SEPTEMBER 2020 Volume 6, Issue 8

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:
Scaling up/out: cost-effective and robust transitioning through the clinic to commercial manufacture

GUEST EDITOR: Jan Thirkettle,
Chief Development Officer,
Freeline Therapeutics Limited

CELL & GENE THERAPY INSIGHTS

Volume 6, Issue 8

Scaling up/out: cost-effective and robust transitioning through the clinic to commercial manufacture

CELL & GENE THERAPY INSIGHTS

FOREWORD

Scaling up/out: cost-effective and robust transitioning through the clinic to commercial manufacture

JAN THIRKETTLE is Chief Development Officer at Freeline, a clinical-phase AAV gene therapy company focused on the development of systemically delivered gene therapies for the treatment of chronic diseases. As such, Jan has responsibility for Programme Management and CMC/Manufacturing. Jan is a Director of the US, Irish and German Subsidiaries. Jan has extensive experience in the development of novel manufacturing platforms including natural product and enzyme-derived NCEs, biologics and gene therapies, and has enabled the launch of medicines in all of these areas. Prior to joining Freeline Jan led the establishment of GSK's Cell & Gene Therapy platform and was responsible for CMC/supply for Strimvelis, the first ex vivo gene therapy to receive an EU Marketing Authorisation Application. He has held pharma industry positions spanning from discovery to commercial manufacturing, but is most passionate about the cross-functional elements of late-state development and new platform deployment. Jan holds an MA in Chemistry and a PhD in Biological Chemistry from Oxford University.

Cell & Gene Therapy Insights 2020; 6(8), 1227–1229

DOI: 10.18609/cgti.2020.135

The fact that we could have filled an edition like this many times over with articles addressing the topic of scale-up/scale-out, or, 'making and supplying a whole lot more' is a testament to how far the cell and gene therapy field has progressed in the last decade. Back in the early 2010s when the first pioneering cell and gene therapies were being commercialized, the big questions being asked of the field were very much still about safety and efficacy, and which of the many sub-modalities might yield these. The pioneering companies were often having to create their own bespoke manufacturing equipment and repurpose techniques from either the biologics field, or more likely the research laboratory to manufacture even a few patients' worth of the therapeutic. Very few, if any of the global reagent/equipment/services players were doing more than watching and assessing their strategy.

Fast forward to 2020 and the focus is very much on manufacturing, and in particular the challenge of manufacturing and supplying at a scale to enable treatment of thousands, even tens of thousands of patients. This is a challenge which cannot be underestimated. Whilst the biology and analytical tools have progressed massively, these therapeutics are highly complex and often interact with patients in very complex ways, which means linking product quality attributes to patient outcomes is still largely empirical. This makes the challenge of scaling-up/-out whilst retaining product quality all the more demanding as it means there can be uncertainty around the impact of making such changes on the performance of the product. On top of this, the classic life cycle dynamics faced by all modalities still hold true, namely the competing challenges of agility (ability to react to new information) which is particularly important in early development, and efficiency (maximizing output) which is particularly important for commercialization. Bridging these two demands without having to make changes that could affect product quality requires a very considered approach to technology selection and the strategy for scaling-up/scaling-out, as well as a real focus on the supporting analytics.

Whilst the main focus for viral product and allogeneic cell therapies is scaling up (more per batch) the autologous products have a very different challenge, namely replicating manufacturing of a patient specific batch in increasing numbers. This scale-out scenario brings particular challenges to be met for the whole supply chain/manufacturing system; challenges which are unprecedented in any previous pharmaceutical modality. The focus that these challenges have had for the last half decade at least is now leading to solutions which are increasingly novel and targeted at the specific needs of these products. In this issue, we have contributions from companies addressing everything from viral expression systems to logistics, and from systematic process development/scale-up to product labelling. Despite the challenges posed by the technical and logistical requirements, and the rapid evolution of the space itself, what is clear is that the serious efforts being made to address them will yield results. History, whether from antibiotics products or the biologics field, teaches us that with investment and time (and a demand for a safe, efficacious product) productivity improvements of orders of magnitudes will be delivered; however, in this case, it won't just require development of new technologies, but innovative ways of thinking around the whole supply chain.

AFFILIATION

Jan Thirkettle
Chief Development Officer, Freeline

DOI: 10.18609/cgti.2020.135

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Thirkettle reports personal fees from Freeline.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

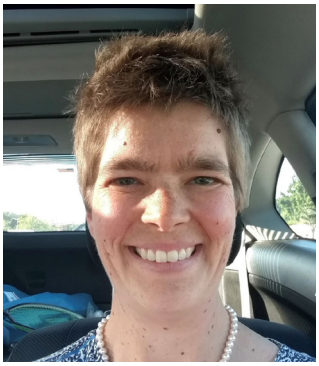
Revised manuscript received: *Sep 28 2020;* ***Publication date:*** *Oct 1 2020.*

EXPERT ROUNDTABLE

Getting a gene therapy product to market: pitfalls and how to prevent them

HETAL BRAHMBHATT
Principal Investigator - Science and Technology Team, Pharma Services, Viral Vector Services, Thermo Fisher Scientific

Dr Hetal Brahmbhatt is a Principal Investigator in the Science and Technology Team, Pharma Services, Viral Vector Services at Thermo Fisher Scientific. She has a PhD in Biochemistry and Biomedical Sciences from McMaster University where she characterized small molecule modulators of apoptotic proteins. In her present role, she contributes to the development of platform processes and technologies for the manufacturing of viral gene transfer vectors.



ELIZABETH SIMMONS
Associate DirectorQC Analytics,Homology Medicines Inc

Beth has a Master's degree from Lehigh University in Analytical Chemistry and Regulatory Affairs with 13 years of industry experience. She has managed small and large Quality Control laboratories including method transfer, qualification and validation teams in both small pharma and CMO settings. During that time Beth has supported 2 facility start ups (Commercial and Clinical) and managed technical teams focusing on small molecule, oligonucleotides, and now gene therapy.

THOMAS GUARINONI
Manager, Downstream Process team, Viralgen

Thomas Guarinoni, MSc was recruited in January 2017 as manager of the Downstream Process team at Viralgen. He was successful in transferring the entire purification process from Askbio to Viiralgen in record time. Thomas Guarinoni obtained his MSc in 2011 at ENSTBB (National Superior College of Biotechnology) in Bordeaux, France.

Cell & Gene Therapy Insights 2020; 6(6), 1237–1247

DOI: 10.18609/cgti.2020.137

Q How important is early stage process development in supporting success in late stage clinical development and commercialization of a gene therapy? What impact can the choices made early on in a product's development have as it moves towards the clinic?

ES: The greatest inefficiencies we have found stem from relying on external vendors to execute your development and manufacturing. We invested early in our internal capabilities and by keeping bioprocess development, analytics, and manufacturing in-house, we continue to learn and optimize our process throughout therapeutic development, whilst being able to scale-up our capacity and control our own schedule.

With internal capabilities we can collect data quickly, drive decisions, and make appropriate changes as needed. This means that our GMP process is essentially the same as our commercial process, which helps us to expedite that whole system.

"Considering what that final process should look like will dictate how your process development should unfold, both upstream and downstream."

- Hetal Brahmbhatt

TG: As a CMO, we of course share the development we are doing with customers. We also benefit from a technology transfer from AskBio.

One of the most important things for achieving success in your development and commercialization is plasmid design and cell line development. If you already have a good toolbox that is well designed and well selected, and a good small-scale model able to generate a great product with a robust process and robust production, half of the job is done. It is also beneficial if you are able to start working as soon as possible on Quality Control (QC) and validated assays. The more knowledge we gain on the process from an early stage, the better commercialization is likely to go.

HB: To really understand how process development supports late clinical stage development, the first question we like to ask people is: what does the process look like at the end?

Sometimes you have clients who want something 'quick and dirty', so their early process is designed in a certain way. But as you start moving towards the clinical phase, the client may realize that the yields are not sufficient, or the process is not completely scalable. They may need to change the manufacturing platform or to consider multiple batches in order to get to those desired yields. So while they may have originally been seeking a quick and easy, 'good to go' process, they suddenly find they need to establish another process and then do the comparability studies afterwards.

The alternative is to take an approach where you know what the late stage will look like, allowing you to develop the process upfront and avoid the need for additional studies later. A lot of the pitfalls that we see are around planning ahead. Considering what that final process should look like will dictate how your process development should unfold, both upstream and downstream.

DOI: 10.18609/cgti.2020.137

Q If you could go back to the early stages of process development for a product when working with a client, what would you do differently, or what would you advise them to do differently? What would you consider to be the most critical considerations for the effective transition to commercial scale manufacture?

TG: Having something that is scalable and already efficient, and not something 'quick and dirty' as Hetal just described, is often a great help for taking your product to the market more quickly. As she mentioned, if you generate a process for phase 1 or 2 that brings you quickly onto the market, but later on you find you are unable to scale-up, or you have to do bridging studies for comparability in between your toxicology batch, your phase 1/2, and your phase 3, then that presents a big issue.

I would definitely try to find a process that is as robust as possible but also as scalable as possible in an easy way. Also, start QC development and validation as early as possible, in order to avoid losing time in reaching the market. That would be my advice for early stage process development.

ES: We primarily focus on rare genetic disorders, so we have a high likelihood that we are going to expedite fairly quickly from early stage straight through to commercialization. To manage this, we focus on development and on a robust analytical platform early on.

We have developed nearly 40 analytical assays to help quantify and evaluate the quality of our products throughout development. By collecting reliable data, we are able to quickly identify opportunities for process improvements and this also helps us gain process characterization information very early on.

Ultimately, this allows us to help with any necessary process changes and making decisions around that, as well as getting ready for process validation at the end. Focusing on our analytics panel early on helps to expedite the whole system.

HB: From our perspective, we see the need to understand the product- or process-related impurities, and how they affect the critical quality attributes (CQAs).

Using adeno-associated viral (AAV) vectors as an example, you could employ a certain purification process but then as you try to enrich for full capsids, a lot of the understanding of how this affects the potency is unknown. It is good to have that information upfront to improve process design.

Q How important are technology choices during the early stages of process development, and how difficult is it to make changes as you approach commercialization?

ES: We focused early in the process on developing a robust platform and we utilize a fully chromatography-based downstream process. This enables us to have easy scalability and prevents us from needing to make major changes through the development process. Instead, we can just focus on small variations that may be implemented quickly.

The greatest benefit of leveraging a platform-based process is that you can utilize previous learnings to expedite the development of new pipeline programs. We have found that for subsequent programs, we can decrease analytical development time by nearly 90%, and process development time by nearly 50%, for each subsequent platform program.

TG: The platform approach is primarily used in the AAV world and for monoclonal antibodies (mAbs) so far, where affinity chromatography, full/empty separation with chromatography, polishing steps, and tangential flow filtration (TFF) are all used frequently.

If you apply this platform to various serotypes you can save a lot of time in terms of analytical mitigation or analytical assay checks. You already know what to expect, so you don't need to start from scratch. The drawback is that you may have a platform working for various serotypes, but some synthetic capsids may have a different reaction to your platform, so you will need to adapt. Overall, though, we've found you always gain knowledge and save time using this approach.

HB: An important consideration in technology choices is once again, what does the process look like at a commercial scale? Is your choice going to be a single-use system, or a hard pipe system? Are you making sure that your process development design fits the needs of the equipment that you have at scale? Are you accounting for any potential interaction of the molecule with the different surfaces involved? If you are using a hard pipe system, are you going to be sanitizing the system in between runs? Do you have sufficient data collected around the cleaning, the validation? You may choose to reuse the column – is that sufficiently built into your process? Technology choices are very important, and knowing the platform upfront is important.

"The greatest benefit of leveraging a platform-based process is that you can utilize previous learnings to expedite the development of new pipeline programs."

- Elizabeth Simmons

Another constraint we see is in considering not just what equipment you are using, but what steps are involved. Take TFF, for example. In a small-scale study where you are working with very small volumes, you may not have the requirement to mix the retentate. However, in a commercial process, you may need to incorporate a mixing step to improve the buffer exchange process. The choice of having a mixer, and having studies done to support that mixing, become really important.

DOI: 10.18609/cgti.2020.137

Q A critical part of a scalable manufacturing strategy is to ensure product quality and safety. How do you develop that scalable analytical strategy for gene therapy?

ES: Analytics always takes longer than you think it will. I can never stress this enough: start before you think you need to!

Regulators are now expecting validation for all dosing methods to help ensure that your dosing strategy is consistent from toxicology straight through to commercialization. Obviously, this front-loads a lot of that analytics work on the dosing method, but by having a robust dosing method you can get critical information about your process performance through all phases of development. It helps both aspects - analytics and process.

The other critical aspect for all gene therapy and gene editing companies is potency, which can be extremely challenging. I always recommend starting potency development as early as possible. Sometimes this might even be before you have a final candidate selected. Here, we try to couple our analytical development folk with our research people, so that they can start working on the biological indicators very early on in order to get a jumpstart on analytical development.

As gene therapy is fairly new, the regulatory guidance changes regularly, so it is key to be flexible with your analytical strategy. Each time a new guidance comes out, it is important to read through it and turn to your regulatory and quality assurance teams to figure out how you are going to navigate any new expectations.

TG: We firstly evaluate the risk to patient safety and the clinical study that the customer will perform. As Beth mentioned, one of the most important considerations is determining the dose you will inject. That has been quite an issue in the past year.

Essentially, the approach is to focus on risk assessment of the QC, and to try to validate as much as possible. You will not be able to validate all of the QC you use in phase 1/2, so you need to focus on what you think is essential.

The dose study, and the dose finding, presents the need to validate the assay for concentration determination, and so on. Regulators have been more and more challenging in this area, and we are seeing quicker acceptance on validation protocols that are much more stringent than in the past.

"Essentially, the approach is to focus on risk assessment of the QC, and to try to validate as much as possible. You will not be able to validate all of the QC you use in phase 1/2, so you need to focus on what you think is essential."

- Thomas Guarinoni

HB: When we talk about analytics, starting from process development, it is of course always nice to incorporate assays that are high throughput or have quick turnaround times.

One thing that we have seen come up again and again is that it is helpful to try to use any analytical methods that are partially quantified in the matrices that your product is going to be in, and this is applicable throughout the purification process as well as at the end. The reason for that is you want to make sure that whatever data you have is still going to hold true and is going to be reproducible to hit your target CQAs.

Q We have touched upon the concept of risk mitigation quite a lot. What is your overall approach to risk mitigation?

TG: Clearly the risk assessments of your process, of the definition of your quality target profile, of QC, and so on must all be done based on the patient. Here, we aim to develop, qualify, and validate all the assays that we think are important, particularly in relation to patient safety.

ES: We utilize a phase appropriate approach to risk mitigation and leverage a strong risk management program to ensure that each process decision we make is evaluated before we actually execute it. We ensure everyone is in agreement about the risk before we move forward.

We are a very data-driven organization: all of our decisions are very much focused on the data we have going into them, as well as the data coming out after we have implemented that change. Therefore, we primarily focus on our robust analytics, both during the manufacturing process and for a drug substance, to make sure we are clearing all potential contamination.

As we gain greater process understanding and have a better understanding of contamination clearance throughout the process, we update our testing panel to adjust accordingly, and focus on areas that require additional information. For example, there may be a new process step that we want to evaluate a little more closely.

As part of this approach, for each process change that we implement, we try to put in an appropriate testing panel that assesses that specific process change. That could be yield before and after or, if we are aiming to clear a certain contaminant, we can test before and after to ensure that after we have implemented the change, the desired affect was achieved. There is always a feedback loop and campaign summary to make sure that our data is telling us what we are hoping to find - if not, then we readjust our strategy.

HB: I would echo a lot of what Beth says. It is very important for a risk mitigation strategy to ensure that you have a very well-defined process characterization in place, so that you know the process you have is robust and reproducible.

Another consideration that we have seen is ensuring you are using raw materials that are suitable for the manufacturing phase you are in. Often, we see raw materials that are not GMP grade being used at a point when they should be, for instance. There are certain raw material testing requirements based on the phase you are in, and it is important to conduct the appropriate studies around this, and to be prepared to do all the raw material testing for either a phase 1 or late stage process in-house.

Additionally, try to avoid any supply chain issues. If there is an alternative product available, ensure you have done the studies ahead of time so that when you actually hit your late stage process, you don't run into supply chain issues, or have material that is not suitable for GMP.

DOI: 10.18609/cgti.2020.137

Q As gene therapies for larger therapeutic indications start to move towards the clinic, what developments or innovations would be on your wish list to enable commercial-scale manufacturing?

ES: My background is predominantly in analytics, so I am very much focused on that space for this question. I would like to see more rapid analytical options to help facilitate real-time process performance, whether this is in-line analytics that you see in some other industries, or better analytical column choices. Anything to give you more diverse options for the analytical test panel.

On the process end, we have been able to achieve a 2,000 liter scale for AAV manufacture. It would really be helpful to see that scale-up for starting materials, as supply is getting increasingly competitive, especially during the Covid pandemic. It's becoming increasingly important to evaluate your starting materials and raw materials early, and to be able to purchase them early enough, too.

TG: If I had to write a wish list for AAV, I would say it would be interesting for the field to share knowledge, as we are trying to do now, with proper case studies. We know that for mAbs or vaccines, there have been some publications around chemistry, manufacturing and controls (CMC), with big pharma sharing their knowledge, approaches, and validations. I think the gene therapy field would benefit from more of this at the CMC level, and from trying to get more standards in place.

It would be beneficial for all the players to try to share and focus upon a standard AAV - from ATCC, for example – in order to evaluate the difference versus the CMO or dose study. This is because assay results do tend to vary from one location to another.

In the field of manufacturing, things are improving in terms of bioreactors, columns, TFF, and so on. I don't see any issue of scalability. I also do not expect that the AAV field gene therapy field will have the same production needs as the mAb world. I wonder how big scale really needs to be in order to provide enough material for treating all the patients with a particular disease, at least as long as the field remains predominantly focused on rare diseases. Perhaps this is a shortsighted point of view, but as long as you treat, say, 10,000 patients in the first year, and then you only need to treat the newborn cases of the disease thereafter, you do not need to have the capability of expanding to a 50,000 liter bioreactor, for example.

This leads me to wonder if the scalability of the gene therapy world will be expanded continuously, or will be limited to expanding process capabilities just enough to be able to cover all of the patients who need a particular treatment. It may be a different story with viral vectors used in cell therapy manufacture, but even there, I believe improvements in infectivity, for instance, will make a difference moving forward.

"It's becoming increasingly important to evaluate your starting materials and raw materials early, and to be able to purchase them early enough, too."

- **Elizabeth Simmons**

ES: There is certainly still a big focus on rare diseases and in that area, I would agree with Thomas that perhaps this degree of scalability is not necessary.

However, when considering starting materials and related business continuity, we are seeing increasingly long lead times to procure them. If you could increase those scales so you are not relying on such frequent purchasing of critical starting materials, then you can hold the generous supply needed to facilitate all of your programs. I would agree, however, that for the actual drug substance itself, I don't think we are going to be in that 20,000-50,000 liter scale.

> "What I would like to see would be ...an ultracentrifuge that could be automated, is scalable, and that you would be able to use for multiple purification cycles whilst still achieving the yield and the CQAs that you desire."
>
> - **Hetal Brahmbhatt**

HB: In terms of what development I would like to see, I am going to answer from a downstream perspective.

As you move towards commercial manufacturing, automation is a required aspect that you have to build into your processes. A lot of the challenges we see with AAV purification are in the enrichment of full vector particles. There are different approaches to do this – you can do it by chromatography, and you can also do it by ultracentrifugation methods.

Both have their pros and cons; ultracentrifugation works really well, but scalability becomes a concern. Chromatography is the alternative controlled approach, but it may or may not give you the same level of enrichment and is often dependent on the starting feed stream.

What I would like to see would be either a technology that enables you to have better chromatographic separation, or an ultracentrifuge that could be automated, is scalable, and that you would be able to use for multiple purification cycles whilst still achieving the yield and the CQAs that you desire.

Q Finally, with commercial-scale production comes higher supply needs. What challenges can arise when trying to ensure consistent security of supply of quality materials for commercial-scale manufacture? What advice would you give on how to address them?

ES: Right now, we are in unprecedented times and the challenge is much more dramatic than it ever has been before. Covid is impacting the entire industry across the globe.

To remedy this, I think it is a matter of beginning to evaluate your material needs, and of purchasing those materials that are high risk and have long lead times early and in bulk. Having some stability data for those starting materials and raw materials will enable you to do this, and allow you to purchase in larger quantities so that you can keep this material for an extended period of time.

For us, the biggest risk mitigation factor is first leveraging our internal capabilities as

DOI: 10.18609/cgti.2020.137

much as possible, and then supplementing them with multiple different vendors where needed. This enables us to control our own supply, thus enabling us to maintain our development timelines across all of our platform programs, even in these unprecedented times.

TG: It is becoming more and more complicated to ensure your supply chain is working correctly when you scale-up a process. Obviously, dual sourcing is one of the options that we should always try to evaluate, although it is not possible when we are talking about things like cell culture media or transfection reagents. Even with chromatography, it may be quite tricky to assume you can use two different resins without encountering issues.

As Beth said, try to buy early and extend lifetime as much as possible for your important products. For example, if I have a product with a one year lifetime, I might be able to perform an internal study, assess the quality attributes of this raw material, and possibly extend it to two years, making my supply a little more secure.

It is a constant collaboration with your supplier - or, depending on your point of view, a constant fight with your supplier! But generally, the collaboration you see is good. Work as closely as possible with your supplier, try to extend the standard shelf-life of the products that are really important to you, and try to dual source whatever and wherever you can.

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *This article is the transript of a Roundtable sponsored by Thermo Fisher Scientific. The original Roundtable can be found here.*

Roundtable recorded: *Sep 9 2020;* ***Publication date:*** *Oct 1 2020.*

DOI: 10.18609/cgti.2020.137

Driving performance through collaboration

Biologics are complex and every process is unique. That's why you want a partner who can respond to your needs—even if what you need is a custom or open architecture approach. With a proven portfolio of solutions that spans from discovery through large-scale commercial production, our specialists have the know-how to adapt to what's next. It's our commitment to you, and **it's what we call Bioprocessing by Design**.

Let's move forward together.
Find out more at **thermofisher.com/bioprocessing**

ThermoFisher SCIENTIFIC

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

SCALING UP/OUT: COST-EFFECTIVE & ROBUST TRANSITIONING THROUGH THE CLINIC TO COMMERCIAL MANUFACTURE

COMMENTARY

Small labels, big challenges: solutions for advanced therapy labeling

Heidi Hagen & Christophe Suchet

Accurate and useful labeling of advanced therapies from starting material to final drug product is critical for patient safety, compliance, and treatment delivery. In this article, the authors present label and label printing best practices for advanced therapies and discuss solutions to major challenges faced by sponsors, stakeholders, and regulators. In the context of this article, the terms "label" and "labeling" refer to in-process and final drug labels, not warning labels, package insert content, or other advisories issued and managed by regulatory agencies. As cell/tissue collection and drug product labeling are complex topics, it is also important to note and this article will focus on a few key areas, outlined below. This article will not cover other important labeling topics, including label stock, adhesives, inks, exact label content and layout (other than referring to ISBT128/SEC), label version control, label design, label testing/qualification, or label size and location.

Cell & Gene Therapy Insights 2020; 6(8), 1183–1195

DOI: 10.18609/cgti.2020.128

Today's advanced therapies are novel and hold great promise, but also pose new challenges that sponsors and stakeholders are rallying to solve. One such challenge is in-process and final product labeling. The requirement for appropriate and approved labeling is not new in biotech and pharma – it is foundational to every drug product and was recently reinforced by the FDA's guidance for gene therapy INDs [1]. Advanced therapies, however, introduce patient or donor cellular raw material into the supply chain, resulting in a much more complex process and treatment journey to produce these therapies (Figure 1).

FIGURE 1

The cell treatment journey. A complex, distributed process delivers innovative treatments.

As a critical patient-product identifier and safety measure, advanced therapy drug product labels must often be created for each individual patient and built in real time, with important and variable information added to the label at key steps in the cell collection and manufacturing process. Healthcare Providers (HCPs), in performing cell collections, now find themselves collecting starting material and are required to comply with cGMP information gathering and labeling processes. Given the patient-specific, real-time nature of advanced therapy labeling, labels can be pre-printed and shipped to clinical sites, which simplifies some aspects of label creation but can also result in storage, matching, and disposal problems. Or manufacturers can choose to have HCPs print labels at the clinical site, which reduces storage and matching problems but can cause challenges related to hardware, hospital IT compatibility, and printing equipment.

With more than 1,060 advanced therapy drug products and clinical trials operating at medical centers world-wide each with a different process and set of Standard Operating Procedures (SOPs), the need for simplified, standardized labeling processes is acute [2]. The catalyst for labeling standardization began with a public health issue related to unsafe handling of donated blood in the 1990s, and the subsequent discovery that traceback capabilities did not exist. The FDA stepped in and initiated what we now consider standard traceability of blood and tissue donation products. Building on this foundation, the International Council for Commonality in Blood Banking Automation (ICCBBA) published the ISBT 128 global standards in 1994 [3]. Cell therapy pioneers adopted

DOI: 10.18609/cgti.2020.128

these baseline labeling standards for human blood and tissue products at the FDA's direction. This initially served the industry well – to a point – and raised the bar on patient safety.

However, as more advanced therapies enter the clinic and approach commercial approval, it has become clear that there is a gap in labeling standards when implemented for today's advanced therapies. The ISBT 128 and SEC standards are in effect for blood and tissue collection or donation activities, but the standards were not established with the expanded and complex advanced therapy supply chain in mind, where traceability from order through manufacturing, treatment and beyond is required [4–6]. As a result, a cross-industry standards updating effort is now underway, which will be discussed later in this article.

Label issues are a major reason for the FDA rejecting or questioning New Drug Applications (NDA) and Biologics License Applications (BLA) [7]. Revisiting proven best practices related to donor material and drug product labeling will help sponsors and stakeholders bridge the gap between existing standards and emerging standards, and enable manufacturers to bring life-saving advanced therapies to patients in need more safely and efficiently.

