commented:

"I have been involved in the research of cardiac regenerative medicine for the past 20-years as a pioneer in this field and solved all the major challenges such as ventricular-specific cardiomyocyte differentiation, purification, large-scale manufacturing and efficient cell delivery. We are confident that our lead pipeline HS-001 can be a curative therapy for severe HF, with the mechanism that transplanted ventricular-specific highly-purified cardiomyocytes engraft to patient's heart and retain for a long-term."

GUANGPING GAO JOINS IVERIC BIO AS CHIEF STRATEGIST

IVERIC bio has appointed gene therapy pioneer Guangping Gao, PhD, as the company's Chief Strategist of Gene Therapy. In his advisory role,

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Dr Gao will help shape IVERIC bio's gene therapy strategy going forward.

IVERIC bio is a biopharmaceutical company focused on developing treatment options for retinal diseases.

Ranked as #4 on *Nature Biotechnology's* list of the World's Top 20 Translational Researchers for 2017, Dr Gao is an internationally recognized gene therapy researcher who has played a key role in discovering and characterizing AAV vectors. Dr Gao has over 30 years of scientific research experience in gene-based treatments.

Dr Gao is currently the Co-Director of Li Weibo Institute for Rare Diseases Research, Director, Horae Gene Therapy Center and Viral Vector Core, Professor of Microbiology and Physiological Systems and Penelope Booth Rockwell Professor in Biomedical Research, at the University of Massachusetts Medical School; an elected fellow of both the *US National Academy of Inventors (NAI)* and the *American Academy of Microbiology*; and the current President of the *American Society of Gene and Cell Therapy*.

Written by Dr Applonia Rose, *Cell and Gene Therapy Insights*