SEVEN PROVEN PRACTICES

Experience demonstrates that certain practices lead to successful labeling and support the ability to deliver safe treatments to patients. The following top seven labeling practices are proven to work across multiple therapies, for both clinical and commercial phase products:

1. Standardized formats
2. Patient privacy and patient identifiers
3. Complete Chain of Identity (COI) and Chain of Custody (COC)
4. Multi-language capabilities
5. On-demand label printing
6. Printing to any existing and approved printer
7. Collaboration with regulatory agencies

Standardized formats

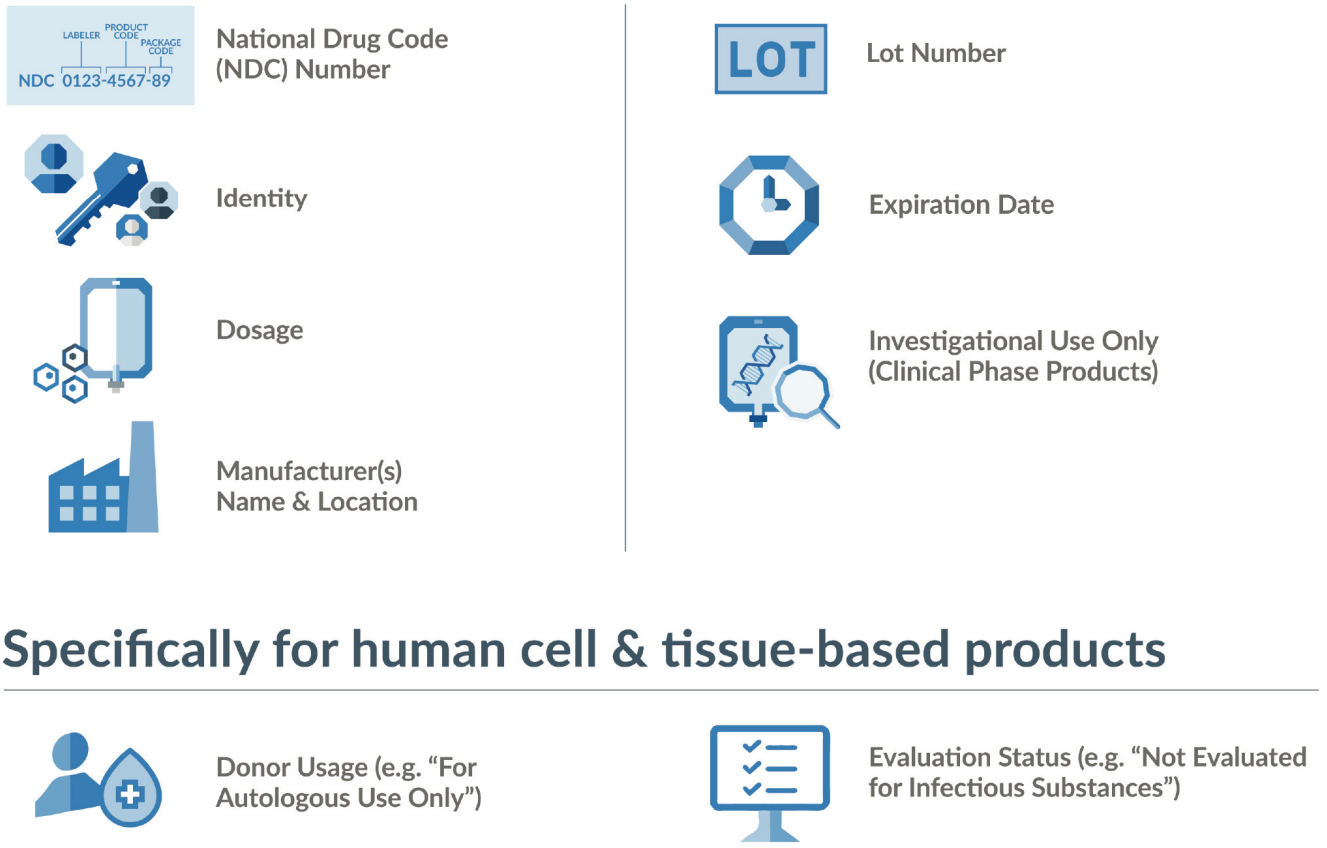
Standardization across materials, products, and processes decreases risk and errors while increasing efficiency and scalability. This is foundational to cGMP and especially important in a distributed ecosystem such as the advanced therapy supply chain, where many partners are not accustomed to cGMP practices. When established standards exist, utilizing them is a key to compliance. The FDA has certain baseline requirements for labeling (see Figure 2 [9–12]), which is part of basic cGMP operations and, as discussed in the introduction, there are additional global standards that currently exist – ISBT 128 and the SEC – for labeling blood and tissue products and providing traceability [4–6,8]. Compliance with these standards is often mandatory, with the primary aim of ensuring patient safety through blood and tissue product traceability.

An additional consideration is the importance of providing label information via scannable barcodes and human readable formats. Space is at a premium on labels, and in this digital age, it is tempting to count on reading barcodes with scanners. But maintaining continuity and integrity of patient identifiers across the entire supply chain is paramount for avoiding product mix-ups. That may mean having the ability to verify information without the benefit of a digital tool.

The independent, non-profit Standards Coordinating Body is facilitating an industry-wide working group to update the ISBT 128 standards for apheresis collection labels and establish minimum label requirements for apheresis cell collections (Figures 3 & 4) [13]. More detail on this initiative can be found later in this article.

FIGURE 2

Finished drug product label.



Key baseline requirements by the FDA ensure consistency and standardization [9–12].

Patient privacy & patient identifiers

As a critical support for patient safety, labels must contain some patient-identifying information. The nature of that information, such as name, initials, or date of birth, is a frequent topic of debate. Alongside patient safety, patient privacy is also important.

Amid privacy concerns, it's important to keep in mind that name, initials, and date of birth are typical patient identifiers used in medical centers and for prescriptions to ensure that the right product is administered to the right patient. This is part of standard practice on drug product packaging, and HCPs are trained to use this information appropriately. This practice is demonstrated in the use of patient information on approved cell therapy product labels such as Provenge® [14], Yescarta® [15], Kymriah® [16], and Zynteglo® [17] (EMA approved).

The European Union's (EU) General Data Protection Regulation (GDPR) has been reshaping the way data is handled across every industry sector, including clinical research, by strengthening and standardizing the protection of personal data. Navigating GDPR and traceability requirements in the EU may require further expert help and collaboration with regulators – a best practice discussed in more detail later.

Concerns over visibility of patient information on external secondary and tertiary packaging (e.g. shipping labels) that is more broadly viewable can be addressed with anonymized information. Additional persistent, transparent patient identifiers can be created and tracked in ways that are consistent with

DOI: 10.18609/cgti.2020.128

patient privacy regulations. One such set of identifiers is commonly referred to as Chain of Identity, or COI, which we'll discuss next.

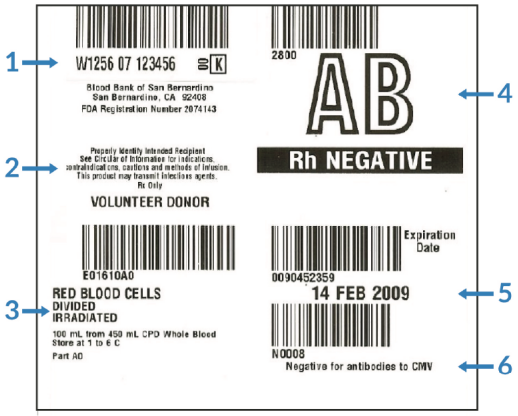
Chain of Identity (COI) & Chain of Custody (COC)

COI and COC are the cornerstone of three important success factors for any drug product – patient safety, regulatory compliance, and operational efficiency (Figure 5). These 'chains' are part of the expanded lot genealogy for advanced therapies, a key aspect of a Quality Management System, and of the traceability required by regulatory authorities [1,18]. Labels are critical for supporting COI and COC at every step of the supply chain from collection to treatment. Labels provide important data

FIGURE 3

ISBT label format.

1. Donation Identification Number
2. Product Information or Collection Date and Time
3. Product Code and Description
4. ABO/Rh groups
5. Expiration Date and Time
6. Special Testing (optional)

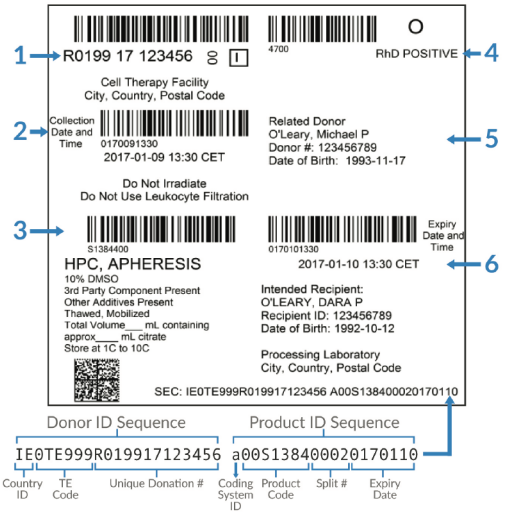


ISBT 128-compliant label formatting guidelines (revisions in process to better address the needs of cell therapy collections) [5].

FIGURE 4

SEC-compliant label formatting guidelines [6].

1. Unique Donation Number
2. Collection Date and Time
3. Product Code
4. ABO/Rh groups
5. Donor Identification
6. Expiry Date and Time



SEC label format.

FIGURE 5

COI and COC defined.

Chain of Identity

A patient's core unique identifier created for the permanent, transparent association of patient-specific data points to tissue and/or cells from order through product(s) creation, fulfillment, and post-treatment monitoring (including collection, manufacturing, administration).

Chain of Custody

Permanent and auditable data capture from the origin of tissue and/or cell collection through product administration. The data identifies the staff that handled the product, actions performed by those staff, and the location/date/time of those actions (who, what, when, where, and how).

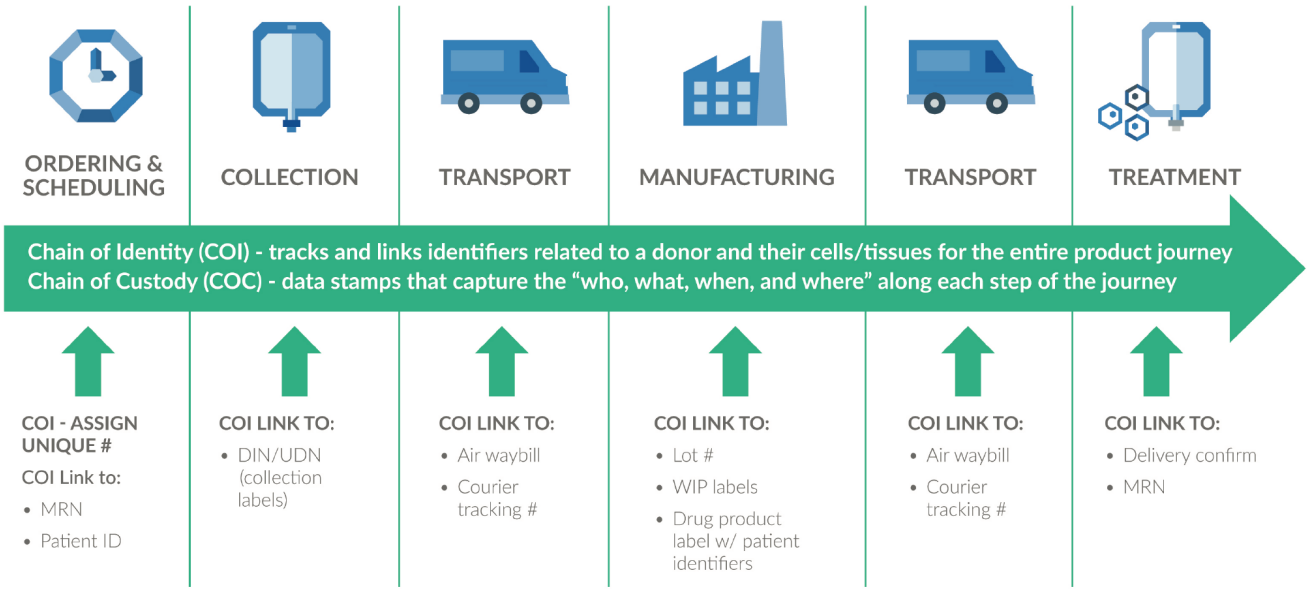
Chain of Identity and Chain of Custody are an essential part of patient safety, compliance, and the manufacturing journey.

and identifiers – via both human-readable and machine-readable formats – which are linked to essential information that ensure the right product is in the right place and undergoing the right process for the right patient (Figure 6).

The established best practice for advanced therapy COI and COC tracking is the use of

FIGURE 6

Priority #1: Patient safety with COI, COC.



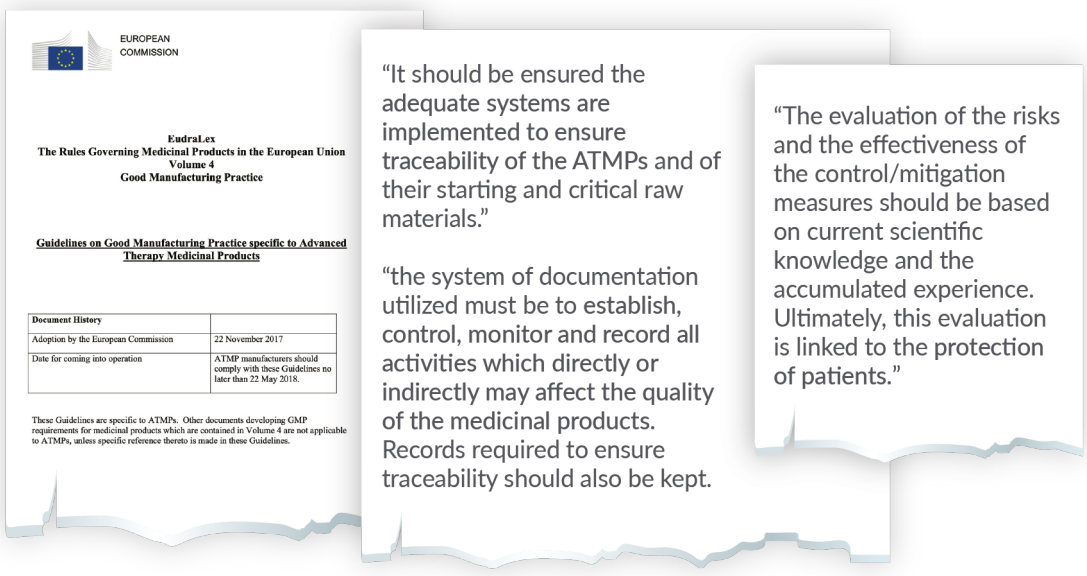
The numerous data points and hand-offs in the product journey make managing COI and COC a complex task. Digital tracking reduces risk and more easily meets compliance requirements.

Expanding traditional Quality Management Systems (QMS) for the advanced therapy supply chain.

DOI: 10.18609/cgti.2020.128

FIGURE 7

Regulatory guidance from the European Medicines Agency, 2017.



Guidelines related to Good Manufacturing Practice (GMP) specific to advanced therapies [18].

a digital system. The numerous touch points and physical locations in the product journey make manual tracking time-consuming and error-prone. It is too risky to leave patient safety to chance in this way. Additionally, routine compliance becomes onerous, and product approval may be a challenge. Regulatory reviewers want an application to demonstrate that traceability is well in hand (Figure 7) [1,18]. From an operational standpoint, the cost and resource utilization to maintain manual traceability is not efficient or scalable beyond a small number of patients or with a simple supply chain process.

Multi-language capabilities

The complex, distributed ecosystem of advanced therapies often means supply chain partners and patients are located in many geographic regions, both within the United States and across the globe. The need for starting material and drug product to cross borders, or to be produced and delivered locally in a region different from that of the biopharma company, presents additional labeling challenges. Labels must be in compliance with local, national and international requirements, depending on the situation, and must also be relevant and useful to those handling and processing the material or product. One important aspect of this is the ability to generate raw material, in-process, and final product labels in multiple languages.

Ensuring patient safety means that personnel at local care sites and other supply chain partners must be able to easily and definitively read key pieces of information on labels, meaning the information must be presented in the appropriate language(s). This is challenging due to the limited physical space available on many labels, the sheer number of possible languages and language formats a company may need to support (left to right, right to left, top to bottom), and the variability in translations. Working with experienced partners and regulators on label design, formatting, and printing helps ensure that the essential information is included, presented in only the correct languages required for that

geography, and that the physical space on the label is used efficiently.

Solutions such as Vineti's Personalized Therapy Management Platform (PTM; Vineti solution) [19] come equipped and ready to support multi-language labels (including character based languages), providing the ability to deploy and maintain standardized, compliant labeling across multiple geographies for local use. Additionally, establishing or procuring approved and standardized phrase libraries ensures accurate, consistent translations every time and cuts significant time out of the process for creating, reviewing, and revising labels. The benefits of proactively tackling multi-geography labeling challenges pay off early, even if there are only a few regions involved. As clinical trials progress and expand, and a therapy moves toward commercialization, it can be difficult to scale one-off or manual processes. Having established multilingual capabilities early on to scale up and out across geographies will save time and money getting therapies approved and to the patients who need them most.

On-demand label printing (vs print & ship)

One of the decisions that advanced therapy manufacturers face is whether to print labels in advance and ship them to the partner sites, or establish the capabilities for partner sites to print labels on-demand, prior to, or during, processing. This seemingly small decision has significant factors to consider, and is a major consideration as therapy delivery scales. For many reasons, on-demand label printing is the recommended and most compliant option.

Most importantly, patient safety is easier to ensure with on-demand label printing. Patient ID verification is linked to label printing and the patient is paired with their labels from the outset. On-demand is also much simpler to manage from a compliance and supply chain standpoint. Label reconciliation is a key cGMP compliance activity to prevent product mislabeling (or product mix-ups) [20]. Every label must be accounted for, and pre-printed labels create additional touch points and opportunities for labels to be lost and unverified. Operationally, pre-printed labels create a whole new 'supply chain' that must be managed, requiring additional and costly resources, and putting additional and unnecessary pressure on an already time sensitive process.

Printing to existing printers

There have been different approaches and challenges related to label printers as well. One option was to deploy dedicated printers for each therapy to partner sites. This may be problematic in some cases, because additional complexity and cost is unnecessarily introduced into the supply chain, there are additional security considerations, and scalability is hampered. Ecosystem partners such as HCPs and clinical sites may have limited space for additional hardware, and deploying dedicated printers to individual partner sites involves installation, training, logistics, and maintenance requirements that are time-consuming, personnel intensive, and expensive.

A more sustainable and scalable model is to utilize existing printers at partner sites for label printing. This is time and money saving

FIGURE 8

FDA's mission.

"The Food and Drug Administration is responsible for protecting the public health by assuring the safety, efficacy and security of human and vetinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation."

The FDA's mission extends to essential safety components of drug products, including labeling [21].

DOI: 10.18609/cgti.2020.128

for both the partner and the drug developer and simplifies one aspect of a complicated process. It is also more secure to utilize hardware already in operation within a site's IT system, and modern cloud-based solutions provide enhanced security and remote management. This ability is a feature of Vineti's Personalized Therapy Management platform (PTM), which provides a turnkey solution for label printing (for more information on PTM, please see [19]).

Collaboration with regulatory agencies

The FDA's mission is "…protecting the public health by assuring the safety, efficacy and security of human and veterinary drugs, biological products, medical devices…" (Figure 8) [21].

One important way the agency carries out this mission is through the requiring and approving of in-process and final product labeling. It is worth mentioning again that label issues are cited by FDA regulators as a major reason for rejecting or questioning New Drug Applications (NDA) and Biologics License Applications (BLA).

Frequent collaboration with regulatory agencies cannot be stressed enough. This helps ensure filing acceptance and avoids the many potential pitfalls for advanced therapy labels. Beginning with IND filing, regulatory guidance puts emphasis on the importance of labeling, further indicating that early focus and collaboration on labeling is smart [1]. If left until late in the process, labeling processes and details may present an unexpectedly time-intensive, complex issue to solve. This holds in the United States, and is also important globally, where there may be multiple agencies involved and differences from region to region. Each drug product will have something unique that will need to be addressed specifically by regulators. It is better to ask questions up front, rather than to wait until the application is filed. Early conversations and feedback during product development can eliminate clinical holds and costly, timeline-breaking re-work – in addition to delays in getting treatment to critically ill patients.

TRANSLATIONAL INSIGHTS: STANDARDS FOR SCALE

As advanced therapies grow in number and reach, sponsors and stakeholders are developing standards to enable a patient-centric drug product ecosystem. The Standards Coordinating Body (SCB) is conducting FDA-funded work to carry out standards directives in the 21st Century Cures Act, Section 3036. As part of its work on advanced therapy standards, the SCB, in partnership with ICCBBA, is building on the existing labeling standards for cell collection products and modifying them to accommodate the current state – for autologous and allogeneic products – to help prepare the industry for future success [22].

This SCB industry working group on labeling is composed of a variety of ecosystem stakeholders and subject matter experts who can provide the perspective and experience needed to develop suitable standards [23]. Some of the standards being evaluated in relation to apheresis collection labels include a working common definition of COI and COC, label content, layout, required data, and data formats. Timelines for gathering final input and publishing the updated standards are being determined in collaboration with ICCBBA. To see the draft standards document, please visit the ICCBBA website [24]. To learn more about this important effort and get involved, please contact SCB [25].

Looking to the future, such standards will become more important than ever. The number of advanced therapies in the R&D pipeline is increasing, with a goal of making more therapies safer and capable of being delivered on an out-patient basis. Scaling will involve expansion more broadly into community-based settings. This accessibility across all

types of medical centers is critical for patients. Therefore, supporting the ability for smaller, more distributed clinical sites to collect starting material, deliver treatments, and manage patient-specific labels is required for greater access. Simple, flexible, compliant label printing is one important piece of this model. By following proven practices and working together to develop and implement standards, advanced therapy sponsors and stakeholders will take a critical step towards a future of greater advanced therapy access for all.

HEIDI HAGEN has been an Operations Executive in the Biotechnology industry for 30 years and is a Co-founder of Vineti. She has overseen the delivery of more than 100,000 doses of personalized cell therapy and has guided numerous therapies through clinical development on to commercialization. She has an extensive and proven track record in leading operations and commercializing innovative technologies ranging from recombinant protein/device combinations to the first active immune cell therapy, Provenge®. Heidi is on the Board of Directors for Vericel Inc., Ziopharm Inc., and Lykan Biosciences. Previously, Heidi was the Global COO for SOTIO, in Prague, Czech Republic with a US office in Boston, MA. Before joining SOTIO, she worked for Dendreon for ten years as Senior Vice President of Operations, and 10 years with Immunex Corporation in a range of roles in drug development and operations management. Heidi has a BS in Cell and Molecular Biology, an MS in Bioengineering, and an MBA from the University of Washington.

CHRISTOPHE SUCHET brings more than 20 years of information technology and senior biopharmaceutical IT experience to Vineti. Most recently, he served as Vice President of IT for Kite Pharma, Inc., where he developed the essential technology systems that helped Kite's first cell therapy receive FDA approval, scale rapidly, and secure the landmark sale of the business to Gilead Sciences, Inc. Prior to Kite Pharma, Christophe served as Vice President of IT at Pharmacyclics Inc., a clinical stage and commercial biopharmaceutical company that grew in two years from no product revenue, to $1 billion revenue, to acquisition by AbbVie for $21 billion. Christophe has served as Genentech's Director, IT Pharma Development Applications, and as IT Director, SAP Center of Excellence & Enterprise Applications. He received his MS in Biology and Economy from AgroParisTech.

REFERENCES

1. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs): Guidance for Industry. Silver Spring, MD. The FDA. January 2020; 4,16–17: https://www.fda.gov/media/113760/download
2. Alliance for Regenerative Medicine. ARM Annual Report & Sector Year

DOI: 10.18609/cgti.2020.128

in Review: 2019. Washington, D.C. Alliance for Regenerative Medicine. 2020; 30: https://alliancerm.org/publication/2019-annual-report/

3. International Council for Commonality in Blood Banking Automation (ICCBBA) ©2020: https://www.iccbba.org/isbt-128-basics/get-to-know-isbt-128

4. International Council for Commonality in Blood Banking Automation (ICCBBA). Introductory Booklets ©2020: https://www.iccbba.org/isbt-128-basics/introductory-booklets

5. International Council for Commonality in Blood Banking Automation (ICCBBA). ISBT 128 Standard: Labeling of Cellular Therapy Products: Version 1.2.0. San Bernardino, CA. November 2018: https://www.iccbba.org/uploads/6c/db/6cdba776ce2ba38a0c21776f-2b7ee95b/ST-004-ISBT-128-Standard-Labeling-of-Cellular-Therapy-Products-v1.2.0.pdf

6. European Commission. Overview, standards documents and implementation guides. © European Union, 1995-2020: https://ec.europa.eu/health/blood_tissues_organs/tissues/single_european_code_en#:~:text=The%20%22Single%20European%20Code%E2%80%9D%20or,type%20of%20tissue%20or%20cells.

7. US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research, Office of New Drugs (OND). Manual of Policies and Procedures, Good Review Practice: Refuse to File. Silver Spring, MD. The FDA. September 5, 2018; 15, 17: https://www.fda.gov/media/87035/download

8. Current Good Manufacturing Practice for Finished Pharmaceuticals: Packaging and Label Control, 21 C.F.R. Part 211 Subparts G and J (2019): https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211&showFR=1&subpartNode=21:4.0.1.1.11.7https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211&showFR=1&subpartNode=21:4.0.1.1.11.10

9. Drugs: General – Labeling, 21 C.F.R. Subpart A Sect. 201.2 (2019): https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=201.2

10. General Biological Products Standards – Labeling Standards, 21 C.F.R. Part 610, Subpart G (2019): https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=610&showFR=1&subpartNode=21:7.0.1.1.5.7

11. Drugs: General – Labeling, 21 C.F.R. Part 201 Subpart B Sect. 201.5 (2019): https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=201&showFR=1&subpartNode=21:4.0.1.1.2.2

12. Regulations Under Certain Other Acts Administered by the Food and Drug Administration – Human Cells, Tissues, and Cellular and Tissue-based Products, 21 C.F.R. Part 1271.9 (2019): https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=1271.90

13. Standards Coordinating Body (SCB). Copyright © 2020: https://www.standardscoordinatingbody.org/

14. Provenge® product label: https://www.drugs.com/pro/provenge.html

15. Yescarta® product label: https://www.accessdata.fda.gov/spl/data/783b1236-8e53-41a7-922a-08bdfb7517ef/783b1236-8e53-41a7-922a-08bdfb7517ef.xml

16. Kymriah® product label: https://www.accessdata.fda.gov/spl/data/32b00de7-ce25-48db-abce-cfe0d65dbcc0/32b00de7-ce25-48db-abce-cfe0d65dbcc0.xml

17. Zynteglo® product label: https://www.ema.europa.eu/en/documents/product-information/zynteglo-epar-product-information_en.pdf

18. European Commission, EudraLex. The Rules Governing Medicinal Products in the European Union, Volume 4, Good Manufacturing Practice: Guidelines on Good Manufacturing Practice Specific to Advanced Therapy Medicinal Products. London, UK. The European Commission. November 22, 2017; 8, 10, 30: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf

19. Vineti's Personalized Therapy Management Platform: https://vineti.com/solution/features

20. Current Good Manufacturing Practice for Finished Pharmaceuticals: Packaging and Label Control – Labeling Issuance, 21 C.F.R. Part 211 Subpart G Sect. 211.125 (2019).

21. Food and Drug Administration. FDA Mission. Contents not copyrighted: https://www.fda.gov/about-fda/what-we-do#mission

22. Food and Drug Administration. Standards Development for Regenerative Medicine Therapies, Section 3036 of the 21st Century Cures Act. Contents not copyrighted: https://www.fda.gov/vaccines-blood-biologics/standards-development-regenerative-medicine-therapies https://www.fda.gov/regulatory-information/selected-amendments-fdc-act/21st-century-cures-act

23. Standards Coordinating Body (SCB). Project: Base Labeling Requirements for Apheresis Products for Regenerative Medicine Manufacturing. Copyright © 2020: https://www.standardscoordinatingbody.org/project-base-labeling-apheresis-products

24. ICCBBA, Documents for Review: https://www.iccbba.org/tech-library/iccbba-documents/documents-for-review

25. SCB, Base Labeling Requirements for Apheresis Products for Regenerative Medicine Manufacturing: https://www.standardscoordinatingbody.org/project-base-labeling-apheresis-products

AFFILIATIONS

Heidi Hagen
Co-founder and Advisor, Vineti, Inc., 633 Howard Street, San Francisco, CA, USA

Christophe Suchet
Author for correspondence:
Chief Product and Compliance Officer, Vineti, Inc., 633 Howard Street, San Francisco, CA, USA
info@vineti.com

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Revised manuscript received: *Sep 10 2020;* ***Publication date:*** *Sep 21 2020.*

Vineti

DOI: 10.18609/cgti.2020.128

Vineti

Software solving medicine's most complex supply chain

Supporting **hundreds of leading medical centers** world-wide

Helps your advanced therapy **reach more patients, faster**

Essential to successful personalized therapy workflows

Built in the cloud with unlimited scale, geographically and therapeutically

The **first** independent orchestration platform supporting commercial U.S. and E.U. cell therapies

Standard-setting approach **proven with regulators**

info@vineti.com | **www.vineti.com**

FASTFACTS

TESSA technology: a new paradigm in AAV manufacturing

Cell & Gene Therapy Insights 2020; 6(8), 1225–1225

DOI: 10.18609/cgti.2020.134

The majority of adeno-associated viral (AAV) vector produced globally is manufactured using triple transfection. But plasmid-based AAV manufacture has drawbacks – the need to transfect cells results in a process which cannot be truly scalable, and yield and particle infectivity could be improved upon. Tetracycline Enabled Self Silencing Adenovirus (TESSA) technology utilizes adenovirus to manufacture AAV in order to achieve reproducible AAV yields at scale with considerable cost savings. Additionally, increased vector quality and infectivity has the potential to deliver safer gene therapies at a lower effective dose.

ROBUST AND REPRODUCIBLE YIELDS

In nature, the AAV produced by adenovirus is more infectious, with considerably higher yields, than what is produced via plasmid-based manufacture. By harnessing adenovirus-based production of AAV and solving the issue of contamination, TESSA technology increases AAV2, AAV5, AAV6 and AAV9 yields by >30-fold (Figure 1). For AAV2, particle infectivity is increased by >2,420-fold (Figure 2) and full:empty ratio is increased from 5 to 70%. Adenoviral contamination levels are reduced by 99.99999–100%.

REDUCED COST

TESSA technology represents a highly scalable platform for AAV manufacture which requires relatively small amounts of virus to operate, and can also be used with existing AAV and a single TESSA vector in combination with one TESSA vector. Therefore, moving away from plasmid-based manufacture leads to reduced cost of goods (COG).

Figure 1

AAV2 genome yield using TESSA vectors is increased 42-fold versus a helper-free approach.

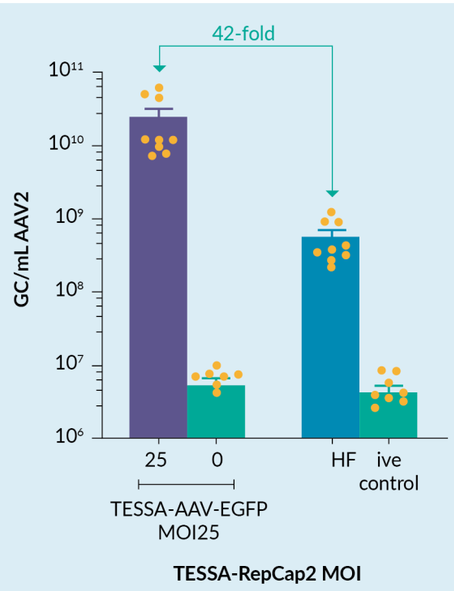
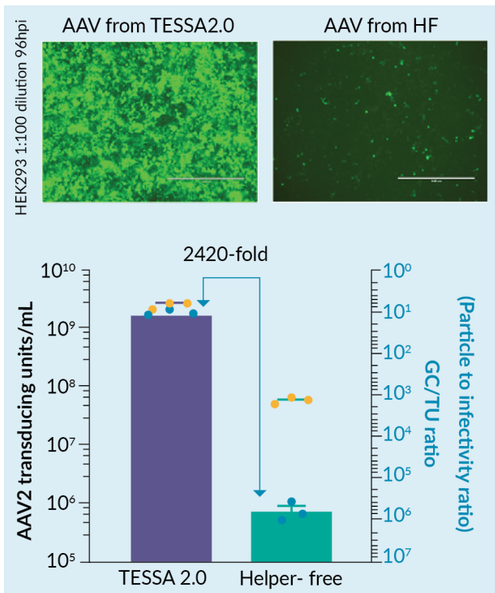


Figure 2

A 2420-fold cumulative increase in AAV2 infectious yield is seen using TESSA vectors versus a helper-free approach.



SAFE AND EFFECTIVE GENE THERAPY

The large increase in AAV2 infectivity has important safety implications. 1 in 6 particles containing a genome are infectious when using TESSA technology, compared to just 1 in 1,200 when using plasmid-based manufacture. As AAV particles manufactured using TESSA technology are more potent, it is possible that this will lower the effective dose of AAV-based gene therapies. This represents a considerable safety advantage and demonstrates that TESSA technology has the potential to produce safer and more effective gene therapies.

OXGENE™

CELL & GENE THERAPY INSIGHTS

SCALING UP/OUT: COST-EFFECTIVE & ROBUST TRANSITIONIONING THROUGH THE CLINIC TO COMMERCIAL MANUFACTURE

SPOTLIGHT

INNOVATOR INSIGHT

Scalable and efficient AAV production process with new Fibro chromatography technology

Laura Adamson-Small, Mats Lundgren & Peter Guterstam

Production methods for adenovirus-associated virus (AAV) vectors have not kept up with the brisk pace of gene therapy development. To manufacture safe and efficacious clinical-grade virus, scalable and cost-effective production processes are needed. Towards this end, we present an efficient process for AAV production and scale-up in suspension cell culture through to purified bulk product. The process was developed by evaluating and optimizing each process step. A novel fiber technology, Fibro, addresses the downstream bottleneck at the capture step by overcoming the diffusional and flow limitations of purification using packed-bed chromatography. Also, a new analytical assay based on surface plasmon resonance was developed for AAV quantitation.

Cell & Gene Therapy Insights 2020; 6(8), 1249–1262

DOI: 10.18609/cgti.2020.138

INTRODUCTION: CHALLENGES IN IMPROVING AAV PRODUCTIVITY & SCALABILITY

The utilization of AAV as a gene therapy vector has increased due to its relatively limited immunotoxicity and wide range of tissue tropism. To date, multiple AAV serotypes targeting different organs including brain, eye, lung liver, skeletal muscle, and heart have been discovered and characterized. Capsid proteins

have been further modified to increase transduction, targeting specificity, and efficacy *in vivo* by methods that include:

1. Directed evolution, which incorporates components of multiple AAV serotypes into the capsid;
2. Random shuffling of capsid sequences to generate new novel capsids; and
3. Adding aptamers to the surface.

AAV vectors are produced through the introduction of helper virus and AAV replication components into the production cell line. When these helper virus sequences are stressed, they trigger expression of both cellular factors critical in AAV production and activate the four different AAV Rep proteins (48, 52, 68, 78). These proteins are critical in AAV production, assisting in multiple functions including limiting replication of the packaging cell, expressing viral capsid proteins, and increasing production of the Cis DNA sequences. The replicated DNA sequences are then packaged into the AAV capsid and harvested from the cells and/or supernatant through the purification process. The ratios of these different viral and cellular helper proteins, as well as the AAV2 replication protein, help to dictate the overall number of particles packaged, the number of particles that contain DNA, and often the integrity or completeness of the genome that's packaged within these capsids.

Currently, multiple methods are utilized to deliver each of these helper components and replication and capsid sequences into the cell. The most frequently used is plasmid transfection, where 2 to 3 different plasmids and DNA sequences are transfected into the cell. In some production systems, these sequences are packaged into recombinant viruses such as herpes, adenovirus, or baculovirus, which then transduce the production cell to initiate the production cascade. In addition, there are multiple cell types that are utilized, including HEK293, BHK, HeLa and insect Sf9 cells. Furthermore, both adherent and suspension platforms are frequently employed in this production process.

While research has continued to improve overall recombinant AAV production, the titers that are reported from the above systems are generally observed to be 5 to 10 times lower in productivity per cell than wild-type AAV, indicating that there are still learnings to be gleaned from the wild-type virus to help drive improvements in recombinant production systems.

Multiple strategies have been employed to improve both AAV production and product quality. Many of these begin with modifying the replication helper or capsid sequences.

One of the first modifications typically introduced to upstream bioprocesses is modifying the ratios of the plasmids or vector components that are going into the cell, with the goal of identifying the ideal amount needed for each specific serotype or Cis sequence to increase production of full capsids. Another strategy employed to improve control of the production cascade is modifying the amount of the replication proteins that are present, either through modifying the start codons, or using alternative constitutive or inducible promoters to better control which of those Rep proteins are expressed, and the timing of their expression within the cell. Furthermore, research focused on codon optimization of both the helper and AAV replication sequences is ongoing. Again, all of these strategies are working towards the end goal of increasing DNA replication within the production cell, improving packaging (or the number of full capsids that are produced in a system), and increasing the viral particle (VP) or capsid protein ratio in order to create a product that is more infectious.

Additional recent research has focused on modulating gene expression within the cell line to create a more favorable environment for AAV production. These studies typically involve evaluation of a panel of genes that are upregulated and downregulated before that gene regulation is correlated with improved AAV production and/or product quality. While there has been a wide range of genes

DOI: 10.18609/cgti.2020.138

reported to be associated with improved AAV production, a number that have garnered particular attention today have been linked to either membrane channel proteins, tumor suppression or regulation, controller transport at the nuclear envelope, or overall DNA replication. However, many of these studies have typically focused on improving just one production cell type or production system, without screening for applicability across multiple platforms.

The approaches described above are mainly aimed at creating a cellular environment and optimal viral gene expression that is more amenable to AAV production, and are typically employed at the raw material improvement stage by modifying either the plasmids or vector starting material in the initial cell banks. In AAV production, these materials are produced and characterized in a GMP-like environment. Following cell expansion with the producer cells that are optimal for AAV production, the next steps involve delivering these packaging components through either plasmid transfection or recombinant viral transduction into each cell. At this point, bioreactors can be further optimized, as can cell culture conditions (including the number of cells in culture media) to further improve AAV production. Depending upon the specific harvest strategy, virus is collected 3 to 7 days after initiating the production cascade. One of the remaining challenges in this field is ensuring the helper replication capsid and Cis sequences are introduced to each cell in an efficient and reproducible manner at the intended production scale.

Currently, the most commonly utilized method for AAV production is plasmid transfection due to its speed in initial material generation and relative flexibility in incorporating the sequence changes previously described. In transfection, DNA is mixed with a chemical that condenses it and creates a positively charged complex that can be endocytosed by the cell membrane. The overall amount of DNA, transfection reagent, and diluent components significantly impacts the quality of transfection complexes. These complexes grow over time as the mixture is incubated, eventually reaching a size where the complex is no longer easily taken up by the target cell. For these reasons, transfection is often cited as a difficult strategy to scale due to its relatively limited reaction time compared to the transfer rate into the production vessel.

Gravity draining of complexes has proven to be suboptimal at larger production scales due to the overall volumes required, as well as the time it takes to drain into the production bioreactor. Meanwhile, other groups have explored pumping a transfection mist, but this has not been associated with increased productivity due to potential damage to the transfection complex during pumping. Additionally, certain media components in the production vessel that are present at the time of transfection can decrease the efficiency of complex fusion and uptake by the target cell. However, despite these challenges, multiple groups have reported successful production using transfection-based methods at scales from 500 to 2,000 liters.

Besides the limited operating window and complex stability, the amount of plasmid required for AAV production has been cited as a limiting factor in the long-term feasibility of this method for delivering packaging sequences into production cells. While plasmid amounts are only in the 20 to 40 mg range in a 10-liter production, the 500 to 2,000 liter scales required for commercial products require grams of plasmid per production run. Additionally, variability can be observed in these methods due to the inherent difficulty in introducing equimolar amounts of the 2 to 3 plasmids used to the packaging cell line, which can then create a heterogeneous replication cascade across the entire production culture. Together, these issues can create limiting factors relating to both scalability and reproducibility of a transfection platform at scale.

An alternative method to transfection, helper viruses are utilized to deliver the replication capsid or Cis sequence into cells. Advantages commonly cited for these systems include the requirement for smaller amounts

of helper virus compared to plasmids, improved stability of the helper viruses, and the fact that when they are placed into culture they demonstrate an improved ability to dispense through the entire production vessel and reach all of the cells. While helper viruses do potentially provide a more scalable method for delivering packaging components to cells, some production platforms still rely on the delivery of 2 to 3 recombinant helper viruses to each cell. This may result in a challenge similar to that faced by transfection: namely, difficulty in ensuring each cell receives an equimolar ratio expression of production sequences. Some baculovirus-based systems have utilized helper virus spread, where they infect with a low MOI (multiplicity of infection) and allow replication through the culture, further increasing the possibility of these systems being even more scalable. However, there are more stringent requirements for viral clearance studies, which are expected to evaluate the efficiency of the purification process in removing the input helper virus.

Finally, there is currently a strong interest in creating a true packaging cell line similar to those utilized in the protein therapeutics field.

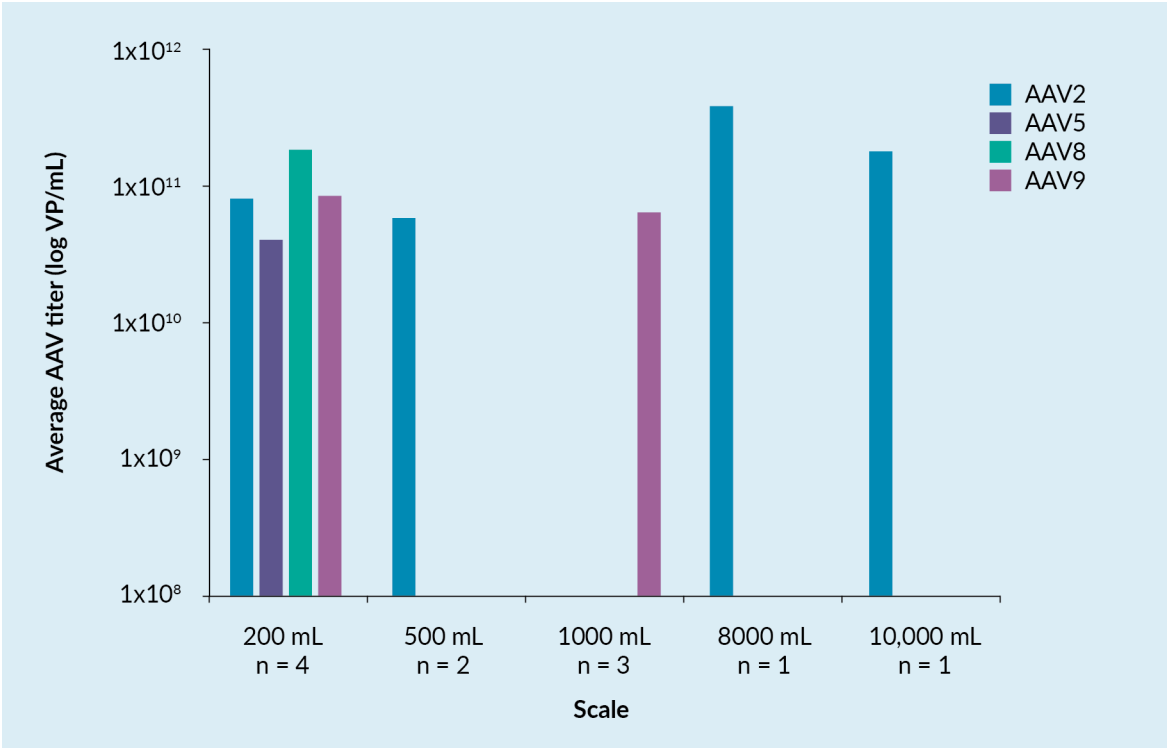
To date, AAV vector developers have utilized various combinations of Cis, Rep, Cap, and/or helper sequences in the production cell. Integration of these components is particularly challenging for AAV production due to the need to carefully control the interaction of those genes involved in the production cascade, which places the onus on ensuring an optimal amount of each Rep protein as well as the adenovirus or other virus-based helper genes.

Multiple induction systems have been tested as a means of modifying which components are expressed and how much – an important step in both improving regulation of cytotoxicity that can come from the adenovirus replication gene, and ensuring an appropriate ratio of gene expression is gained during production.

Further challenges in developing a packaging cell line have been reported due to

FIGURE 1

AAV productivity in different scales.



DOI: 10.18609/cgti.2020.138

potential instability of the integrated ITR (inverted terminal repeats) due to its secondary structure. This instability can occur during cell expansion, during the banking process, or in cells that are expanded for production. In addition, some of the initial studies related to packaging cell lines that have been developed have reported a lower percentage of full capsids, or higher levels of genome truncation or package host cell DNA in the system. Despite these limitations, though, there are multiple groups utilizing this strategy, and considerable effort is being put into improving the platform to create a true packaging cell line in the AAV field.

Finally, it should be emphasized that careful evaluation of product quality should be paired with any bid to improve the upstream bioprocess. Modifications to the helper virus or production sequences have direct implications for overall productivity, but they also control the quality of the packaged genome or therapeutic sequence in terms of the amount of packaged impurity (e.g. host cell DNA, plasmid, or vector DNA sequences) in the infectivity of the protocol. By improving understanding of the virus biology and the production cascade involving helper, AAV, and cellular proteins, this interplay may be utilized to drive AAV systems closer to the cellular production capacity we currently see with wild-type AAV viruses.

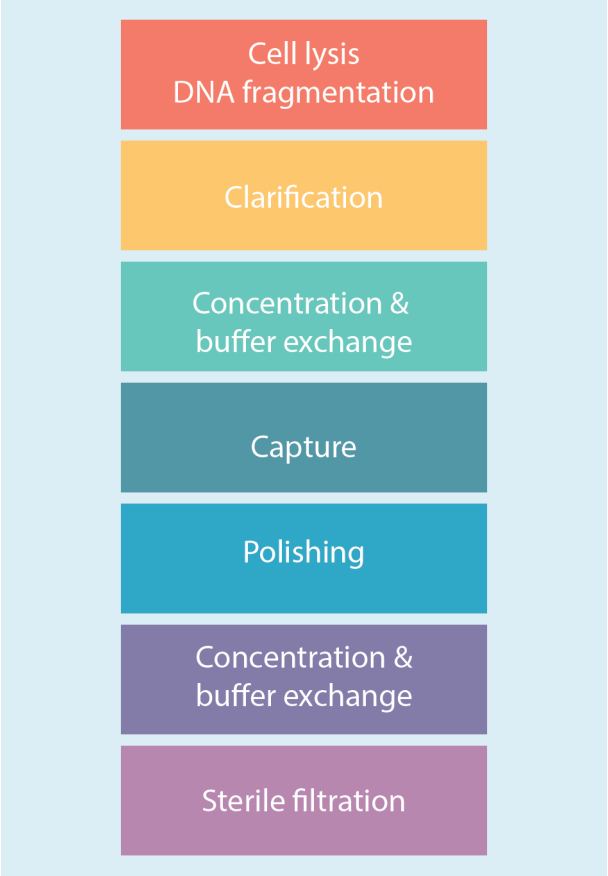
OPTIMIZING SCALABLE AAV PRODUCTION: A CASE STUDY

Upstream bioprocess

Cytiva recently developed a scalable upstream AAV bioprocess based upon triple plasmid transfection into HEK293T suspension cells. AAV2-GFP (containing a green fluorescent protein marker to aid in monitoring propagation) was the initial serotype selected.

The upstream process starts with the working cell bank, which is thawed before expansion in a number of shake flask steps. This is followed by virus production, which is carried out in the Xcellerex™ XDR-10 stirred tank single-use bioreactor, scalable to 2,000 liters.

FIGURE 2



Optimization of the cell line involved adapting the HEK293T cell line to suspension culture. (It may be noted that parallel development of a HEK293 cell-based process is underway, in light of recent debate around the large T antigen and its possible regulatory implications). A chemically defined cell culture medium was used to avoid the regulatory complications associated with animal-derived components such as serum. Next, the cell density and expansion procedure was optimized before creation of the cell bank.

Design of Experiments (DoE) approaches were utilized in optimizing the transfection procedure. A number of different parameters were explored, including cell density, volume of transfection, various different plasmids and the concentrations and ratios between them, PEI (polyethylenimine)/plasmid ratio, incubation time prior to entering the bioreactor,

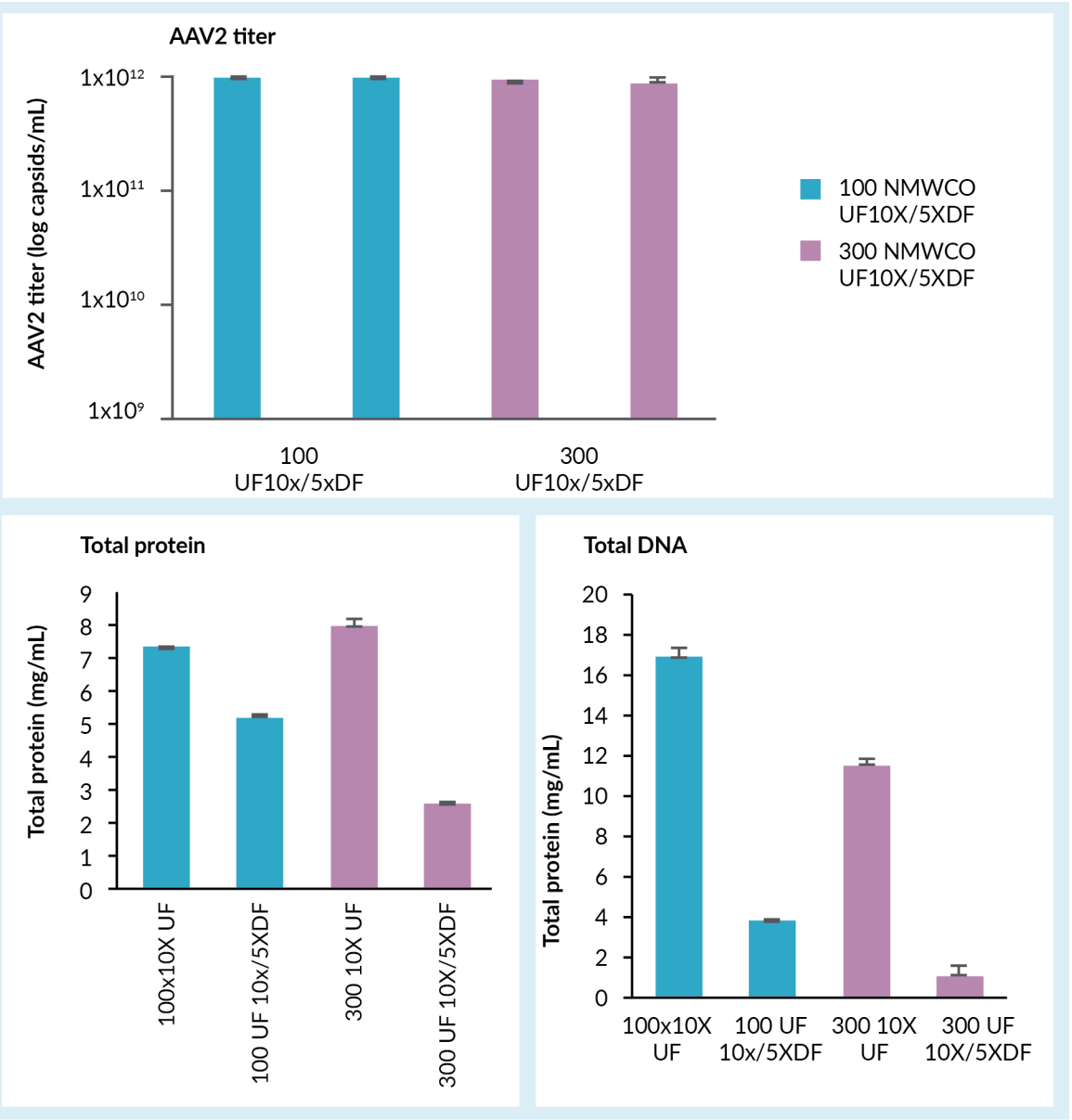
temperature, different supplements (including sodium butyrate), and time of harvest post-transfection.

Figure 1 demonstrates results at different scales with the various AAV serotypes investigated to date: AAV 2, 5, 8, and 9. At small scale (in 20 mL shake flasks) relatively high productivity of approximately 1.0E+11 virus particles was observed. Subsequent runs at larger scales up to 10 liters demonstrated consistent productivity and as noted earlier, there have been previous successful examples of scale-up to considerably greater volumes in single-use stirred tank bioreactors. However, given the simplicity of this particular process, the yields may be considered encouraging.

Downstream bioprocess

Figure 2 outlines the downstream process that was developed. The primary consideration

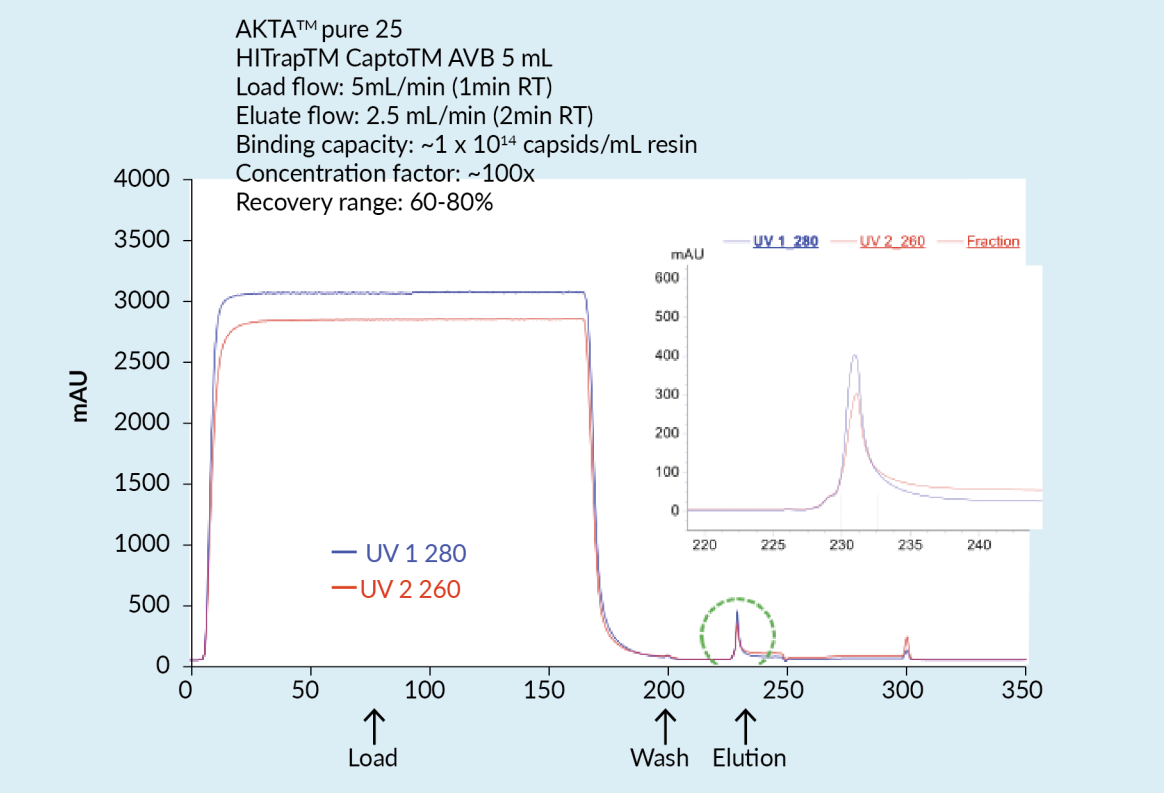
FIGURE 3



Top graph: no virus losses using 300 kDa NMWCO. Recovery was 75 to 80%. Bottom graphs: better impurity removal using 300 kDa NMWCO.

DOI: 10.18609/cgti.2020.138

FIGURE 4



was scalability of the technologies used. Technologies that are difficult to scale (e.g. centrifugation, precipitation) were avoided.

Evaluation and optimization of the downstream purification steps naturally began at small scale and with a focus on the cell lysis and DNA fragmentation step. A combination of 0.5% Tween™ 20 (to lyse the cells) and 40 U/mL Denarase™ (to digest DNA) was employed. This step took place in the bioreactor at 37°C with a mixing time of 4 hours.

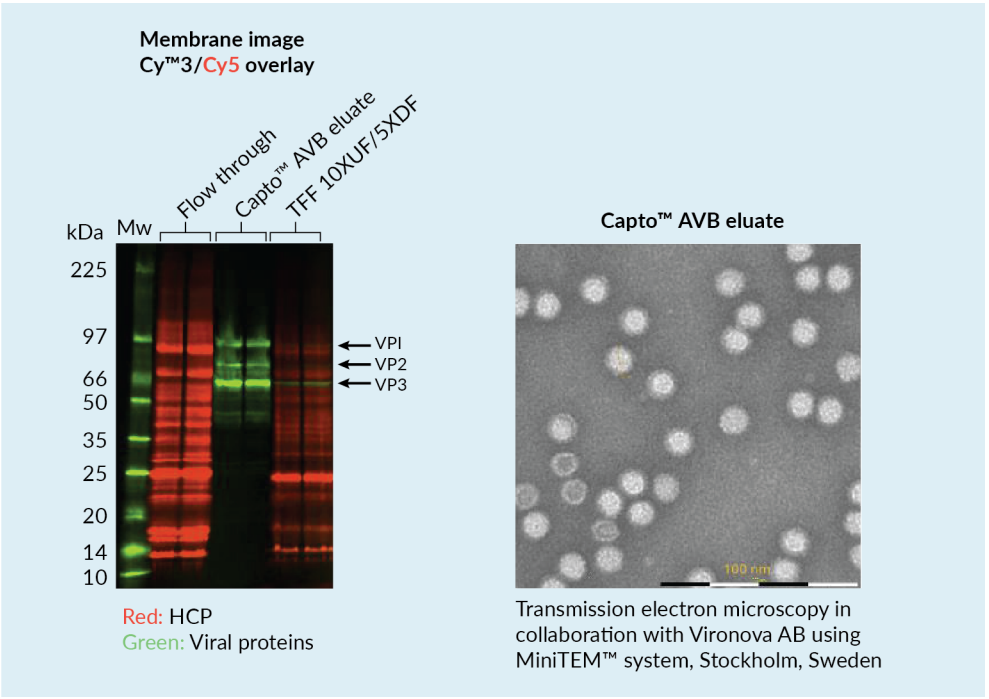
A normal flow filtration step followed using ULTA™ capsules with a range of different cutoffs (5 µm + 2 µm + 0.6/0.2 µm HC) and flow rates (30 to 50 LMH). Recovery from this step was approximately 75% to 80%, with the usual slight variability between runs observed and the inevitable loss of some virus (e.g. through it sticking to cell debris, etc.)

A concentration and buffer exchange step followed, which was done by tangential flow filtration using hollow fiber filters. Figure 3 demonstrates results with cutoff of the filter at both 100 kDa and 300 kDa. 100 kDa is frequently used in a bid to minimize virus loss – however, similar recoveries were observed here with both 100 kDa and 300 kDa, suggesting the potential benefit of being able to reduce some impurities using the larger pore size of the hollow fiber filter. Additionally, both total protein production and total DNA removal were improved with 300 kDa cutoff.

The capture step involved affinity chromatography utilizing Cytiva's Capto AVB affinity resin. Figure 4 shows the chromatogram. Material was loaded onto the column, followed by washing and elution. The small green circle is the elution peak – i.e. where the virus is leaving the column. Some of the conditions used are also included.

This proved to be a very efficient purification method with high impurity reduction achieved from a single chromatography step. Figure 5 shows a membrane image with host cell proteins in red and viral proteins in green. To the right ('TFF 10XUF/5XDF') is

FIGURE 5



the material loaded onto the column, which came from the hollow fiber filtration step in the previous unit operation. Some faint green bands may be seen – it is possible to discern VP1, VP2, and VP3 – but there is of course still a lot of host cell protein in this material. However, in the eluate ('Capto AVB eluate') most of those host cell proteins have been removed, leaving a clean preparation of the AAV virus. 'Flow through' demonstrates what became of the removed host cell proteins. The accompanying electron microscopy image is of the eluate following this single affinity chromatography step utilizing Capto AVB, showing the remaining virus particles.

While efficiency is of course important, it is equally critical to build robustness into the process. With this in mind, ongoing development work is focusing on aspects such as empty-full capsid separation and polishing any remaining impurities. The particular strategy under development involves using high resolution anion exchange resins (for example, Capto™ Q ImpRes) although a number of other alternatives are currently being explored.

Analytics

Analytics are critical for bioprocess success, but they are also very time-consuming – an all-too-familiar issue for anyone who has worked in virus production.

Box 1 lists the analytical tools that were utilized to follow the above process, including a novel technology based on Biacore™ SPR (Surface Plasmon Resonance) technology, which was developed for determination of virus titer. Assays used included infectious titer and total virus titer assays and, of course, a number of assays for host cell impurities and vector characterization.

Challenges faced with these methods include the fact that some are lacking low enough limits of detection, especially when used with early-stage samples from the harvest and the NFF samples. Additionally, they can sometimes be affected by detergents or buffer components, their accuracy depends to a large extent on the sample impurity level, and there is a lot of assay variation.

In a bid to overcome some of these challenges and limitations, a new quantification assay for AAV2 was developed using the

DOI: 10.18609/cgti.2020.138

Biacore™ T200 instrument. This instrument carries a sensor chip to which is bound an anti-AAV2 antibody. The antibody is immobilized by amine coupling. As material flows over the chip, interaction between the AAV and the antibody may be detected. Figure 6 shows the calibration curve for this assay. Well over 60 samples have been run between these two calibration curves (it is difficult to see that there are in fact two curves in the figure, demonstrating the stability of this new assay). Figure 7 shows the comparison between the novel Biacore™ assay and ELISA on various process samples, further demonstrating its sensitivity and efficiency (it is also easier to run than ELISA). This technology is now being implemented for other AAV serotypes.

FIBRO CHROMATOGRAPHY IN AAV PROCESSING

Fibro is a novel, single-use chromatography tool with the potential to alleviate several of the current challenges in AAV downstream processing, including those related to speed and process efficiency. However, it may also positively impact other, more general manufacturing pain points, such as speed to market, scalability, and Cost of Goods.

The Fibro technology is mainly applicable to the capture step of AAV downstream bioprocessing. Currently, the capture step typically provides good recovery, although it can be improved, but it is also a relatively slow process step.

Fibro technology enables one to address both the capture and the prior concentration step.

Concentration typically has a recovery of approximately 80% and like capture, it is widely considered to be relatively slow and time-consuming. The reason for introducing this step is to concentrate the feed material and to minimize the loading time in the subsequent capture step. In some cases, a buffer exchange is also done in this concentration step, usually by TFF.

BOX 1

Virus infectious titer

- Transduction assay: flow cytometry

Virus titer

- Viral genomes: qPCR
- Viral capsids: ELISA, SPR (Biacore™ system)
- Full-empty ratio: qPCR/ELISA, analytic IEX, TEM

Host cell impurities

- Total protein: BCA assay
- Total DNA: Picogreen™ Assay
- HC DNA: qPCR
- HCP: ELISA

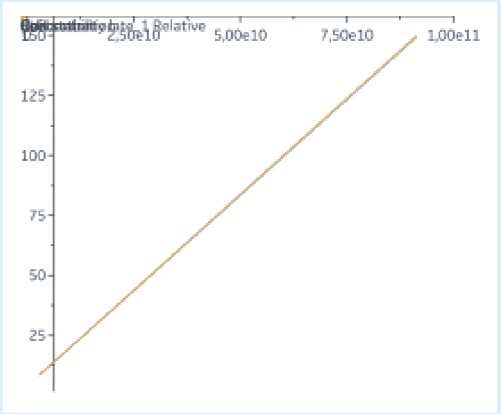
Characterization

- SDS-PAGE, Western blotting
- TEM
- SEC and IEX HPLC

Capture steps using affinity resins are usually associated with very long loading times. This is due to the fact that AAV titers are relatively low, and the flow properties of the resins mean that extended residence times are required – usually 1 to 3 minutes. Consequently, large volumes of feed material may take a great deal of time to load. Indeed, columns are sometimes oversized to minimize time spent on this loading phase. Recovery in the capture step is negatively impacted by this

FIGURE 6

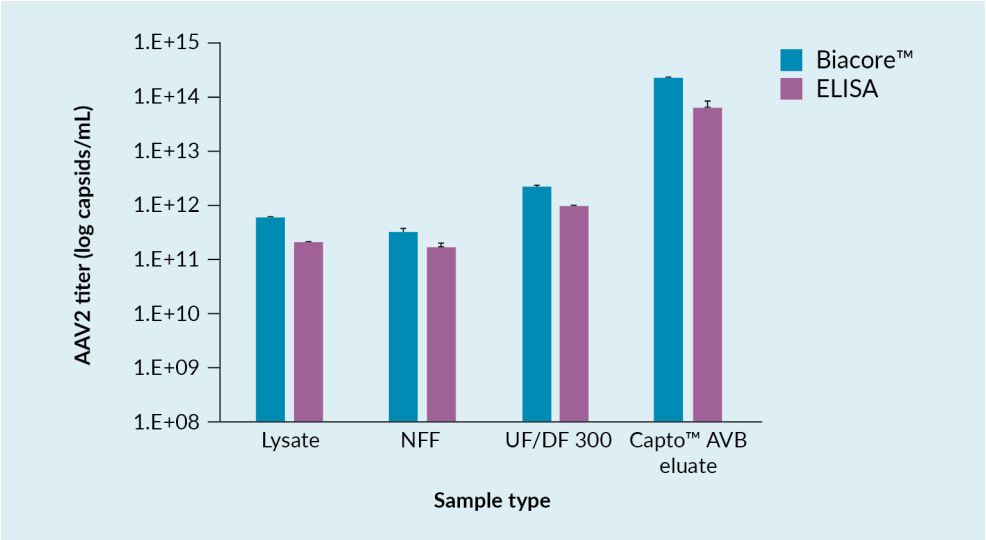
Calibration curve.



- ATCC reference standard
- Calibration range: 0.3 to 9.2 x 10^{10} vc/mL
- Between the first and second calibration curves, 66 samples were analyzed

FIGURE 7

Biacore™ and ELISA analysis of process samples.



long process time as is the low pH elution, which is usually used for affinity ligands.

Fibro is an electrospun cellulose material that has relatively large pore size, allowing the ligands that are immobilized on this format to be accessible directly, without any need for diffusion. Therefore, residence times of only a few seconds are needed.

There are alternatives available on the market – bead resin, for example. Bead resin has a much larger surface area, but the majority of this surface area is not accessible by the relatively large AAV. Other materials are not dependent on diffusion. However, these do not have the same surface area, and they have a different structure without the same, even pore size distribution that Fibro features. Fibro has a large surface area and also has a high binding capacity, which is key for AAV.

In the process scenario demonstrated in Figure 8, this non-diffusion dependent base matrix reduces the typical residence time of ≥1 minute for a classical affinity resin to 1.3 seconds – a loading rate that is 46 times higher, which results in significantly increased productivity. Capitalizing upon this short residence time, 1 liter of clarified harvest feed, which is not preconcentrated, can be loaded in a 400 mL unit and processed in 1 hour. This speed in turn makes TFF dispensable.

Table 1 shows different residence times and different flows. The capacity is dependent on the flow, but it will remain high even at a short residence time of 1 to 2 seconds, only starting to decrease at approximately 0.5 seconds. The right-hand column of the table shows the corresponding time to process 2,500 MV (membrane volumes), which further demonstrates the speed with which one can process clarified feed material even without a preconcentration step.

In order to provide context in terms of the quantity of Fibro material required to purify a large-scale run: 1 liter of feed material on a 400 microliter Fibro unit corresponds to 500 liters of feed material needing a few hundred milliliters of Fibro material.

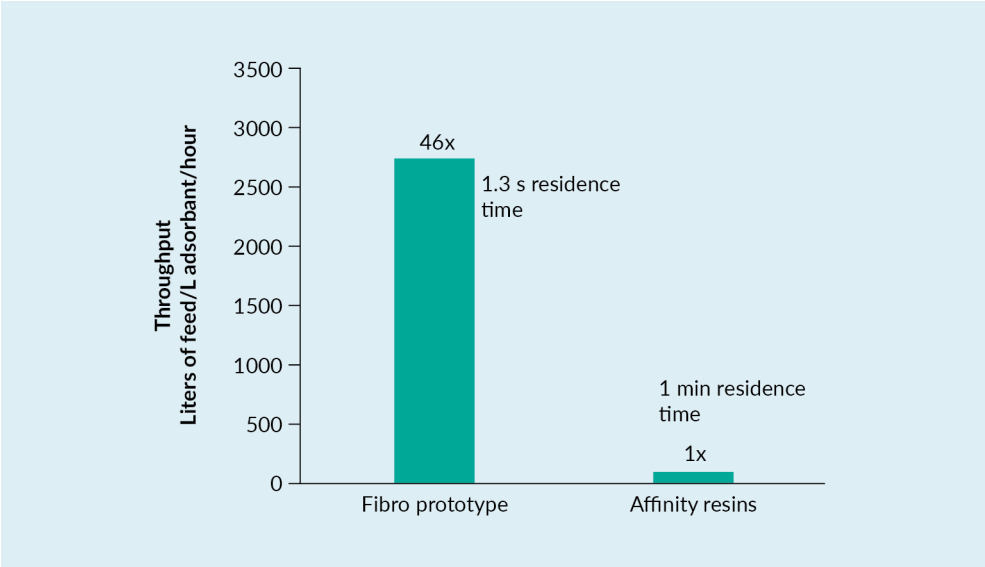
A number of different units are being developed that range in size from the lab-scale 400 microliter HiTrap™ unit up to the 2.4-liter Large Fibro unit. The Medium Fibro unit (160 mL) is capable of processing 500 liters of feed material in a single run. Each unit is compatible with corresponding hardware that is suitable to cope with the flows involved.

In summary, Fibro offers a number of advantages for the AAV capture step, including speed, capacity, recovery, and the efficiency and convenience of a single-use format.

DOI: 10.18609/cgti.2020.138

FIGURE 8

Process loading rate comparison.



The fast flow negates the requirement for a prior sample concentration step. Rapid cycle times positively impact maintenance of both virus integrity and infectivity. Rapid processing is also positive for the recovery, and by avoiding the need for a TFF step, any losses associated with that step are removed. Fibro units are prepacked, with a simple setup in the manufacturing facility, and offer short process development timelines due to the speed of every cycle.

Note that Fibro units for AAV are still under development.

AFFINITY VS MULTIMODAL LIGANDS FOR AAV CAPTURE

Affinity ligands are now well established on the market for most AAV serotypes. A key strength is their prowess at impurity removal, due to their high specificity in binding the target AAV.

Affinity ligands do have drawbacks. These include the challenging elution conditions with low pH – a particularly important consideration for runs with resins because of lengthy timeframes involved. Additionally, those affinity ligands currently available on the market do not discriminate between full and empty particles. The elution conditions usually raise the risk of the AAV sample aggregating and finally, protein-based affinity ligands have limited cleanability. However, efficient impurity removal with affinity ligands may in some cases be considered unnecessary because one may simply introduce a subsequent full/empty separation step, which will also remove many impurities.

Multimodal ligands are not as effective as affinity ligands at impurity removal, but they do hold the advantage of very mild elution conditions. There is an opportunity to enrich full AAV, depending on how one runs the elution and collects the elution peak. Multimodal ligands are acceptable with high

TABLE 1

Residence time (s)	Flow (MV/min)	Capacity (capsids/mL)	Approx. process time for 2500 MV
0.5	120	6.1×10^{13}	25 min
1	60	2.0×10^{14}	50 min
2	30	3.4×10^{14}	90 min

conductivity during binding, meaning that no buffer exchange is required prior to loading. They minimize the risk of aggregation. Being synthetic, they may also be cleaned under very harsh conditions. Finally, they have broad cell type coverage.

There also exists an opportunity to circumvent multimodal ligands' limited impurity removal capabilities, even if one wishes to avoid a subsequent full/empty separation step: residual HCP and DNA can be removed through the addition of a gentle flow through Capto™ Core 400, following a capture step with a multimodal ligand.

CONCLUSION

Significant improvements have been made to vector productivity and genome packaging by modifying both viral and cellular sequences to better support AAV production. However, with the large range of production systems still used to manufacture AAV, these improvements may need to be reoptimized with each platform to better control the production cascade. Further challenges remain in the scalability of these methods.

As these platforms continue to improve, early assessments of product quality will increase the field's understanding of the link between viral gene expression, replication, and improving AAV platforms.

The optimization of processes and the continued emergence of improved upstream and downstream steps, tools, and assays such as those described above, will play a key role in evolving traditional AAV virus production into fit-for-purpose commercial gene therapy manufacture.

AFFILIATIONS

Dr Laura Adamson-Small

Upstream and Analytical Development Lead, UCB Biosciences

Dr Mats Lundgren

Customer Applications Director, Cytiva

Dr Peter Guterstam

Product Manager, Next Generation Resins & Technologies, Cytiva

DOI: 10.18609/cgti.2020.138

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *This article is based on the transcript of a webinar held on the 30th July 2020, which can be viewed here.*

Publication date: *Oct 1 2020.*

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

SCALING UP/OUT: COST-EFFECTIVE & ROBUST TRANSITIONIONING THROUGH THE CLINIC TO COMMERCIAL MANUFACTURE

PODCAST INTERVIEW with:

Adrian Lee-Mohan, Senior Vice President, QuickSTAT & Quick Specialized Healthcare, and **David Murphy**, Executive Vice President, Life Science and Cell & Gene Commercialization, Quick Specialized Healthcare Logistics.

Delivering cell and gene therapy: evolving logistics considerations

Cell & Gene Therapy Insights 2020; 6(8), 1215–1224

DOI: 10.18609/cgti.2020.030

Q Adrian, this year you celebrated 30 years with Quick, meaning you have been ever-present during the emergence and development of the advanced therapies industry as we know it today – can you reflect upon that long journey, and share your key concerns as you look at the ATMP field today?

ALM: It has been a long and eventful journey, and I feel like I have had several completely different careers in that time. I wouldn't pretend that it has not been very hard work, but it has always been rewarding and varied. Just when you think you are close to knowing most things, along comes a whole new field of medicine, and the learning process starts over.

With regards to the ATMP field, at Quick we have been at the forefront alongside the customers, and I believe we have effectively learned together. My main concerns would probably relate to the establishment of protocols for commercial distribution by the sponsor based on clinical phase experiences, without the inclusion of the logistics provider.

What we have experienced is that what works on a small scale, clinical phase is not always practical, or possibly even necessary at a much larger commercial phase. Therefore, I think that clinical and commercial need to be aligned from the get-go, and the logistics provider should absolutely be involved at the earliest possible stage.

Q David, you spoke to Cell and Gene Therapy Insights back in the Fall of 2017 about the role of shipping and logistics in the commercialization of cord blood-based therapies – can you share your thoughts on how that particular field and its supply chain has evolved since then, and what issues it still faces on an ongoing basis?

DM: 2017 seems like a decade ago even though it has only been three years, because this industry, including cord blood therapies, has evolved so rapidly. The ongoing development of allogeneic therapies is exciting to observe, but there are important differences between autologous and allogeneic therapies that must be acknowledged and understood.

> "...clinical and commercial need to be aligned from the get-go, and the logistics provider should absolutely be involved at the earliest possible stage."

The technical details associated with autologous therapies are slightly more challenging, whereas allogeneic is more structured and scheduled. You can easily schedule collections at cord blood banks or blood centers throughout the country, or the world for that matter, traditionally sending these collections with high integrity to contract manufacturers throughout the world.

With autologous therapies, it is all based upon scheduling a patient, which can be a challenge. These are often very ill patients so

DOI: 10.18609/cgti.2020.030

there could be delays, or they could be too ill to even go through an apheresis process. To work with this level of potential variability we have worked very hard with the industry for many years. For example, back in 2010 and 2011 when the first cell therapies were approved, we were right there to support the developers and we continue to do so today.

Turning to COVID-19, we have seen interesting events happen in the last four months in terms of challenges. We have had to deal with a shrinking airline industry, a trend that we do not think is over, as we expect that the airlines are going to continue to right-size.

We have made incredible efforts to save the day in many cases for this industry. We have chartered a lot of airplanes and made a lot of long drives. It has really been interesting and through this crisis management, I think it has validated what Adrian and I have put together on a case-by-case basis to clients; it has validated our processes.

You never know what is going to happen next year. We can only continue to work hard with our clients, be creative, and find solutions.

Q Adrian, you are UK-based – what are your expectations currently in terms of the repercussions of BREXIT, and how can or will you prepare for them?

ALM: This has been the biggest question we have faced over the last four years, which has been preoccupying both our minds and the minds of our clients. It has been overshadowed since March, and COVID-19 has taken the number one spot. However, it is still very much an issue, and one that we still don't really know the answer to.

As we all know, until the end of 2020, there will be no change. But that deadline is now fast approaching, and we will not be in a position to accurately assess the potential repercussions until the final trade agreement between the EU and the UK has been established. We don't know how close to the 1st of January 2021 this will emerge.

Personally, I was hoping for considerable alignment in the medical field, and I was cautiously optimistic that any repercussions would be contained. But in recent months we have seen the two sides lay out their frameworks for negotiations, and they do seem to be at loggerheads. Certainly, in the rounds of talks that have been held so far, both parties have sounded fairly intransigent in their negotiation red lines. We will continue to hope for a last-minute agreement, but so far, we do not have any progress.

In the UK, Quick is an official customs broker. We clear all of our own inbound shipments at all of the key airports. We are electronically linked and connected to HM Revenue & Customs, so any import or export customs functions that are needed with effect from January 1st next year should be smooth and rapid.

Additionally, we have been increasing our staffing levels in this key area, so that we are able to respond to the additional pressures and potential changes that are coming.

Q What are the key components that you feel make for a good logistics provider for the advanced therapy sector?

> "The technical details associated with autologous therapies are slightly more challenging, whereas allogeneic is more structured and scheduled."

DM: Without getting overly technical, the reality is that experience matters. Between Adrian and I, we have nearly 63 years with the Quick Group.

We have been working in this area for 35 years, and that makes a big difference – we have acquired a great deal of experience working with organs, tissue, blood, and the pharmaceutical industry. In many ways, the requirements for cell therapies and organ transplant are very similar – the expectations, the chain of identity and custody, and the required temperature integrity are all very similar.

Tenure in our organization is incredible. It is very common to have groups of people in all our centers of excellence and control towers, that have 20 or more years of experience in special logistics throughout the medical community.

When you work so closely with these medical organizations, you must demonstrate honesty and integrity. You must be frank with them, particularly when you are dealing with risk-mitigating situations. The industry in general has changed, in a good way, in that the interaction between vendor and pharmaceutical or biotechnology company is now very open and honest. I am very encouraged about that, because whenever possible we should be on the front line with our clients.

Transportation is critical to the success of these therapies, and at the end of the day, we are all working for the same patients. Every patient deserves our most concentrated effort.

Q How do you evolve as an organization to meet the changing demands of this sector?

DM: One of the most important questions received from us by our clients is around scalability. We are involved and engaged with these organizations from a clinical perspective, and on into a commercial perspective, and it is important for the client to understand our scalable abilities.

On the clinical side, we are dealing with perhaps 15–30 patients as you go through the clinical stages. But of course, the goal is to get to a commercial position. Suddenly you could be talking about tens of thousands of patients, and each patient requires three shipments at a minimum, and potentially up to 10 or 11 shipments. You can understand the concern that clients may have in terms of our ability to scale to those levels.

In 2010, when the first FDA-approved therapy was developed, we had to demonstrate our ability to scale to 90,000 patients per year. It was a daunting task, and this is when employees with 20 years of experience is huge advantage.

We are also seeing changes in the airline structure. Some airlines will likely not be in existence for much longer – or at the very least, they are going to be a skeleton of an air carrier compared to what they were six months ago.

DOI: 10.18609/cgti.2020.030

In these situations, clients deserve options, and those options need to be very in-depth and practical. If you are going to experience potential delays and you want to mitigate risk, you must be able to offer other options that are perhaps very expensive. But it is still an option because we are focused on the patient. If we all continue to focus on the patient, I think it is going to be a very exciting ride.

Q Autologous cell and gene therapies are now a global commercial reality. What would you pick out as your top three key learnings on the logistics side, that may be drawn from the experience of the trailblazers in this area?

DM: As we discussed earlier, for both the autologous and the allogeneic therapies, there are strong similarities to the transplant community, which we have been handling for between 35 and 40 years. We are the pioneers in transporting organs and lifesaving drugs for the industry, and what we have learned over the years is very valuable to the solutions that we provide today.

The other important thing is to engage – engage with your logistics provider both early and often. When you start talking, even before the clinical stage begins, identify where the manufacturing is going to be done, and then look at the logistics. Learn what expectations you should have of your service provider. You might think you have it all spelled out, but when it comes to logistics it is not always that simple, and you may have gaps. If you do this early, you can flush all these issues out, mitigate risk, and come up with creative ideas on how to approach things.

This becomes even more important as these therapies grow globally. We have done an outstanding job between Adrian's team and the center of excellence in the UK, and our team in the US. We have handled some very complicated logistics solutions in Europe and in North America. The next evolution is going to be in Asia, and we are certainly prepared for that.

Lastly, from a biotechnology or pharmaceutical company perspective, logistics partners need to be viewed as a true partner. We act as a partner, we work with our clients as partners, and we must have the same mindset. This is what we are experiencing today in this industry, and it is a breath of fresh air.

Another thing is that we need to be good stewards of these therapies. We need to be able to educate our airline partners, our ground handlers so that they are as aware of the therapies they are handling as we are.

Q How is Quick seeking to develop its solutions further to support autologous therapy chains on a worldwide basis?

ALM: For Quick, this would really focus on what is effectively a reset of the supply chain involved in delivering these therapies, and in meeting the specific demands of each individual client.

> "...engage with your logistics provider both early and often."

We are still in a relatively early phase, particularly for CAR-T. We must fundamentally build an entirely new supply chain model for the cell and gene field and the CAR-T field.

We must take into consideration the scalability, as well as the demand for dedicated one-patient-one-product moves – including all of the chain of custody and chain of identity considerations, non-X-ray implications, redundancy planning, and so on. We have to review every available air and road option, and much more besides.

Faster and more streamlined solutions will probably first and foremost be provided by developing the supply chain, in a three-pronged approach. Firstly, that would be by completely retooling the ground network to withstand the pressures and meet all the logistical criteria. Secondly, it would be by working with the airlines and the ground handlers to educate their resources around these products and working with them to potentially develop new services or solutions. And thirdly, working with international regulatory bodies to explain to them why they should potentially make exceptions to some of the existing legislation for products in this field.

We have successfully done some of these things in several cases. For example, from a regulatory body perspective, we encountered a border control authority issue around export, meaning that potentially lifesaving medicine could not be exported in a timely manner within the product's viable lifecycle. We had to work together with the sponsor, and the authorities, and ourselves, in a collaborative approach, to find and bypass this issue. We did this very successfully, and it was very rewarding for all parties.

We have achieved the same thing from a service perspective. Working with an airline and the relevant ground handlers, we discussed issues in great depth and looked at all options. We managed to produce a bespoke handling service that meant we could minimize some of the risks around the failure of freight to be loaded onto the aircraft, by creating an enhanced ground ramp access solution. Again, it was a collaborative approach among multiple parties, and it made a real difference.

We are proud of doing this, and we are excited to push those boundaries and come up with entirely new solutions just by trying to think slightly differently. Can we approach this from a different angle, where can we get better collaboration from, where can we push the airlines, or push the handlers, to do more? That is exciting for us.

Q There seems to be a strong focus right now within the industry on working more closely and diligently to alleviate the increasing pressure on apheresis centers and clinical point of care that comes from this rapid growth of the cell and gene therapy field. What roles can logistics, tools, and service providers play in this effort?

> "The significance of timely collections and deliveries of the apheresis materials, and the product, is critical."

ALM: This is a rapidly growing market, and we are in the main part relying on hospitals and apheresis centers. These institutions have had to find the capability to slot entirely new

DOI: 10.18609/cgti.2020.030

processes into their already hectic clinical schedules. On top of that, they have had to adapt to additional responsibilities.

They may not necessarily be used to handling things such as non-X-ray, and chain of custody and chain of identity processes. They have needed education and help, and it is our job to ensure that we guide them through those processes and assist with the relevant documentation and training. That is something we can do together with the sponsor, through interaction with the hospitals and the sites.

The significance of timely collections and deliveries of the apheresis materials, and the product, is critical. Not only for the patient's sake, but also for the functionality of the institution itself. They do not have endless flexibility. They have tight timeslots, and they need us to produce and perform to what we have agreed.

My second point would be around transparency throughout the logistics process. This is key for all parties, and can be through the logistics provider's information tools, or through a wider platform that links all the stakeholders. As an example, we are currently working with clients who engage third-party organizations to link the key milestones that we might be reporting on, and the key data. That is between the client systems, our systems, and an overarching information platform. In some cases, this can involve booking system for the hospitals, too.

Lastly, as David mentioned, I cannot stress enough the importance of having experienced, specialized, and dedicated team members within the service provider. This is crucial to the sustained scalability of any project.

Q As we have touched upon already, allogeneic cell therapies are clearly on the rise. Many make light of the supply chain logistic challenges that this therapy faces, compared to for example autologous therapies. Where do you see potential issues arising that this field will need to address as it continues to grow?

ALM: Allogeneic therapies can be manufactured in larger batches, from unrelated donor sources, and as such the supply chain is one way, one-journey led. There is more predictability on the source material than there is with autologous, and there is more consistent availability for collection.

Autologous therapy is slightly different. It is vein-to-vein, and therefore has a supply chain that is circular – it has got multiple legs. It is a single source material, often a lengthy manufacturing process, and there is less consistency, or availability for collection. There are also differences in the temperature: normally a single temperature for allogeneic therapies, but often multiple requirements for autologous.

There are some key differences, but in reality, when it comes down to the critical elements of the supply chain, we don't identify too much of a difference at all. Chain of custody and chain of identity are critical in both. Strict temperature controls and the equipment we must provide, produce, and validate to achieve that – this applies to both. The non-X-ray requirements will most likely apply to both as well.

Most significantly, the delivery is effectively to a patient for surgery. We still have the same logistical challenges that apply in terms of hyper-strict timings, and highly detailed individual site requirements, and so on. To our eyes, there is not a lot of difference between the two.

Q Finally, could you sum up for us your, and Quick's, chief goals and priorities over the coming few years?

DM: In a unique way, COVID-19 has validated the processes that we have worked very hard on, and the procedures and logistics plans. What we have learned is that everyone has a role, and it is important to keep our staff healthy and safe. This has served as a reminder that we should continue to focus and concentrate on that.

Our chief goal from a client perspective is to reinforce the early dialogue with a logistics provider. It is particularly important now, in the days that we are living in.

Our investment strategy continues to be very strong, and we have to bolster our staff in Europe and North America. We must demonstrate to every client that scale is important to us, and we want to make these relationships long-term. These are key objectives for us in 2020, and on into 2021.

We will continue to add value through some of our internal functions, for instance, enhancements of our IT systems. As an example, we have just unveiled a redesigned web portal which is very exciting to us and our clients.

The most important thing going forward is to continually refine our quality management program. This is something that could be a pain point if left unaddressed. You must have a very robust quality program.

We are enhancing our ground support in targeted cities. Many of these therapies are being administered by world-class oncology clinics throughout the country, so many organizations are using the same hospitals, clinics, and apheresis centers. Where we feel that it makes sense, we will put our own assets and resources in those cities to help with the process. This takes some planning and investment, and it is something we are committed to doing.

We also recruit and train on-site assets, meaning that if a client is really interested and has the volumes to substantiate it, we will put people on-site, to help them with some of the logistics issues they may have to navigate on a local basis.

Finally, for nearly 40 years we have been patient-centric, and that will continue. I think that is probably the biggest asset that we have – the singular mindset that everything we do is for the benefit of a patient. We will continue with that mindset.

BIOS

David Murphy

David Murphy is a 30-year veteran of The Quick Group of Companies, holding various leadership roles in Quick's Life Science division. Over the past 8 years, David has served as Executive Vice President of Quick's Life Science Division, and works closely with major healthcare organizations to develop specialized logistics solutions to safely transport human organs, tissue, blood and blood products for transplant or research. He also works with biotech and pharmaceutical companies to plan and implement transportation strategies for personalized medicine; including cell, gene and immunotherapy

DOI: 10.18609/cgti.2020.030

treatments. He develops scalable transportation solutions that preserve the product integrity of these life-saving shipments, and most importantly, the overall safety of patients. He helps to ensure adherence to the strict regulations of the life science industry and the chain of custody at every shipment milestone. David was instrumental in the logistics planning of the first FDA approved cancer vaccine, and subsequent commercialization roll out.

Adrian Lee-Mohan

Adrian Lee-Mohan has been with the Quick Group since 1990, and has held various management roles in operations, finance and sales. He develops strategic relationships with pharmaceutical and biotech companies throughout Europe, in order to provide comprehensive logistics solutions for their global supply chain, ensuring product integrity and patient safety. Adrian has extensive experience in clinical trial logistics, cold chain management and packaging solutions. His focus is on personalized medicine, establishing sound supply chain models for Cell/Gene/CAR-T/Immunotherapy, from all phases of clinical trials through to commercialization.

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Article written in Partnership with Quick.*

Interview conducted: *Sep 4 2020;* ***Publication date:*** *Oct 1 2020.*

Quick
A Kuehne + Nagel Company

PERSONALIZED LOGISTICS
FOR CELL & GENE THERAPIES

Unique Patient. Customized Therapy.

Logistics for Cutting-Edge Treatments.

As the trusted leader in the industry, Quick delivers unmatched logistics expertise, temperature control, chain of identity, chain of custody and certified quality control processes. Our personalized logistics solutions help ensure product integrity and patient safety for your cutting edge treatments.

- Global leader in personalized medicine transportation
- Preferred partner for the first FDA approved immunotherapy
- APH to infusion, clinical to commercial, scalable solutions and supply chain planning
- Temperature control and procurement of packaging systems for product and patient safety
- Regulatory expertise, quality and GDP protocols, full chain of custody
- Robust IT systems with real-time tracking and temperature monitoring
- Ability to support commercial growth with scalable IT systems and global footprint.

Learn more

or go to: https://quick.aero/ask-the-experts/

Life Science Solutions from the Quick Group

www.quick.aero

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

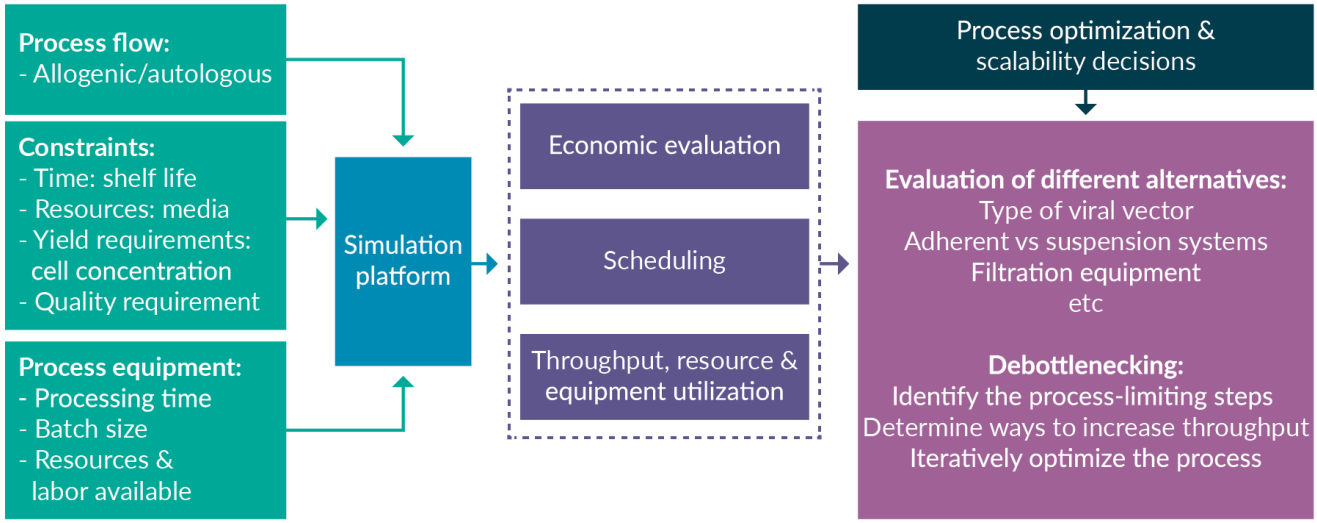
SCALING UP/OUT: COST-EFFECTIVE & ROBUST TRANSITIONIONING THROUGH THE CLINIC TO COMMERCIAL MANUFACTURE

EXPERT INSIGHT

Scale-up vs scale-out: evaluating manufacturing scalability of CAR T cell therapy using process simulation tools

Apoorva Katragadda, Iftekhar Karimi & Xiaonan Wang

Graphical abstract



Cell & Gene Therapy Insights 2020; 6(8), 1047–1056

DOI: 10.18609/cgti.2020.116

INTRODUCTION

Personalized medicine is gaining a lot of attention in the pharmaceutical industry as it is treating diseases that previously had no cure [1]. Unlike traditional pharmaceuticals, where the drugs are produced in bulk, these drugs are patient-centric and have to be manufactured for each patient or each sub-group separately. Recently, personalized medicine especially in the form of virus-based cell therapies witnessed exponential growth. After the FDA approval of the first chimeric antigen receptor (CAR) T cell therapy [2], the cell and gene therapy sector has become a fast-growing segment of the pharmaceutical industry. Recently, Tecartus, a CAR T cell therapy, has been approved for adults with mantle cell lymphoma (MCL) who have not responded to or relapsed following other kinds of treatment [3]. More of these products are awaiting approval from the FDA in 2020 [4]. However, since it is a relatively new field, the manufacturing processes are yet to be standardized or optimized at commercial scale. The scalability is an open question requiring investigation due to the high cost of manufacturing and associated uncertainties. Hence, there is a need to address the scalability issues in the manufacturing of CAR T cell therapies in order to meet increasing demand.

CAR T cell therapy can be broadly classified into two types: allogeneic and autologous. For allogeneic therapies where the cells are taken from one donor and used for multiple patients, the product is manufactured in large batches (200–2000L) in order to meet the dose requirements. For autologous therapies where each patient's sample is collected and injected back to the same patient, each sample is manufactured separately as a single batch [6]. With the increasing popularity of CAR T cell therapy products, there is a challenge to increase throughput. This can be done by either scaling-up or scaling-out. "Scale-up" refers to increasing the size of the equipment from the early clinical stage production, whereas "scale-out" refers to using multiple equipment of the same size as used in the early stage. As explained above, due to fundamental difference between allogeneic and autologous therapies, we need to evaluate both options to decide which one fits for a given therapy [7]. Until now, the majority of research has been focused on finding different scale-up considerations for specific unit operations in the manufacturing process and evaluating them based on the experimental data [8]. However, when demand increases, the main challenge lies in the optimal design and scheduling of the manufacturing process. The manufacturing process as a whole needs to be evaluated and bottlenecks should be identified in order to have optimal manufacturing scale-up/scale-out to satisfy the demand. Therefore, the scalability is determined by the entire complex manufacturing process of CAR T cell therapy.

PROCESS DESCRIPTION:

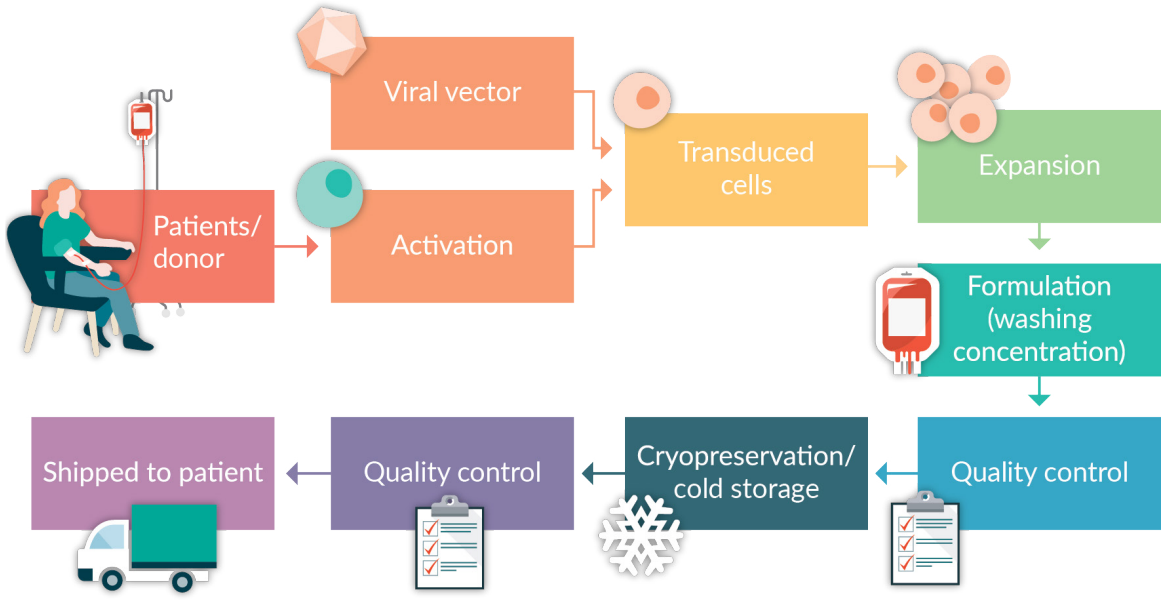
A generalized autologous or allogeneic CAR T cell therapy manufacturing process is indicated in Figure 1. It can be divided into three steps: upstream, downstream, and fill/finish. For scaling-up and scaling-out, one must understand each unit operation in the manufacturing process. Since this field is evolving, the process should be evaluated, and the capacities have to be determined before investing.

- Upstream: In the upstream process, the samples, i.e. cells are collected from patients/donors and further engineered. The upstream process consists of two parts: the viral vector preparation and sample modification. The viral vector is a key raw material needed in the modification of samples in CAR T cell manufacturing. Lentiviral vector has been commonly used in clinical trials of CAR T cell therapies [9]. The key difference in autologous and allogeneic therapies is that in allogenic therapy, the virus is the ATMP whereas in the case of autologous therapy the virus is just used in the gene modification of the patient's cells, where

DOI: 10.18609/cgti.2020.116

FIGURE 1

Generalized manufacturing of CAR T cell therapy.

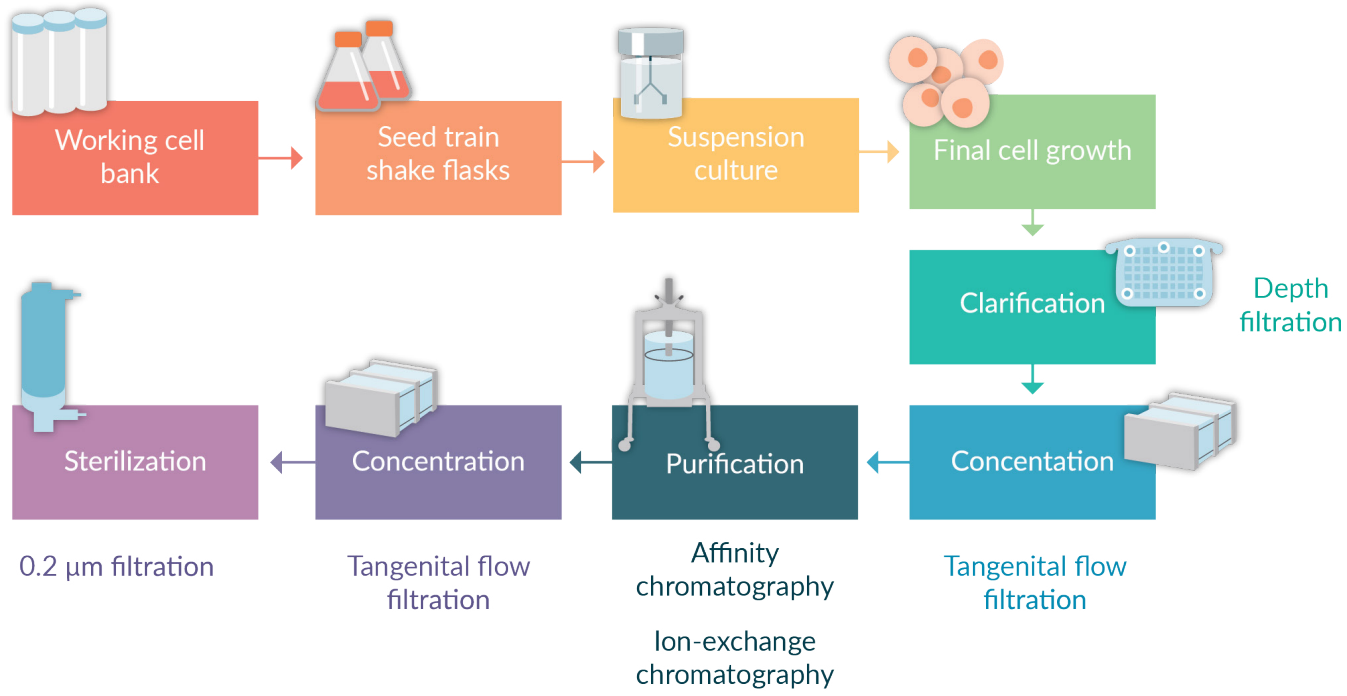


the modified cells are the ATMP. The viral vector production is a very complex and time-consuming process (Figure 2). It is normally produced in bulk for both allogeneic and autologous therapies as a common raw material for all the samples. The viral vector is produced essentially using defined cell lines. For viral vector in a CAR T application, the primary cell line in use is a human cell line (HEK cells). The production starts with a working cell bank (WCB) that is first removed from the cryogenic storage container. These cells are thawed and washed. They are then transferred to T flasks for expansion as the current practice. In the case of autologous therapy, where the viral vector is raw material, the throughput can be increased either by scaling-out and use multiple shake flasks, or scaling-up using bioreactors. The expansion step is followed by purification and concentration of the viral vector. After the viral vector is ready, the sample modification takes place. The sample cells collected from patients/ donors undergo washing and activation. After the gene delivery, where the samples undergo viral transduction, is carried out, the modified cells are expanded. The expansion depends on whether it is allogeneic or autologous therapy. Since in autologous the sample volume is low, scale-out is the option used to process more samples. In the case of allogeneic, the cells can be expanded either in shaker flasks or bioreactors based on the batch size requirement and the downstream throughput. There are a lot of alternatives for scale-up in the upstream process of cell therapy. Currently, many companies such as GE Healthcare (Cytiva), Sartorius and Pall have provided hardware solutions for scale-up for different batch sizes.

- Downstream: After the expansion is complete, the cells are harvested through a series of filtration/centrifugation to achieve the required cell density. Since there is no standardized process until now, the process design depends on the density required. One of the possible processes is given in Figure 2, where sequential concentration and purification are carried out. Here, the downstream process of the viral vector is more complex, than the downstream processing of the CAR T. Hence, capacity planning should take into consideration

FIGURE 2

Schematic of viral vector production and purification.



both the requirements [10]. Currently, the cell concentration and washing are carried out in centrifugation tubes (≤500 mL). However, the transition to scalable and closed systems is crucial for scale-up/out [11].

- Fill/Finish: After the downstream purification of the product, the cells are prepared for the filling and freezing steps. As the shelf-life of the cells is very short (30 mins to few hours), the fill/finish process should be quickly completed. The cells are either cryopreserved or reformulated for short-term storage at 2–8°C. Cryopreservation is more practical option as it gives flexibility for scheduling.

PROCESS SIMULATION

In any process development, technological alternatives available for each step of the manufacturing process are evaluated. For any cell and gene therapy, until now the main efforts have been spent on determining the efficiency and effectiveness of various alternatives based on experiments. However, this is less effective in developing an optimum process design as the dependence of each decision on the overall cost is not taken into consideration. One of the major bottlenecks is that the manufacturing is cumbersome and manual and applying the concepts of process engineering would help in increasing productivity and the economic viability of the product. There are two ways in which digital tools can be employed in order to complement the real experiments. First is the development of detailed mechanistic models which would require fundamental understanding of the process mechanism in order to develop the governing equations. Another way is the data-driven *in silico* modelling which would use experimental data. In the case of CAR T-cell therapy, which involves biological systems, the complexity involved is too high and the process understanding is limited. In addition, due to the GMP requirements, there is high quality

DOI: 10.18609/cgti.2020.116

of process data available. Therefore, real experiments can be supported by *in silico* experiments to accelerate and improve the process development. Simulation tools enable approximate imitation of the operations in the manufacturing process and have been used for traditional pharmaceutical manufacturing to evaluate different equipment, scenarios, economic performance, and throughput analysis of the process. Therefore, we can naturally extend the use of simulation tools for the process development of CAR T cell therapy manufacturing as well. The use of simulation tools will help us get yield insight which was not possible with experimental analysis of each process step. From the simulation, we can identify the total capital investment, manufacturing cost, cycle time, and capacity of the manufacturing. Also, the modelling of the manufacturing plant plays a very important role in production planning and scheduling. The simulation will allow scheduling by abiding by the resource and capacity constraints. It effectively serves as a fast and inexpensive alternative to actual experiments.

In the past, simulation tools have been used for various process design and scheduling purposes, such as throughput analysis and chemical processes debottlenecking [12]. They have also been proposed in biopharmaceutical process, such as the manufacturing of monoclonal antibody (mAb), to use simulation tools for process optimization [13]. However, in the case of cell therapy, simulation tools have not been fully adopted. The scalability of CAR T cell therapy has been investigated with respect to individual equipment's but not yet the whole process.

The current "gold standard" is to adopt a simulation environment to model the entire process and make decisions based on the outcome. The different aspects in which simulation can be used in the manufacturing of CAR T cell therapy are:

- Process design: The manufacturing process of CAR T cells consist of equipment alternatives with different yields and processing times. Simulation helps select the best technology for each processing step in the manufacturing process. Also, there is added complexity due to the biological variability, i.e. each batch has different processing times. Simulation of the CAR T cell manufacturing also considers the uniqueness of complex biological systems.

- Economic evaluation and what-if analysis: The simulation tools perform thorough economic analysis and project the cost calculation for the required scale of production. The tools can also be used to analyze different future scenarios and compare the costs associated. Therefore, economic cost can be used as a metric to decide the optimal process design for commercial CAR T cell manufacturing [14].

- Bottleneck identification: Once different technology alternatives are compared and the process flow sheet is decided, the scalability decisions must be made. Since the costs related to the CAR T cell therapy equipment are tremendous, it is critical to understand the capacity and time utilization of each equipment i.e. proportion of time each equipment is used in one plant cycle, and optimize the throughput with minimum investment. The main goal for scaling up or scaling out is to increase the annual throughput [15], the general formula of which is given below:

$$\textit{Annual throughput} = \textit{batch size} \times \textit{number of batches}$$

In the case of allogeneic CAR T therapy, where the samples are prepared in bulk, throughput can be increased through scaling-up. While in the case of autologous therapy, in the majority of steps each sample is manufactured separately, to increase the throughput, scale-out by installing parallel units becomes a better option. However, since the process is a result of interactions among various unit operations with different processing times and capacities, scale-up/

scale-out of the entire manufacturing process is not straightforward and has to be decided through debottlenecking techniques.

While we try to increase the throughput, we might run into bottlenecks from either equipment or resources. The bottlenecks encountered can be either time bottleneck or capacity bottleneck. The debottlenecking process is carried out as an iterative process evaluating the effect of each decision made on the entire process. Once we increase the capacity of certain equipment might lead to a new bottleneck and the process continues until we achieve the required throughput.

Different metrics can be used to identify the bottlenecks such as time utilization of the equipment, capacity utilization, etc. For example, in the process of debottlenecking, we compute the capacity utilization of all the equipment for the plant running at maximum capacity. The equipment with almost 100% capacity utilization is the limiting one as the throughput cannot be increased further. Therefore, the capacity is increased via scale-up for bulk manufacturing or scale-out for separate manufacturing, in order to remove the bottleneck and increase the throughput. After the changes are made and throughput increased, the utilization is re-calculated to find other bottlenecks. Therefore, the scalability of the manufacturing process is based on the debottlenecking of the manufacturing process iteratively until the required throughput is achieved.

The debottlenecking process will result in optimal scale-up/scale-out based on whether it is allogeneic or autologous therapy. All the possible scenarios should be considered before making the production decisions. Since, in the future, there is a high possibility that a single manufacturing center will cater to both allogeneic and autologous sample production, the scalability decisions should be based on the future demand for both types. Hence, this becomes a multi-product plant simulation problem where we keep the various scenarios in mind.

Challenges in using simulation tools: In the previous sections we have highlighted the advantages of using simulation tools and how they can deal with challenges of CAR T cell therapy manufacturing scalability. However, simulation tools do not standalone and have to be used in complementary to the ongoing experiments. The two main steps of implementing simulation tools in the system are data collection from experiments and model validation. Data is needed for developing *in silico* models which is used to describe the biological processes realistically. For the validation, the credibility of the *in silico* models should be demonstrated. Therefore, the *in silico* experiments and real experiments would be complementary and would help in making the process design more efficient.

The use of simulation tools and digital technologies comes with following challenges:

1. Scale-up limitations: Scale-up for CAR T cell therapy is not straightforward and involves a lot of complexities. Since it is cell culture based process, cell-density based scale-up is more relevant than volume-dependent scale-up. Also, maintaining cell product's critical quality parameters such as density, purity etc., is very crucial while scaling-up. Therefore, scale-up decisions are governed by the above mentioned constraints and should be considered in simulation tools.

2. Data: High-quality data is necessary to build realistic simulation models. Since the CAR T cell therapy is very new and far away from being standardized, *in silico* models cannot capture the whole process. However, once the field advances, simulation has the potential to serve the role of selecting the best technology, and enable process optimization and scheduling [16].

3. Regulatory approval: The simulation tools in order to be employed needs regulatory approval. The USA Food and Drug Administration (FDA) has clearly mentioned the technical information needed for the approval of digital tools. After the regulatory approval, the next process is verification, validation and uncertainty quantification (VVUQ) [16]. The verification

DOI: 10.18609/cgti.2020.116

step determines if the simulation model fits the mathematical description, validation is achieved by comparison of simulation results and real-world data and uncertainty quantification studies the effect of inputs uncertainty and assumptions on the simulation results.

INDUSTRY 4.0 AND DIGITAL TWIN

Industry 4.0 is the industrial revolution that links the physical production and operations with smart digital technology, big data and ML in order to establish an holistic ecosystem [17]. New level of interconnectivity through IoT and access to real time data has revolutionized various sectors such as process industry, manufacturing etc . In other words, it results in the convergence of physical and digital spaces. One of the important steps in the Industry 4.0 is the development of a digital twin. Which is a virtual replica of a process or product. Digital twin is an integrated digital model of a physical system which provides important insights on the system performance. Digital twin uses the process data from experiments into deep process understanding and enables efficient decision-making. Digital twin has a lot of advantages in the field of CAR T-cell therapy. The ability to support real time monitoring of the process is crucial for any cell therapy as it supports visibility and helps in quality control. Also, the use of digital twin enables automation and helps in replacing the cumbersome manual manufacturing process. The integrated system of manufacturing process and its digital twin is established through soft sensors and would lead to better process control [18]. Another aspect where digital twin has unique advantages is quality control [17]. The batch failure due to the failure of quality assurance and/or contamination is a critical issue in cell culture derived products. Having a real-time monitoring of the process through digital twin helps in better monitoring and avoiding such problems.

Software review

The above-mentioned computer experiments can be done in many simulation environments. Here we give a review of the commercial simulation software that can be used for cell and gene therapy manufacturing [19].

1. Bio-G: The software is a real-time modeling system for biopharmaceutical manufacturing. It is very flexible with the capability of connecting to any database and updating data in real-time for the analysis. This software accounts for different challenges such as variability associated with human cells. The key advantage is that it gives realistic and faster results than other modeling frameworks with real-time simulation [20].

2. SuperPro Designer: This software is a process flowsheet based on a database containing a variety of unit operations. Based on the input data, the software calculates materials and energy balances for each process step. It has a built-in database for raw materials and consumables, meanwhile accepting the user databases [21].

3. BioSolve: It is an Excel-based modelling tool with pre-populated unit operations for process design. It is static and mainly used for the economic evaluation of the process. Therefore, it is a good tool to compare different technologies for a given process step [22].

4. AspenOne: Like Superpro, it is a flowsheet-based software by AspenTech. It is mainly used for the design and optimize the traditional pharmaceutical ingredient (API) manufacturing. Compared to Superpro, it does not have a wide range of unit operations relevant to CGT manufacturing [23].

5. Discrete event simulation (DES) Software: The above-mentioned software all include mathematical modelling, i.e. mass

TABLE 1

Comparison between different simulation software indicating their advantages and disadvantages

	Bio-G	SuperPro Designer	BioSolve	AspeOne	DES	Bio4C™ ProcessPad
Real-time	✓				✓	✓
Equipment modelling	✓	✓	✓	✓		✓
Dynamic	✓				✓	✓
Static		✓	✓	✓		
Biological variability	✓					✓

and energy balances in unit operations. However, only DES can be used to model discrete and dynamic manufacturing process to visualize flow to the materials. It has an advantage over the spreadsheet-based simulation as the latter is deterministic and does not consider the variability. Hence, DES becomes useful when we do not have sufficient data for the mathematical modelling of the equipment. Examples of DES software are EDEM, ExtendSim, Simul8, etc. [24].

6. SimCad Pro: This software can simulate process-based environments such as manufacturing, logistics, healthcare etc. It is a DES tool capable of modelling any process flow-type environment. Also, unlike other DES software, it supports real-time monitoring using devices like RFID tags [25].

7. Bio4C™ ProcessPad: It is a software suite by Merck combining process analysis, process control and plant-level automation. It has both offline and browser-based modules that can analyze data from databases offline and conduct real-time monitoring of the bioprocessing plant, respectively. This software is more than just a simulation tool, but aims to implementing Industry 4.0 in bioprocessing field. It includes a wide range of unit operations and provides alternatives for each step of the manufacturing process [26].

Apart from the well-established software mentioned above, other software are currently being developed which can cater to the specific needs of CAR T cell therapy. Some of these software are K-ComBioPro (a computational Bioprocess Design simulation tool), Siemens smart biomanufacturing tool, etc. In summary, there are a variety of simulation software available for modelling the CAR T-cell manufacturing, with various levels of complexity and details included. Table 1 below gives a direct comparison of the advantages and disadvantages of these products for choosing the proper software based on specific requirements.

CONCLUSION

In this review, we emphasized the importance of process simulation in tackling the scalability issues in CAR T cell manufacturing and the challenges involved. It is understood that the scalability of the process is not straightforward and requires analysis of the entire process. The importance of simulation tools is three fold:

1. Fast and inexpensive way to optimize the design process through comparison among different alternatives,
2. Economic evaluation, and
3. Scalability decision support based on debottlenecking of the process.

There are different types of commercial simulation software available, each one with different levels of details and assumptions. The choice of software should be based on the requirement. Moreover, simulation of the

DOI: 10.18609/cgti.2020.116

process will also aid the implementation of a digital twin which is crucial to meet the unique requirements of CAR T cell therapy. The digital representations would include all the details of the system by mirroring the process in the virtual space to gain the same benefits of accessing the system. In the case of CAR T, it would mean the representation of the entire production system in real-time aided by sensors and smart devices. The production systems can be integrated with logistics and good visualization of the entire process is enabled. A digital twin will add great value in the case of CAR T cell therapy considering real-time information on the quality and state of the sample are important at all the steps of the manufacturing and transportation. Therefore, simulation is the first step in the direction of transforming the cell and gene therapy manufacturing and implementing Bioprocessing 4.0 through digital twins.

REFERENCES

1. Tang J, Hubbard-Lucey V, Pearce L, *et al.* The global landscape of cancer cell therapy. *Nat Rev Drug Discov*. 2018; 17(7), 465–466.
2. Zheng P, Kros JM, Li J. Approved CAR T cell therapies: ice bucket challenges on glaring safety risks and long-term impacts. *Drug discovery today* 2018; 23(6), 1175–1182.
3. FDA, FDA approves brexucabtagene autoleucel for relapsed or refractory mantle cell lymphoma. 2020.
4. Senzel L, Ahmed T, Ma Y, Chimeric Antigen Receptor Therapies. *Immun. Conc. Trans. Med.* 2020; 349–359.
5. Vormittag P, Gunn R, Ghorashian S, Veraitch FS. A guide to manufacturing CAR T cell therapies. *Curr. Opin. Biotechnol.* 2018; 53, 164–181.
6. Contributor, B. Scale-Out Biomanufacturing – A Paradigm Change to Scale-Up. 2018; Available from: https://bioprocessintl.com/sponsored-content/scale-biomanufacturing-paradigm-change-scale/.
7. Brady M, Varley M. TO SCALE-UP OR SCALE-OUT? THE CHALLENGE OF COMMERCIALISING CELL THERAPIES. Cambridge Design Partnership; Available from: https://www.cambridge-design.com/news-and-articles/blog/commercialising-cell-therapies.
8. Cacciuttolo MA , Arunakumari A. Scale-up considerations for biotechnology-derived products. *Pharm. Process Scale-Up* 2001; 95.
9. Levine BL, Miskin J, Wonnacott K, Keir C. Global manufacturing of CAR T cell therapy. *Mol. Ther.-Meth. Clin. Dev.* 2017; 4, 92–101.
10. Wang X, Rivière I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. *Mol. Ther.-Onc.* 2016; 3, 16015.
11. Rowley JA. Developing cell therapy biomanufacturing processes. *Chem. Eng. Progress*. 2010; 106(11), 50–55.
12. Koulouris A, Calandranis J, Petrides DP. Throughput analysis and debottlenecking of integrated batch chemical processes. *Comp. Chem. Eng.* 2000; 24(2–7), 1387–1394.
13. Toumi A, Jürgens C, Jungo C, Maier B, Papavasileiou V, Petrides D. Design and optimization of a large scale biopharmaceutical facility using process simulation and scheduling tools. *Pharm. Eng.* 2010; 30(2), 1–9.
14. Jenkins MJ, Farid SS. Cost-effective bioprocess design for the manufacture of allogeneic CAR-T cell therapies using a decisional tool with multi-attribute decision-making analysis. *Biochem. Eng. JournaL.* 2018; 137, 192–204.
15. Petridis D, Koulouris A, Siletti C. Throughput analysis and debottlenecking of biomanufacturing facilities: a job for process simulators. 2002;
16. Geris L, Lambrechts T, Carlier A, Papantoniou I. The future is digital: in silico tissue engineering. *Current Opinion in Biomed. Eng*. 2018; 6, 92–98.
17. Tao F, Cheng J, Qi Q, Zhang M, Zhang H, Sui F. Digital twin-driven product design, manufacturing and service with big data. *Int. Jour. Adv. Man. Tech*. 2018. 94(9–12), 3563–3576.
18. Kritzinger W, Karner M, Traar G, Henjes J, Sihn W. Digital Twin in manufacturing: A categorical literature review and classification. *IFAC-PapersOnLine*. 2018; 51(11), 1016–1022.
19. Jagschies G, Lindskog E, Lacki K, Galliher P. Biopharmaceutical Processing: Development, *Design, and Implementation of Manufacturing Processes* 2018;
20. Bio-G. Bio-G solutions and expertise. Available from: https://www.bio-g.com/solutions/.
21. Intelligen, I. SuperPro Designer Product Features. Available from: https://www.intelligen.com/superpro_features.html.
22. Biosolve. BioSolve Software. Available from: https://biopharmservices.com/software/.

23. AspenTech. AspenONE Product Portfolio. Available from: https://www.aspentech.com/en/products/full-product-listing.

24. Simul8. Available from: https://www.simul8.com/.

25. CreareaSOFT. SimCAD simlation software. Available from: https://www.createasoft.com/process-simulation-software.

26. Merck, Bio4C™ ProcessPad Software.

AFFILIATION

Apoorva Katragadda
Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore, 117585, Singapore

Iftekhar Karimi
Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore, 117585, Singapore

Xiaonan Wang
Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore, 117585, Singapore

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *The authors thank the MOE AcRF Grant in Singapore for financial support of the projects on Precision Healthcare Development, Manufacturing and Supply Chain Optimization (R-279-000-513-133) and Advanced Process Control and Machine Learning Methods for Enhanced Continuous Manufacturing of Pharmaceutical Products (R-279-000-541-114).*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: 15 Jun 2020 Revised manuscript received: 18 Aug 2020; Publication date: Sep 3 2020.

DOI: 10.18609/cgti.2020.116

CELL & GENE THERAPY INSIGHTS

SCALING UP/OUT: COST-EFFECTIVE & ROBUST TRANSITIONIONING THROUGH THE CLINIC TO COMMERCIAL MANUFACTURE

SPOTLIGHT

INTERVIEW with:
Ryan Cawood, CEO and Founder, OXGENE

Ryan founded Oxford Genetics in 2011, after earning a first class degree in genetics and a PhD from Oxford University. The idea behind the company was to simplify and standardise the process of DNA engineering using a proprietary DNA plasmid platform called SnapFast™ that allowed researchers – for the first time – to assemble complex sections of DNA as simply as 'molecular Lego'. Ryan used his background in genetic engineering and virology to guide and grow the business through a series of strategic changes that explored how further development of the SnapFast™ platform through in house research and development could help overcome multiple challenges in the development of new biologics.

This culminated in a rebrand to OXGENE in 2019, as the company redefined itself as a leading solutions provider, using a combination of proprietary technologies to address multiple pinch-points on the journey through design, discovery, development and manufacture of a novel biologic.

A necessary transition: why viral vector production for gene therapy needs to evolve

Cell & Gene Therapy Insights 2020; 6(8), 1163–1170

DOI: 10.18609/cgti.2020.127

Q Can you give us some background on the story of OXGENE, and its technological focus to date?

RC: I founded Oxford Genetics, rebranded to OXGENE, nine and a half years ago. I was doing a PhD in gene therapy at the University of Oxford and making some quite complicated gene therapy vectors, which got increasingly challenging to construct as they got bigger and involved larger and larger pieces of DNA. By the time I finished my PhD, I was convinced that there was better way to build DNA. I founded the company on that central premise. The concept was to build DNA like Lego; to build consistent DNA blocks that had reproducible behaviors, and then assemble more complicated pieces of DNA from those predefined blocks.

At that point, I didn't know much about running a business, or how the industry worked. I originally thought we'd be a product-based company that would sell the pieces of DNA we made. That worked pretty well, but it became clear that the company was never going to grow much beyond that unless we changed the business model. We started to use the pieces of DNA we made to custom-build larger sections of DNA for customers. And then behind the scenes, we also started using that same platform to build our own technologies, and to invest in our own research and development. This grew into three different areas: antibody discovery, gene therapy manufacturing strategies, and CRISPR engineering. We have evolved as the markets have moved, and today most of our research is in the gene therapy area.

Q Why the strong focus on gene therapy, and why now? What is your take on how viral vector manufacturing needs to evolve, particularly in terms of its scalability demands?

RC: It's a really exciting time for gene therapy. When I was doing my PhD, the industry was struggling. There weren't many clinical trials going on, and there was little investment in the sector. I'm really pleased that as we've been developing our business, the industry has completely changed, thanks in large part to some clinical success stories in patients, which is excellent to see. We've followed the industry as it's expanded, which is also why we've invested more heavily in that part of our business.

> "...the industry is essentially using technologies – for the most part – that were established 20 or even 25 years ago."

In terms of the current state of play in gene therapy manufacturing, the industry is essentially using technologies – for the most part – that were established 20 or even 25 years ago. It's almost like making a cake. Every time you want to make a cake, one person puts all the ingredients in, mixes them up, and hopefully the cake comes out well in the end. Right now, to make a gene therapy vector, you have to transfect multiple plasmids into the cells, add the transfection reagents and hope for the best. It's not particularly reproducible; sometimes it

doesn't work, sometimes the yields are low, and it's very difficult to then scale the process up.

Our approach to viral vector manufacture needs to change.

> "Our approach to viral vector manufacture needs to change."

Q Turning to adeno-associated vector (AAV) manufacture in particular, what do you see as the critical factors in achieving this field's twin key goals of improved yield and quality, and how is this reflected in OXGENE's platform?

RC: Yield and product quality are inextricably intertwined. Some diseases require systemic gene therapy treatment, meaning that you need extremely large quantities of AAV per patient. But if you're going to deliver large quantities of virus systemically, you need the quality of your viral vector to be very high and very potent. Otherwise you're delivering material that either doesn't work or may cause toxicity. If you can improve yield and quality together, you can reduce cost of goods and have a product that's more active on delivery.

Q Can you go deeper into OXGENE's philosophy and approach to simplifying AAV production processes – for example, in terms of reducing the number of transfection steps required?

RC: Our philosophy is that the only way in which you will truly be able to scale AAV manufacturing is to completely remove the dependency on plasmids and the transfection process. This is partly because of the number of input ingredients you need, but also because the process itself is limited by cell density, is hard to scale, and comes with prohibitively high costs. We've been developing multiple technologies that all focus on reaching that objective.

One particularly exciting new technology we're bringing to market is TESSA, which stands for Tetracycline Enabled Self Silencing Adenovirus. If we think of how AAV is produced in nature, it only replicates when it's in the same cell as an adenovirus; that's why we call it an adeno-associated virus. In this natural setting, the AVV produced is of exceptionally high quality; almost every AAV particle has an AAV genome packaged inside it. But when we produce AAV using plasmids, for some serotypes only 2–5% of the particles actually contain a genome.

We wanted to reproduce 'natural' AAV replication, and to do that, we needed to use an adenovirus. But why aren't people doing that already? The main reason is that when you use adenovirus to manufacture AAV, you make about as much adenovirus in the end as you do AAV. This is potentially a major safety issue, and means you have to work really hard to purify the AAV and remove all the adenovirus. We knew this was the challenge we'd have to overcome, so we developed a way to halt the adenovirus lifecycle halfway through. This means that the adenovirus can go into a cell, convert that cell into a viral vector manufacturing machine,

> "...we've managed to get the yield from our lentiviral packaging cell lines pretty close to that of our transient transfection process."

then shut itself down. It can provide the help to make AAV, but doesn't make any more adenovirus. In terms of suppressing adenovirus production during an AAV manufacturing run, TESSA is somewhere in the region of 99.999% to 100% effective.

Once we developed this adenovirus, we thought about how to use it to manufacture AAV. We could replace the helper function in the AAV manufacturing process, but you'd still have to deliver two other plasmids: one with Rep and Cap and one with the ITR-flanked gene of interest. So we thought perhaps we could add the AAV Rep and Cap genes into the adenovirus as well, thereby removing another plasmid from the process. That has been tried before, but without success. However, because of our molecular Lego platform, we could make lots of different viruses in different configurations to find the one that worked best.

We can now deliver everything you need to manufacture AAV, with the exception of the ITR-flanked gene of interest, in a single virus; and there are many other transfection-free methods of delivering this.

Q Are there any aspects or features of the OXGENE platform that are designed specifically to solve bottlenecks in large-scale AAV vector manufacture?

RC: As we discussed before, the main challenges for AAV manufacture are maintaining – or improving – AAV yield and quality in large scale production. So far, the degree to which our TESSA platform improves AAV yield is serotype dependent. For some serotypes we've observed a ten-fold improvement, and for other serotypes we've seen a 100-fold improvement; that's just in the number of virus particles that are coming from the cell. What is almost more interesting is that when we look at those particles, they're also in some cases up to 2,000-times more infectious. As well as these improvements to yield and quality, we've also seen a significant increase in packaging efficiency. For AAV2, this has increased from about 2–5% to around 70%. Going back to how much AAV you'd then need to deliver to the patient, there may potentially be significant safety benefits to this as well.

Q Shifting the focus to lentiviral vectors (LVVs) production, can you outline this particular platform and how it addresses issues that relate to LVVs specifically?

RC: We've been developing packaging and producer cell lines for LVVs for about three and a half years, and we're now offering these out for evaluation. These cell lines allow you to

reduce the number of plasmids you need to transfect into the cells from four down to one in the case of the packaging cell line, or none for the producer cell line. The market expectation is that lentiviral packaging and producer cell lines will be the solution to scalable lentiviral vector manufacture, which is why we've focused our attention on this, perhaps more conservative, approach to manufacturing lentivirus than we've taken for AAV.

The fact that there are clinical products that use lentiviral vectors being used to treat patients speaks to the success of the industry. However, you need significantly fewer lentiviral particles per patient than you do for AAV, because lentiviral vectors are most commonly used for *ex vivo* cell therapies. And because you're then transducing the cells *ex vivo*, you don't require the 1 x 10^{12} viral particles per kilogram you might need to treat a patient with an AAV based gene therapy.

Lentiviral production is also slightly different, because we have a precedent to follow, in that retroviral packaging and producer cell lines have been around since the mid-to-late 1990s. Creating a stable producer cell line means that all the genetic components of the viral vector are integrated into the cell's own genome, so you no longer need to perform a transfection step to produce lentiviral vectors expressing your gene of interest. Now this is much simpler for retrovirus than lentivirus, because there aren't that many genes, but the number of genes in HIV-based lentiviral vectors – some of which are toxic to cells – make this a bit more challenging.

Q The traditionally high cost of LVV remains a major concern for the cellular immunotherapy field in particular – how does OXGENE's platform seek to aid in cost of goods reduction?

RC: If you run a bioreactor to produce lentiviral vectors, about 40% of the cost of goods comes from plasmids and reagents. If you can cut that cost by using a producer cell line, then you immediately make a significant saving on production. That's just in terms of your costs going in, not even considering the process improvements. For example, transfection limits batch size, and increases the complexity involved in actually making the virus. Simplifying this process improves reproducibility. That said, the main challenge for lentiviral packaging and producer cell lines is that viral yields are generally slightly lower than with the transfection process, leaving a trade-off between scalability and overall yield. So far, we've managed to get the yield from our lentiviral packaging cell lines pretty close to that of our transient transfection process. It's slightly lower for the producers, so we're busy optimizing and improving that now – but it's already at the point of commercial viability, because it would be cheaper to use this cell line than consistently produce large quantities of lentivirus by transient transfection.

> "...we are doing really exciting things in terms of genetic engineering and developing new approaches..."

Q Why is collaboration so important in viral vector bioprocess development, and how is this reflected in OXGENE's approach?

RC: We've been fortunate to have a number of different collaborations with some significant players in the gene therapy industry over the last 3–4 years. We might think we are doing really exciting things in terms of genetic engineering and developing new approaches, but end-user companies have a different perspective, and their feedback has been invaluable. If you're going to attempt to throw out the existing process, it's crucial to understand just how far you can push the boundaries, and the only people who can tell you that are the therapeutic companies. They've taught us a huge amount, and we hope to have many more collaborations in the future. It is the best way to learn what the industry needs, and the best way to make progress.

Q Can you sum up both your own and OXGENE's chief goals and priorities for the coming 12 months?

RC: We have just been through the process of refitting a new facility of around 7,000 square feet, which will allow us to expand our process development capabilities. Bringing our new viral vector manufacturing technologies to market is our number one priority for the year ahead. We want to get these to the point where we've done all the validation our customers will want to see, and made sure that the data is available for them in the event that they want to file those technologies with regulatory bodies.

Beyond that, we want to continue to grow the company. For the last 3 or 4 years we've been growing at around 160% a year, which has been great. This year is obviously going to be more challenging than others, due to the Covid-19 pandemic, but so far we are optimistic that we can continue our progress.

AFFILIATION

Ryan Cawood
CEO and Founder, OXGENE

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author holds patents or has patents pending relating to TESSA technology. The author has no other conflicts of interest.*

Funding declaration: *The author is an employee of and shareholder in Oxford Genetics Ltd.*

ARTICLE & COPYRIGHT INFORMATION

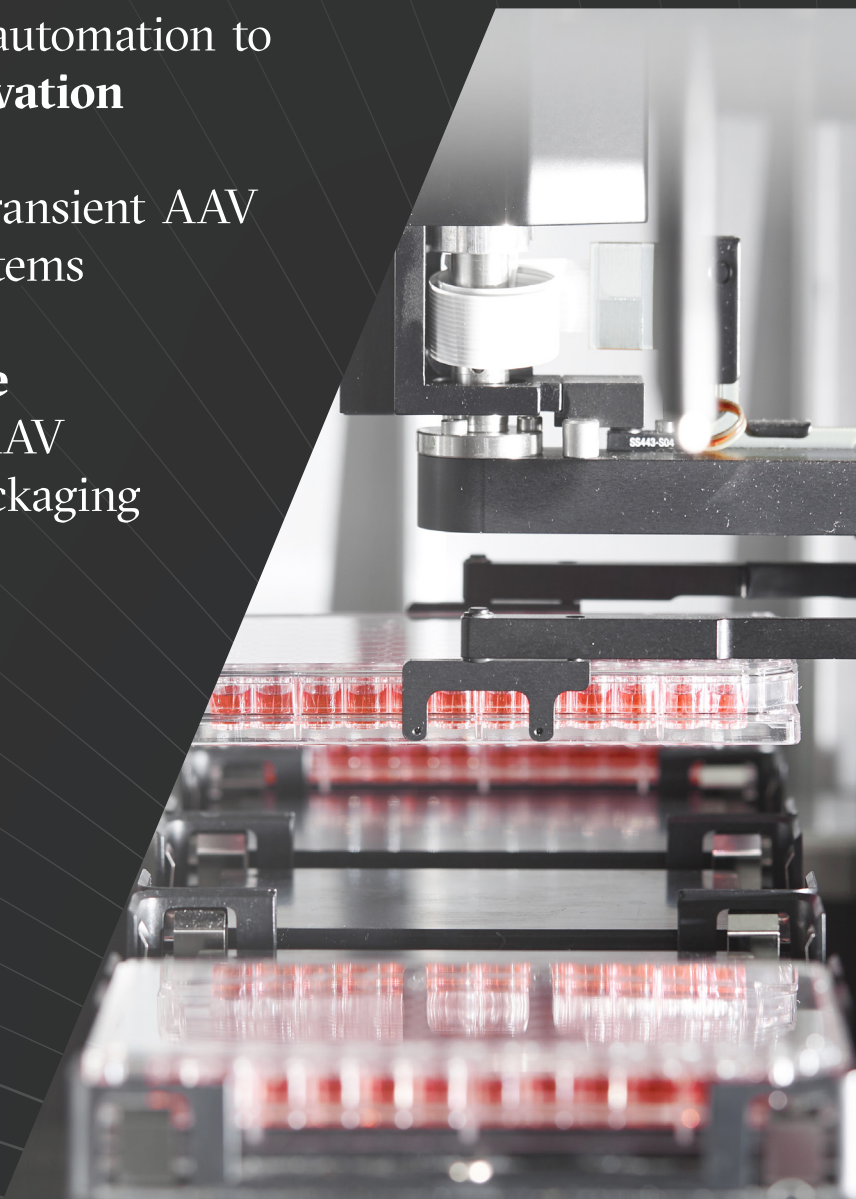
Article source: *This article is written in partnership with OXGENE.*

Interview conducted: *Aug 20 2020;* ***Publication date:*** *Sep 29 2020.*

OXGENE

GENE THERAPY AT THE EDGE OF IMPOSSIBLE

- At OXGENE, biology meets automation to deliver groundbreaking **innovation**
- We offer **industry leading** transient AAV and lentiviral production systems
- Now launching fully **scalable technologies**: plasmid-free AAV production, and lentiviral packaging and producer cell lines



Accelerate your discoveries at
www.oxgene.com

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

SPOTLIGHT SCALING UP/OUT: COST-EFFECTIVE & ROBUST TRANSITIONIONING THROUGH THE CLINIC TO COMMERCIAL MANUFACTURE

COMMENTARY

The power of small: success of small academic/healthcare units within the cell & gene therapy industry

Benjamin D Weil & Owen Bain

Cell and gene therapy (CGT), within the biopharmaceutical industry, continues to gain traction with the success of chimeric antigen receptor (CAR) T-cell therapies from Novartis and Gilead, stem/stromal cells from Takeda (formerly TiGenix), limbal stem cells from Chiesi, oncolytic virus from Amgen, and many more. However, these novel therapies were not born in pharma, they originated and were clinically developed within small academic GMP manufacturing sites. This commentary reviews the impact and significance of small academic/healthcare units as a launchpad for the innovation and success of CGT ventures, from the perspective of two senior managers at the Centre for Cell, Gene & Tissue Therapies (CCGTT) at the Royal Free Hospital, London, UK.

Cell & Gene Therapy Insights 2020; 6(8), 1207–1214

DOI: 10.18609/cgti.2020.132

INTRODUCTION

The ATMP field is rapidly progressing with more novel therapies entering the CGT field than ever before. The route from discovery to late clinical trials, however, is littered with complex hurdles to overcome, fraught with numerous far-implicating early decisions which often determine success and

commercialization further down the line. Typical drug discovery within large pharmaceutical companies requires high throughput screening of potential targets to develop new therapeutics. However, this research & development (R&D) model is not conducive to cellular immunotherapy, and internal drug development has not worked well for advanced therapy medicinal products (ATMPs) within pharma, to date.

As a result, the vast majority of original research and novel cell/gene therapies have arisen from academic institutions. There is now growing acceptance and understanding in the importance of translational focus for early research within academia. Translational medicine is integral to the success of CGT; at the Royal Free Hospital – within a very small, highly skilled team – we adopt lab-scale preclinical research, design and validate closed bioprocess systems with Good Manufacturing Practice (GMP) compliant materials, author Chemistry and Manufacturing Controls (CMC) documentation for regulatory submission, and now manufacture commercial-scale GMP drug product. This development timeline has occurred within a 2-year period; a feat contract manufacturing organizations (CMOs) and pharma alike are not adept to complete. And yet, it is not an isolated achievement, but one which has been mirrored throughout small academic units across the CGT industry.

WHY CELL/GENE THERAPY?

ATMPs, particularly autologous therapies, do not fit the existing traditional pharma model of large batch manufacturing with pharmacy product dispense. Early stage ATMP clinical trials predominantly treat a small number of patients, targeting rare indications. Often the skill sets required for this manufacture are not met within pharma: an amalgamation of pharmacists and chemists vs biologists and translational scientists. As Table 1 shows, many products have been taken successfully to market that were originally developed from academic units.

Academic manufacturing units promote success for the university, for the patient group, and for (likely) commercial spin-out. But how can translational success, from drug development to clinical trials (and beyond), be achieved in such a relatively short timeline?

SME knowledge of the drug product will be unparalleled, however, their experience with GMP manufacturing and bioprocess futureproofing is often limited. Within the CGT field, C(D)MO capability is limited, and by selecting this route at such an early stage, a company will often lose the ability to take ownership of product manufacture and the knowledge gained which is vital for successful future development of a commercial product. Additionally, contract research organizations (CROs) can manage trials and the regulatory burdens, but little internal product development is feasible through CROs/CMOs pathways.

Small academic manufacture units have widely been able to marry these three operations: the product knowledge and technical skill of small and medium-sized enterprises (SMEs), the GMP production prowess of CMOs, and clinical expertise of CROs. As a result, often the transition from early research, through qualification and validation studies, clinical trial application submission to clinical manufacture can be performed under one roof. This can significantly reduce the cost, time, and complexity, de-risking the 'valley of death' between research and late phase trials.

Not only this, but small GMP units are forming direct relationships with SMEs to provide expertise and facility space for early clinical manufacture, in addition to internal product development. Multiple units are now using this as a symbiotic relationship, where both parties benefit as an alternative model. The healthcare unit will benefit from leasing out excess capacity, and the biotech company is able to move rapidly through their product timeline. This 'hotel' model allows a company to manufacture within an existing facility under their existing quality management system and license. The company is therefore

DOI: 10.18609/cgti.2020.132

TABLE 1

Companies with cell and gene therapy products and their associated academic links.

CGT product	Commercial entity	Academic roots
Strimvelis®	Orchard Therapeutics (previously GSK)	San Raffaele Telethon Institute for Gene Therapy
Holoclar®	Chiesi Farmaceutici	University of Modena
Alofisel®	Tigenix/Takeda	Katholieke Universiteit Leuven/ Universiteit Gent
Kymriah®	Novartis	University of Pennsylvania
Imlygic®	Amgen (BioVex)	University College London
Yescarta®/Tecartus™	Gilead (Kite)	National Cancer Institute
Zynteglo®	bluebird bio	Paris Descartes University
Zalmoxis® (now withdrawn)	MolMed	San Raffaele Biotechnology Department
Pending clin. trials (multiple autologous CAR-T products)	Autolus	University College London
Pending clin. trials (clonal neoantigen T cells)	Achilles Therapeutics	University College London
Pending clin. trials (allogenic umbilical cord derived – MSCs)	Orbsen Therapeutics Ltd	NUI Galway
Pending clin. trials (gamma-delta T cells)	GammaDelta Therapeutics	King's College London
Pending clin. trials (AAV based gene therapies)	Freeline Therapeutics	University College London
Pending clin. trials (T cell therapy)	Adaptimmune	Oxford University
Pending clin. trials	BioNTech	Mainz University
Pending clin. trials (allogeneic CAR-T cells)	Pfizer/Allogene	University of California
Pending clin. trials (Car-T cells)	Juno/Bristol Myers Squibb	Memorial Sloan-Kettering Cancer Research Center/ Seattle Children's Research Institute
Pending clin. trials (allogenic CAR-T cells)	Cellectis	Institut Pasteur
Spherox®	Co.don	University of Heidelberg

able to train up and retain its own staff to manufacture within the unit, so technical expertise is not lost once the company outgrows the small facility. At which point, clinical development of the product is de-risked sufficiently to enable expansion to a large facility where scale up/out can be achieved, such as the Cell and Gene Therapy Catapult Centre. This pathway significantly reduces the time that would otherwise be taken for commercialization.

THE ACADEMIC UNIT PARADIGM

So how is this amalgamation of skills achieved within one site and how is the environment within these small units conducive to cell therapy specifically?

There are numerous situational and objective advantages, which can be broadly divided into four categories: expertise, the approach to risk, finance opportunities, and logistical advantages.

Expertise (of product type)

Many academic/healthcare manufacturing units were born through bone marrow processing laboratories or similar hematological procedures. These treatments precede the definition of ATMPs brought into force by the EC regulation 1394/2007. However, FACT and JACIE accreditation (required for their operation) align well with current cell therapy regulation. As a result, processing standards, as well as staff training, facilitate working with live biological products. The experience from working with viable cellular products is interwoven with the flexibility and bioprocess challenges required for ATMP manufacture. Technical staff are adept at aseptic manipulation of small-scale cell therapies.

Project diversity for ATMPs is high with many unique bioprocesses (in Phase 1 especially), so industry is dependent upon academic groups to conduct high risk early research which requires skilled, specialized staff. The structure of teams within academic teams are

cross-functional, with each member responsible for multi-task projects with broad job descriptions. Due to their academic background, many staff have a strong scientific education – over half the senior managers at the CCGTT hold a PhD or equivalent qualification – and possess a diverse skill set given their wide remit and knowledge base. The speed of communication, and the ability to implement change quickly and effectively, is increased by direct access to highly trained individuals who each possess actionable, decision-making capability. As the remit of the facility is focused on early stage clinical trials, there is a wider diversity of products that lends itself to a breadth and depth of knowledge across the field in order to successfully implement these therapies.

Risk

A high success rate within ATMP pipelines at small academic units is now often expected, frequently with a 'make it happen' approach. In no small part, this is due to the higher risk tolerance in comparison to industry. Minimal criteria to demonstrate safety is prioritized, with no Process Analytical Technology (PAT) integration or reduced regulatory affair demand. The initial procedural setup is rapid: from first contact to information transfer, establishing agreements and documentation; from bioprocess design to GMP manufacture and clinical testing. The communication of stakeholders throughout the development and production process is vastly simplified than through an industrial platform and permits the acceleration of project uptake and deliverable timelines. For quality control of materials, each reagent/consumable is risk assessed and its impact on quality and safety reviewed (as per Guideline on GMP specific to ATMPs: EudraLex Volume 4). There is much less tolerance for a risk-based approach outside of academic settings.

The speed of process development (PD) to GMP is impressive, and an academic environment suits cell therapy. Variation is acknowledged and anticipated, as culturing biological material is inherently variable, particularly when the starting material for the resulting product is procured from the patient. CGT is robust in terms of procedure: there are no chemical pathways with associated impurities to remove, no misfolding of proteins, no heterodimers, and as such, critical process parameters are less strict/less heavily defined. Excipients are comparable across the field, particularly those for cryopreserved products and academic and healthcare labs provide accessibility to product licensed materials.

Logistic relationships

Academic units are advantageously connected, often both literally and figuratively, to a hospital or treatment center; frequently located on the same campus. This relationship permits the ability to get reagents from pharmacy with ease, but more importantly, facilitates a direct link to the clinical site and patient groups. For products with a limited shelf life, drugs can be directly transferred from the academic unit to the patient much simpler than from a large centralized manufacturing facility. For example, the GOSH cell therapy manufacturing facility exists within Great Ormond Street Hospital; the Centre for Cell, Gene & Tissue Therapies is housed within the Royal Free Hospital, London; and the Advanced Therapies Facility in Birmingham is connected with not only the NIHR clinical research facility, but also the Queen Elizabeth Hospital. This proximity enables starting material procurement, product manufacture, and point-of-care treatment to occur on the same site; an ideal model for autologous products. There are also opportunities to support point-of-care manufacturing within this model in the near future.

Finance

The prevalence and success of academic manufacture units is indicative of their success. Part of this accomplishment is due to the diversity of finance streams available. Academic institutions

DOI: 10.18609/cgti.2020.132

often have access to funding opportunities through grants to pursue research and innovation which are not permitted to commercial entities. In the UK, Research councils such as the MRC, BBSRC or NIHR can be applied for to support healthcare research and translation (although access to funding will vary internationally). Additionally, synergy with spin-out companies and SMEs allow for applications to be made for private or small business support, such as Innovate UK grants (who also support translational research). By merging the boundaries between clinical research and commerce, support for internal and external development of products can be provided in a more lucrative fashion (Box 1).

Commercial success

Academic small manufacturing units have access to knowledge and staff which benefit ATMPs, and access to funding multiple funding streams. They have a relationship with risk that permits rapid, high-success rate translation, and proximity and ties to hospitals which assist in procurement and product delivery. Although the field is still in its infancy, due to these advantages, many successful products have been developed through this platform. As a result, an increase in commercial interest and opportunities have been created which has enabled mutually beneficial relationships between SMEs and academic units to flourish. Unlike CMOs, academic units provide stake-holders with the ability to retain product control and oversight, whilst increasing their workforce's knowledge and improving their product pipeline and further procedural development – the trifecta of SME, C(D)MO and CRO backing. For the GMP unit, working with an SME provides access to much needed capital, additional staff and expertise depth (Table 2).

BOX 1

Academic MHRA-licensed GMP manufacturing facilities in the UK

Multifunctional cell and gene therapy manufacturing facilities

- GMP Cell Therapy Manufacturing Facility, Cellular Therapeutics Ltd (University of Manchester)
- Centre for Cell and Gene Tissue Therapeutics, Royal Free Hospital London
- King's College London, Rayne Cell Therapy Suite (RCTS) and the Cell Therapy Unit (CTU) with the NIHR Welcome Trust King's Clinical Research Facility

Dedicated gene therapy manufacturing facilities

- University of Oxford, Clinical BioManufacturing Facility (CBF)

Dedicated cell therapy manufacturing facilities

- Great Ormond Street Hospital (GOSH), Great Ormond Institute of Child Health
- John Goldman Centre for Cellular Therapy, Imperial College London
- Moorfields Eye Hospital, Cells for Sight Stem Cell Therapy Research Unit
- National Institute for Health Research (NIHR) Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London (GSTT BRC)
- Newcastle University, Newcastle Cellular Therapies Facility
- RoslinCT (spin-out from the Roslin Institute)
- University of Birmingham, Advanced Therapies Facility
- University of Manchester Cleanroom Facility

Data from the Cell Therapy Catapult's Cell and gene therapy GMP manufacturing in the UK report, 2019.

TABLE 2

Comparison of SME manufacture through a CMO versus academic GMP manufacturing unit.

CMO manufacture	Academic GMP unit manufacture
Devolution of control	Retention of process ownership; increased PD and drug knowledge
Financial burden	Reduced cost for increased value
Complexity of tech transfer	Minimal demand; retention of manufacturing knowledge
No benefit to staff competence	Increased workforce competence; training records and GMP skills gained
Barriers to process change	Transition to scale up/out simplified
Slow chain of communication	Ability to solve problems in real time
Slow GMP transition	Rapid GMP transition
No access to CMO quality system	Access to Quality Assurance (QA)/Quality Control (QC) from GMP unit

A common business model is: the generation of IP through academic research, early venture capital (VC) or comparable investment, initiation of a spin-out company, staff recruitment followed by operational activities to build from an academic unit (see Figure 1). Academic units are not likely to take products to market themselves, but act as a critical launchpad to get therapies into trials sooner.

Many of these spin-out companies are part owned by universities, which benefit young academics, the institution, and the CGT field alike. Through the likes of Syncona in the UK (Syncona is a FTSE250 company, focused upon life sciences investment to translate and deliver innovative healthcare treatments for patients. They found, build and fund healthcare companies to commercialize science in areas of unmet clinical need), it is now commonplace for promising research to be spun-out from academia to form small biotechnology companies, with limited hurdles to get through and the retention of expertise. For example, Freeline Therapeutics and Autolus, two companies born from humble academic research, have rapidly become world leaders within the field and now are publicly traded companies. The integration of SMEs within academic units has not only been hugely influential upon the success of each party, but CGT globally.

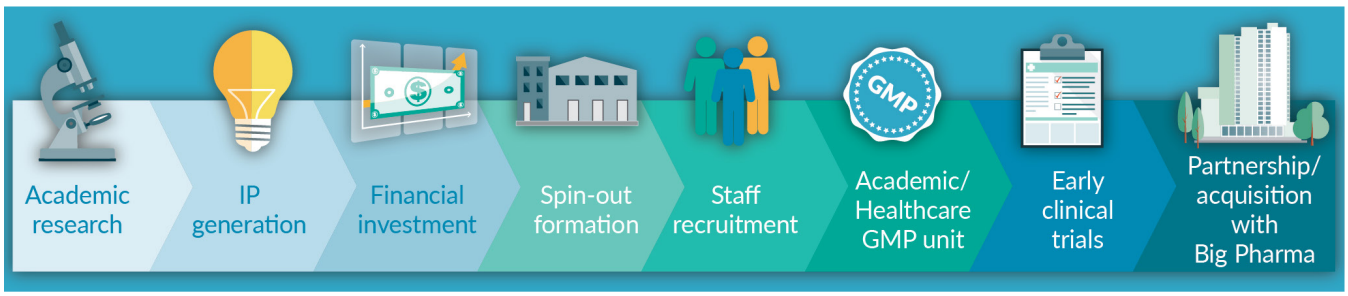
Furthermore, the majority of ATMPs commercialized are now being spearheaded through large pharmaceutical companies. Their original research and manufacture was conducted at an established licensed facility, already in place within close proximity to the clinical need. Then, thanks to successful early clinical operations, de-risked products owned by SMEs can be commercialized and operations expanded through partnerships with larger companies who possess the infrastructure to meet global demand.

THE FUTURE FOR CGT

Academia and small healthcare units continue to shape progress and success within the CGT field. The pandemic, SARS-CoV-2 virus, has caught many unprepared and with insufficient strategy for rapid response.

FIGURE 1

Business model flowchart for the commercialisation of academic research within cell and gene therapy.



DOI: 10.18609/cgti.2020.132

However, the urgency of COVID-19 treatment has put a spotlight on the small academic units, supported with big Pharma partnership.

The Oxford COVID-19 vaccine team has worked at unprecedented rates to design and take a novel drug through clinical development, GMP manufacturing, and first-in-human trials. This accelerated rate to initial clinical manufacture, within the Clinical BioManufacturing Facility at Oxford, has occurred within a small manufacturing facility through academia. Industry does not have access to the same expertise, risk profile, treatment options and funding as academic healthcare groups. Following early clinical success, partnership with AstraZeneca will enable expansion and a globalized manufacture strategy. This powerful development platform is acting as a springboard to many new products coming through the CGT industry.

Longer term, there are still challenges to address for small academic GMP facilities: implementation of large-scale allogeneic medicines, the resilience of academic groups under extreme funding pressure, and future proofing knowledge and expertise within small units (without the benefit of commercial memory). As the field continues to expand, another challenge is confidentiality for multiple commercial entities working alongside each other in smaller facilities with limited space and resource allocation. Additionally, due to international challenges with funding, the procurement and manufacture of new raw materials for which no pharmacopeia – grade variants are available, and the abundance of capital and increasing interest from big Pharma (Novartis, Gilead, GSK, Takeda, Roche etc.), the current 80–90% ratio of products born from academic sites may decrease across the next decade. Nevertheless, CGT growth is still intrinsically linked with the diverse and rich product pipeline generated through small academic/healthcare units, particular within cellular therapies for oncology.

For further reading, or a perspective of academic/healthcare GMP units from Europe and the United States, please see [1–4].

REFERENCES

1. Bersenev A, Fesnak A. Place of Academic GMP Facilities in Modern Cell Therapy. *Methods Mol. Biol.* 2020; 2097: 329–39.
2. Digiusto DL, Melsop K, Srivastava R, Tran CT. Proceedings of the first academic symposium on developing, qualifying and operating a cell and gene therapy manufacturing facility. *Cytotherapy* 2018; 20(12): 1486–94.
3. Iancu EM, Kandalaft LE. Challenges and advantages of cell therapy manufacturing under Good Manufacturing Practices within the hospital setting [published online ahead of print, 2020 Jul 11]. *Curr. Opin. Biotechnol.* 2020; 65: 233–41.
4. Viganò M, Giordano R, Lazzari L. Challenges of running a GMP facility for regenerative medicine in a public hospital. *Regen. Med.* 2017; 12(7): 803–13.

AFFILIATIONS

Benjamin D Weil
Author for correspondence
Department of Haematology, University College London, Rowland Hill Street, NW3 2QG, London, UK
and
Centre for Cell, Gene & Tissue Therapeutics, Royal Free Hospital, London, UK
ben.weil@nhs.net

Owen Bain
Centre for Cell, Gene & Tissue Therapeutics, Royal Free Hospital, London, UK

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors' work influences their opinions on small GMP academic units in the UK.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Aug 25 2020;* ***Revised manuscript received:*** *Sep 17 2020;* ***Publication date:*** *Sep 24 2020.*

DOI: 10.18609/cgti.2020.132

CELL & GENE THERAPY INSIGHTS

SCALING UP/OUT: COST-EFFECTIVE & ROBUST TRANSITIONIONING THROUGH THE CLINIC TO COMMERCIAL MANUFACTURE

SPOTLIGHT

INTERVIEW

Driving NK cell therapy manufacture to commercial scale

LEONARD SENDER joined Nantkwest in 2016 as its Senior Vice President of Medical Affairs for Pediatric, Adolescent and Young Adult Oncology. Dr. Sender also serves as Executive Director of the Pediatric, Adolescent and Young Adult Cancer Breakthroughs 2020 Program for the Chan Soon-Shiong Family Foundation. Previously, Dr. Sender served as the Medical Director of the Hyundai Cancer Institute at CHOC (Children's Hospital, Orange County). Prior to that, Dr. Sender served as the Medical Director of Clinical Oncology at the University of California's NCI designated Chao Family Comprehensive Cancer Center. Dr. Sender collectively brings more than 25 years of experience treating pediatric, adolescent, and young adult and adult cancer patients, and has been the recipient of a $10 mm Hyundai Hope on Wheels grant to study the genomic basis of pediatric and adolescent and young adult cancers to test the hypothesis that genomic knowledge can enable clinical decisions related to treatment options. In 2010, he founded two entities critical to the development of the emerging adolescent and young adult (AYA) oncology subspecialty as President of the Society for Adolescent and Young Adult Oncology (SAYAO) and Editor-in-Chief of the Journal of Adolescent and Young Adult Oncology (JAYAO). Dr. Sender received his MD from the University of the Witwatersrand in Johannesburg, South Africa and subsequently trained in pediatrics at the University of California, Irvine and in pediatric hematology-oncology at Children's Hospital Los Angeles.

Cell & Gene Therapy Insights 2020; 6(8), 1179–1182

DOI: 10.18609/cgti.2020.129

Q What are you working on at the moment?

LS: Right now we are working on a number of products. One of them is a PD-L1 t-haNK, which is our natural killer cell (NK) product. This is derived from the NK cell NK-92, and is the latest version that allows us to take advantage of the chimeric antigen receptor (CAR) construct, to make what we call an NK-CAR against PD-L1. It is a very powerful cell therapy.

PD-L1 t-haNK is a high affinity NK cell, so it has the high-affinity CD16, as well as having the CAR construct against PD-L1. We have an active trial for metastatic pancreatic cancer, both in first-line maintenance, as well in second-line and greater therapy. This cell is an off-the-shelf allogeneic product, and we've developed the capabilities to produce potentially thousands of doses so that we can treat patients.

Q Can you give us some further background on NantKwest's NK cell therapy platform and in particular, its bioprocess development story to date?

LS: As I mentioned, this is a NK cell that is off-the-shelf, and it was discovered and derived from a patient sample in 1992. The intriguing feature of the cell is that it has maintained a very activated form. They normally activate in inhibitory receptors on NK cells but this one is already activated, which means it is ready to kill tumor cells that it recognizes. The platform has evolved over time, firstly to the standard activated NK (aNK) cell, which was originally NK-92, and then to haNK (high affinity NK).

Only a small percentage of the population has high-affinity CD16 receptors, and CD16 is needed for antibody-dependent cellular cytotoxicity (ADCC). Therefore, haNK was genetically modified to have high-affinity receptors to make it even more productive at killing when using ADCC.

We have further developed the technology and the cells: taNKs are NK cells engineered to incorporate chimeric antigen receptors (CARs) to target tumor-specific antigens found on the surface of cancer cells, while t-haNKs are an innovative combination of our haNK and taNK platforms in a single cell. We've initially chosen to target PD-L1 with our t-haNKs. You can't make a PD-L1 CAR-T, because you can't have them activated all the time. However, one of the unique features of NK cells is we can have multiple doses, and also repeat those doses over time – we are able to give this to patients repetitively and very safely. Our randomized trial in pancreatic cancer is going to investigate the use of these PD-L1 t-haNKs, looking at both their ability to kill the tumor and also other effects, such as affecting the myeloid-derived suppressor cells.

> "PD-L1 t-haNK is a high affinity NK cell, so it has the high-affinity CD16, as well as having the CAR construct against PD-L1."

This is our main NK cell platform, but we are able to make many different CAR constructs

DOI: 10.18609/cgti.2020.129

against many different tumor antigens. We will have others coming down the pipeline that are being generated right now to follow PD-L1.

"The only way NK cells can work in the long-term is if you can successfully cryopreserve them..."

Q Can you talk further about the key requirements and considerations for the technology with regards to scalability?

LS: Some of that is proprietary, but essentially, we have also developed the technology and scalability to produce billions of cells. The dose that we give per infusion is 2 billion cells. We have successfully mastered the manufacturing capabilities of large bioreactors to produce at this scale, and we have developed the ability to irradiate and cryopreserve those cells.

The most significant change we made was the ability to cryopreserve the cells. The only way NK cells can work in the long-term is if you can successfully cryopreserve them, and then ship them around the country and make them on-demand as an off-the-shelf product. We have successfully done that with a good chain of cold custody so we can transport these cells to clinics, and then allow the clinics to infuse them.

The other advantage of cryopreservation is that we have the ability to use this as an outpatient therapy. CAR-T has to be given in a hospital – normally in the intensive care unit – because of the adverse events that can occur, such as cytokine release syndrome. We do not see a cytokine release syndrome with these cells because they work in a different way, so this can be done very successfully as an outpatient procedure.

Q What scale-related challenges are you facing as you continue to move through the clinic and towards commercialization?

LS: We are probably producing more NK cells than anyone else on the planet. As we build our plants, we are figuring out the sort of units that we will need when we go to scale, which for us is being able to make enough cells for 40,000 patients.

Right now, we want to produce enough cells for clinical trials. But eventually, the goal is to get to the thousands of patients who may benefit from this therapy. That will require further scaling, and we think we have figured out how to do this so that we can eventually make trillions of cells.

Some of the things we are doing in development will be unique to us. In order to scale, we will be developing some new machinery, doing things that have never been done before. Addressing issues of scalability is what we are focused on right now.

From a supply chain point of view, I think the biggest problem right now is the Covid pandemic. There are stops and starts for every company and it is very difficult to rely on anything, as any part of the supply chain can be interrupted and experience shutdowns and surges. Living in the time of Covid has certainly made everything much more complicated.

Q Is there anything you can share about the technology you require to operate at commercial scale?

LS: Number one is that we have had to come up with innovative approaches as to how we can irradiate the cells. To do this at scale, there are no machines that exist *per se*. To get to where we freeze as many cells as we do, we have had to come up with innovative machinery, and figure out how to do rate-controlled freezing at scale. This is ongoing right now and we are learning as we go. Everything we are doing is leading to new machines, new development, and we are also working with other companies.

Q Can you sum up for us both your own and NantKwest's chief priorities for the 12-24 months ahead?

LS: I am an SVP for medical affairs, and I am also an oncologist, so I am very close to patients. As someone who has a background in bone marrow transplantation and cellular therapy, this is a very important opportunity to do something fantastic for these patients.

Our main goals are around using two types of cells that we are developing for treating cancer. One is this off-the-shelf product we have discussed, the NK92-based platform. The other is one we will be launching later this year, which is a primary NK cell derived from peripheral blood. We have developed the ability to individually make products for patients, and this is called a ceNK (cytokine expanded NK) cell. We have made one of these that is allogeneic, and one that is going to be autologous. We're very excited about that, and we are working towards getting that product into clinical trials in the latter part of this year.

AFFILIATION
Leonard Sender
Senior Vice President of Medical Affairs for Pediatric, Adolescent and Young Adult Oncology at Nantkwest

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *Dr Sender is employed by NantKwest.*

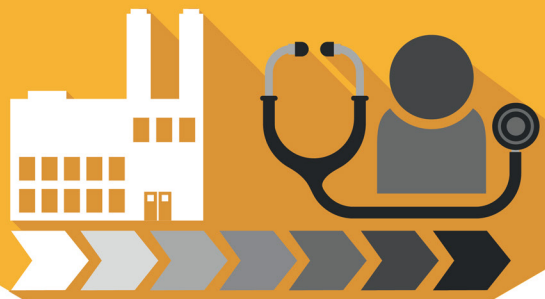
Article source: *Invited.*

Submitted for peer review: *Aug 25 2020;* ***Revised manuscript received:*** *Sep 14 2020;* ***Publication date:*** *Sep 23 2020.*

DOI: 10.18609/cgti.2020.129

CELL & GENE THERAPY INSIGHTS

Supply Chain Channel



BIOPRESERVATION & COLD CHAIN MANAGEMENT

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN CHANNEL: Biopreservation & Cold Chain Management

SEPTEMBER 2020

Volume 6, Issue 8

INTERVIEW

How will COVID-19 impact the cord blood banking sector?
Wouter Van't Hof
663–669

INNOVATOR INSIGHT

Biopreservation and cold chain biologistics risk points in the cell and gene therapy workflow
Todd CJ Berard & Aby J Mathew
1363–1380

INNOVATOR INSIGHT

The supply chain: key considerations for biological starting material for ATMPs production
Elisabet Aguilar
1135–1140

EXPERT INSIGHT: STARTING MATERIAL OPTIMIZATION

Utilization of risk-based approach to characterize starting material for autologous CAR-T manufacturing
Jean Stanton
1067–1078

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: BIOPRESERVATION & COLD CHAIN LOGISTICS

CHANNEL CONTENT

INTERVIEW

How will COVID-19 impact the cord blood banking sector?

WOUTER VAN'T HOF holds a PhD in Cell Biology from Utrecht University in the Netherlands, and has over 15 years of biotech experience in the USA in translational research and development of adult stem cell therapies, including bone marrow stromal cells (MSC) and HPC, cord blood. He is currently Cord Blood Bank Director of the Cleveland Cord Blood Center (CCBC). Under his direction, CCBC obtained FDA approval for the manufacture and distribution of HPC, Cord Blood under federal license, as one of only eight nationally licensed cord blood banks in the USA. As Cord Blood Bank Director he oversees Laboratory Operations, including CMC, Process Validation, Aseptic Processing, and GMP compliance. In addition, Wouter leads the Cell Therapy Incubator (CTI), a new CCBC initiative facilitating internal and external programs for broader development of cord blood cell-based therapies in regenerative medicine. From 2002 to 2013, he was a Director at Athersys, Inc., with responsibility for technology transfer, product and process development, preclinical safety, and was deeply involved in regulatory discussion for clinical study design and management of a GVHD prophylaxis trial. He was the scientific lead on the completed Phase 1 safety study in HSCT support for the MultiStem Product. During his academic career, Dr Van't Hof was an Assistant Professor of Cell Biology in Medicine, Department of Medicine, Division of Pulmonary and Critical Care Medicine, and Assistant Professor of Genetic Medicine, Institute of Genetic Medicine, Weill Medical College of Cornell University.

Cell & Gene Therapy Insights 2020; 6(5), 663–669

DOI: 10.18609/cgti.2020.078

Q What are you working on right now at your cord blood bank?

WVH: My main responsibility in my day job as Cord Blood Bank Director at the Cleveland Cord Blood Center is ensuring that the laboratory operations for cord blood processing, archiving and distribution are functioning properly, and remain in sync with the upstream cord blood collections and the downstream quality review for timely product release for hematopoietic cell transplant. As the only public cord blood bank in the State of Ohio, we procure cord blood at 5 collection sites: two in Cleveland, OH, two in Atlanta, GA, and one in San Francisco, CA. The other locations were chosen to increase collection of cord blood units from minority groups that remain underserved in the cord blood inventories. Since its start in 2008, CCBC has collected more than 70,000 cord blood units. On a daily basis these collected units are shipped to the processing facility in Cleveland, OH, for centralized processing, cryopreservation and frozen storage. CCBC currently has over 10,000 frozen clinical-grade units in inventory and listed in the NMDP and WMDA searchable databases. To date, we have shipped more than 670 cord blood units to transplant centers throughout the USA and in 17 countries world-wide. We are one of only 8 FDA licensed cord blood banks in the USA and remaining compliant and dealing with regulatory inspections requires ongoing attention. Here I am very fortunate that our small organization does an outstanding job in systematically and efficiently dealing with licensure and accreditation expectations. I am very proud of our CCBC staff, some of whom have been with CCBC from the very early days in 2008 and 2009, much longer than my own involvement. With the compliance programs on track and well monitored, the door opens for me to work on areas of organizational need and professional interest.

Q Can you describe those mentioned incremental cord blood needs and interests?

WVH: Another core aspect of our mission is to make cord blood that is not used for processing available for research and development. Our center has provided over 11,500 research-grade units to investigators in both academic and biotechnology organizations. We believe this is crucial, both for the future of our cord blood bank and for the industry. Along this line, CCBC, which is a non-profit entity, established two social enterprise subsidiaries in 2020, to focus on the next iteration of its mission. Enabling the use of donated cord blood, beyond HCT, into the bigger realm of regenerative medicine is a major objective of these new efforts. So, the other part of my day job is leading the Cell Therapy Incubator (CTI), one of the two new CCBC subsidiaries. The CTI is housed in a

"Ultimately, we want to support manufacture of clinical grade CD34 or cord blood derived cell products."

DOI: 10.18609/cgti.2020.078

"Transmission of infectious agents from tissue or blood cell donors to recipients is a major regulatory concern, and COVID-19 would fall right under that. So if coronavirus would be detected in cord blood, we may be asked to include coronavirus testing for product release and reject at risk units. That could have a great impact on units produced since late 2019."

separate facility with GMP capability, built for execution of programs that increase utilization of collected cord blood, and/or support development of new cord blood based cell therapy products or technologies. Initial CTI projects are producing non-clinical grade isolated CD34 cells from cord blood. We see increased demand and opportunity for this as a consistent source material in the biotech environment, with a growing number of companies developing cord blood derived NK cells, Tregs and other specialty products. Ultimately, we want to support manufacture of clinical grade CD34 or cord blood derived cell products. For now, the nonclinical production arm boosts higher utility of collected cord blood, which is a cornerstone of the CCBC mission. In these beginning stages of the subsidiary, I am pursuing new collaborations and contracts. With new research funding opening up for COVID-19, we are receiving many requests for our clinical grade materials, so that is keeping us busy at the moment.

Q Can you outline the technical procedures in your cord blood cell banking and processing work, including those relating to biopreservation?

WVH: Our processes are based on US regulatory and international accreditation compliant procedures and technologies, standard in the industry, with certain specific iterations. We only collect cord blood via umbilical cord puncture in utero, rather than from the delivered placenta, using a single use FDA cleared collection bag set containing CPD anticoagulant. This approach minimizes contamination risk during collection. It also allows for collection at shorter time after delivery, with better chance of obtaining the required volume and cell numbers. If those requirements are met, collected cord blood is then processed. CCBC uses the AXP AutoXpress™ System from Thermogenesis. This is a semi-automated process using centrifugation to separate cord blood into three separate fractions, red blood cells, white blood cells, and a RBC/plasma fraction obtained by volume reduction of the white cell fraction. The white blood cell fraction, containing the desired hematopoietic stem and progenitor cells, becomes a minimally manipulated product, specified as HPC, Cord Blood. This

"...physical cord blood quarantining is a very important aspect of inventory protection and is now demonstrating its relevance in the context of the current COVID-19 pandemic."

final product is formulated in a 25 mL volume, supplemented with 10% DMSO and 1% dextran. The freezing bag itself is divided into a 5 and a 20 mL part, both sealed to allow future use separately, where desired. Importantly, before freezing, each HPC, Cord Blood unit is placed into a sealed overwrap bag to minimize any cross-contamination risk during storage. We must keep in mind that product sterility testing is not completed until 2 weeks after freezing, and that there is a 5–10% baseline for cord blood contaminations, mostly related to collections. This physical cord blood quarantining is a very important aspect of inventory protection and is now demonstrating its relevance in the context of the current COVID-19 pandemic.

As is standard in our field, cryopreservation must be initiated within 48 hours of the time of cord blood collection. The start is defined as the insertion of the canister with the processed unit into the automated, controlled-rate freezing element of the BioArchive® System from Thermogenesis. BioArchives® are big liquid nitrogen units or dewars that accommodate long-term storage of up to about 3,600 units in liquid nitrogen at -196°C, with continuous monitoring. Associated computer modules assign a specific address to each frozen unit inside the freezer inner storage structure, allowing for controlled retrieval. Each stored product is tested for purity, identity, sterility, and potency. Upon batch record review, units complying with donor eligibility and product requirements are released from administrative quarantine and made available for search by transplant centers. We ship the majority of our units through the National Marrow Donor Program logistics system. There is obviously much more underlying detail, but this is the gist of the technical aspects around our inventory and its use.

Q You mentioned COVID-19. Can you frame for us the potential threat it presents to the cord blood banking field?

WVH: As for anybody else, all of our staff are directly impacted by the federal, state and local stay at home directions. We have implemented a minimal staffing strategy to ensure sufficient staff presence on site to monitor and manage our liquid nitrogen storage systems, and to accommodate any cord blood unit requests for transplant. This has worked out well, and we have continued to ship out units efficiently, a few of those within 24 hours of receiving the request. This process requires final review by operations, medical and quality staff members, most working from home, but it is good to see we can handle this under the current societal constraints caused by the pandemic.

DOI: 10.18609/cgti.2020.078

With respect to our products, the general threat of COVID-19, as with any tissue, blood or cell contaminant, is in theory very serious and could endanger all ongoing collections, our future products, and their use. We don't think that's what is actually transpiring. At this time, (early May 2020) outside of the risk for staff in delivery wards, the reality looks like the cord blood industry might hopefully be spared from major harm. COVID-19 is widely understood to not migrate from the mother via the placenta to the cord blood, minimizing risk for the baby. This is a very different scenario from the Zika virus threat a few years back. Absence of COVID-19 in cord blood also means it remains safe to collect and process donated cord blood and it justifies continued cryopreservation of cord blood collected during the active pandemic. Our cord blood collection sites have mostly remained open and actively collecting. Processing at our center in Cleveland has also managed to continue under minimal staffing strategies, with appropriate and workable social distancing procedures. We have been encouraged by production rates remaining very similar as to prior to the pandemic. This may not be the case for all public cord blood banks.

A hidden threat could be that with emergence of more sensitive tests, this coronavirus might in the future actually be found in cord blood stored during the pandemic, with different associated risks. First, in each BioArchive® unit, all frozen units are submerged in a singular liquid nitrogen supply, not in the liquid nitrogen vapor phase. In theory, over the commonly long storage times of cord blood, virus could leak from contaminated frozen cord blood bags, compromising an entire inventory within a shared BioArchive® unit. This is a possible, but very unlikely risk scenario. It would require virus to survive long-term in liquid nitrogen and pass through the walls of two different types of bags. As mentioned earlier, all cord blood units are individually wrapped within protective overwrap bags, so the risk for spread of infectious organisms from contaminated bags and subsequently into 'clean' products is really very minimal. In terms of use of the products, coronavirus contaminated units obviously would not be acceptable for transplant, especially in immune-compromised recipients. Transmission of infectious agents from tissue or blood cell donors to recipients is a major regulatory concern, and COVID-19 would fall right under that. So if coronavirus would be detected in cord blood, we may be asked to include coronavirus testing for product release and reject at risk units. That could have a great impact on units produced since late 2019. On a side note, but related to this topic, current prophylaxis strategies for transplant include more potent combinations of antibiotic, antifungal and antiviral agents. This allows transplant physicians nowadays more aggressive risk-benefit considerations with 'risky' cord blood products, within reason, and especially for patients in immediate critical need. Such considerations are made, for example, for cord blood carrying CMV risk. There also appears to be different responses to COVID-19 between children and adults, and the risk-benefit balance may have different answers for different age groups. Again, these are theoretical considerations, I am not a transplant physician, and in no way should

"...protecting our bank from the COVID-19 impact will involve closely following new regulatory guidance and ... technology development..."

this be construed as a defensive or self-serving statement, advocating risky strategies to benefit use of potentially coronavirus-tainted cord blood. On the contrary. But things do change over time, and a current contamination risk, perceived or not, may become less of an acute problem – for example, with advancement of antiviral agents.

Q What's the current consensus of opinion in terms of whether COVID-19 is having, or will have, an impact, and what steps are you and others in the field taking now to respond?

WVH: If anything, believe it or not, the coronavirus pandemic might actually result in broader use of cord blood as a transplant strategy. Procurement of adult hematopoietic stem cell products, which are bone marrow or mobilized peripheral blood derived, is impacted more directly by coronavirus. The involved collection procedures for adult grafts include more virus exposure risk between donors and collectors. Adult HSCT products are mostly used 'freshly', within short times (hours to days) after collection and with less opportunity for testing prior to transplant. Donations for bone marrow and peripheral blood have gone down, in large extend due to travel restrictions not allowing donors to get to collection centers. However, cord blood, as a frozen and tested product, obtained prior to the COVID-19 pandemic, remains readily available for safe use without virus transmission risk. It is too early to tell how this will play out, as all transplants have largely been put on hold by the worldwide lockdowns and stay at home directions in March and April of 2020. Any new trends in cord blood transplant as a consequence of COVID-19 will unlikely become evident before transplant centers open back up again sometime in 2020. Meanwhile, protecting our bank from the COVID-19 impact will involve closely following new regulatory guidance and keeping an eye on technology development for coronavirus testing in people and products. Finally, the scrutiny from our past regulatory and accreditation reviews, and numerous inspections, has taught us how to implement appropriate documentation and control systems. This will ensure required safety and activity of our cord blood products, even for those manufactured during a global threat of an infectious virus. I must tell you that some of these inspections are no picnic, and again, kudos to our dedicated staff. But under current circumstances, it is encouraging to know that we are following due process, to the best of our knowledge and in line with industry standards. We are controlling what is within our area of control. This awareness provides a great morale boost under strain, be it caused by Zika, COVID-19 or any future challenge.

AFFILIATION

Wouter Van't Hof
Cord Blood Bank Director, Processing Facility Director, Cleveland Cord Blood Center, OH, USA
wvanthof@clevelandcordblood.org

DOI: 10.18609/cgti.2020.078

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *May 11 2020;* ***Publication date:*** *Jun 5 2020.*

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: BIOPRESERVATION & COLD CHAIN MANAGEMENT

INNOVATOR INSIGHT

Biopreservation and cold chain biologistics risk points in the cell and gene therapy workflow

Todd CJ Berard & Aby J Mathew

Cell & Gene Therapy Insights 2020; 6(8), 1363–1380

DOI: 10.18609/cgti.2020.150

INTRODUCTION

The current state of regenerative medicine is a transformational period for cell and gene therapies. In addition to Novartis' Kymriah®, Kite Pharma's Yescarta® and Tecartus™, Spark's Luxturna®, AveXis' Zolgensma®, and bluebird bio's Zynteglo® blazing the commercialization trail, there are over one thousand Phase 1, 2, and 3 cell and gene therapies (CGT) in pipeline development [1]. Although this bodes well for patients, clinicians, industry, and investors, some unique aspects of cell- and gene-based therapies versus traditional pharmaceuticals or biopharma has highlighted the myriad of "new" manufacturing, clinical, and commercialization, challenges our industry now faces [2,3]. Independently, each one of these challenges presents its own unique set of risks. Furthermore, when lined up in sequence and aggregated together in the manufacturing chain, if each portion is not optimized and risk-mitigated, the subsequent impact to the CGT product may be a compounding of the risks; and the sum total of

all parts of the workflow will suffer. These beginning-to-end manufacturing risk points warrant appropriate assessment, and they are recommended to be addressed with the same diligence and priority as the therapies themselves, if the promise of Regenerative Medicine is to be fully realized. Fortunately, much has been learned regarding optimization of a number of key critical process parameters (CPP), and those looking to improve these parameters can leverage what has already been learned. This overview represents targeted lessons learned based on numerous experiences with CGT partners. Although intended to share feedback from experiences that may not always be detailed in the literature, it is not intended to address every aspect of the CGT workflow.

REPRESENTATIVE CELL IMMUNOTHERAPY WORKFLOW

Figure 1 is one representative CGT manufacturing workflow. Similar workflow representations, and related points of risk, have been outlined within a number of publications [4–8].

In common CGT manufacturing workflows, starting source material is obtained; and then is processed, selected, and/or isolated. Often, the material undergoes a biopreservation step (cryopreservation or hypothermic preservation), and transported to a manufacturing facility; where activation, transduction, expansion, and/or final formation take place, before additional transport/storage for clinical application. This workflow highlights several biopreservation and biologistics areas where CGT may be challenged:

1. Ensuring high quality starting material;
2. Optimizing viable functional recovery, and minimizing variability and risk, in process development throughout the workflow chain;
3. Determining appropriate conditions for source material, intermediates, and final product – non-frozen or frozen (and, optimizing the biopreservation steps by utilizing Biopreservation Best Practices [5]); and
4. Exploring and implementing enabling tools and technologies throughout the workflow.

Such tools might consist of: novel CGT processing and packaging technologies; next generation closed systems for fill, finish, and packaging; class-defining biopreservation media; high capacity-controlled rate freezers; cryogenic storage systems; 'smart' cold chain management systems (shipping containers, tracking, and reporting); and automated, water-free thawing equipment technologies. [The normothermic culture state of the cells is also a variable that can impact the quality of the cell product, however that is not a focus of this overview.]

ENSURING HIGH QUALITY STARTING MATERIAL

The importance of obtaining high quality starting material has been previously highlighted [4,5,7]. An early challenge in the CGT manufacturing workflow is ensuring high quality, and consistent, starting material. Cell-based manufacturing and therapies present a unique challenge that does not exist to the same complexity or criticality as with non-cell-based therapies – that difference being the needs, the vulnerabilities, and variability of, living cells. Cells embody an intrinsic variability of normal conditions, response, and function, that can influence the therapeutic efficacy. As such, CGT manufacturing should take into account the inherent variability of starting cell-based materials, as well as the processing methods for these living cells, that will eventually impact the quality of the therapeutic product.

The potential variability and quality of CGT starting materials have been an increasing focus of CGT concern, and has been discussion points of Cell & Gene Therapy Insights experts [4,7]. Those discussions have

DOI: 10.18609/cgti.2020.150

also presented evidence-based pathways for increasing the non-frozen or frozen stability, and/or minimizing variability, of cell/tissue starting materials [4–9].

NON-FROZEN OR FROZEN? CELLULAR RESPONSES TO COLD

It is important to ask a basic question: How can cell viable recovery and function be preserved throughout the manufacturing workflow, in order to facilitate efficacy? It is recognized that low temperatures can slow metabolic activity, reduce oxygen demand, and decrease degradation; but it may be beneficial to understand the benefits and limitations, in order to support biopreservation optimization and risk management of the process/product.

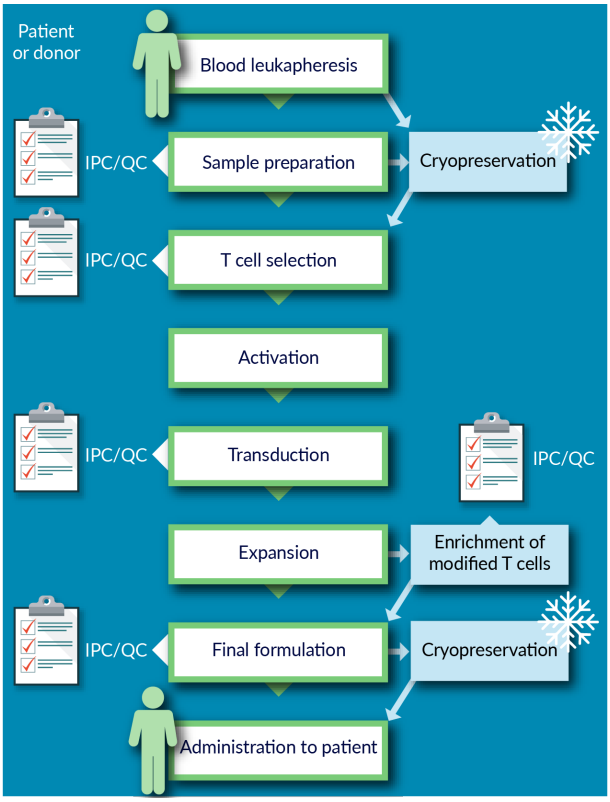
Figure 2 shows three states of cell/tissue application temperature (as primarily utilized in CGT manufacturing and biopreservation), and the relationship between temperature and cellular metabolic activity. At normothermic temperatures and conditions, the cell metabolic function should operate as designed to support activity at the cellular, tissue, organ, and organism levels. Under normothermic conditions, cells maintain homeostasis through a multitude of mechanisms, including ion pumps on the cell membrane and intracellular organelles. Ion pumps tightly regulate vital intracellular and extracellular ionic balance, which also impact osmotic balance, cell volume, etc. [5].

As temperatures decrease to hypothermic temperatures (below 37°C normothermic), lipid membranes undergo phase transitions: a type of structural change that results in loss of fluidity and continuity. Hypothermia induces phase transitions in the lipid membrane that lead to pore formation and loss of integrity. This leads to an influx and outflux of ions and small molecules due to the cross-membrane concentration gradients [9]. Under hypothermic conditions, there is deceleration of ion pumps and reduced ATP synthesis by mitochondria. Ion pumps then have a reduced capacity to regulate intracellular ions, leading to a myriad of issues. This further impedes restoration of ionic balance in the intracellular milieu. This disrupts the overall ionic balance, resulting in dysfunctions in intracellular cell signaling, salinity, osmolality pathways, osmosis, and cell volume, that previously relied on a tightly regulated cell balance. Osmolality and ionic distortions can induce mitochondrial stresses, which can initiate a cascade of adverse events within the cell by increased reactive oxygen species (ROS) and free radicals generation, and lipid peroxidation. When combined with membrane phase transitions, these phenomena can lead to membrane blebbing and other irreversible membrane injuries, among other mechanisms of cell damage and cell death [5,9,10].

Furthermore, in the absence of oxygen and normothermic conditions, glycolysis becomes the main source of limited ATP generation instead of oxidative phosphorylation, resulting in acidification of the intracellular

FIGURE 1

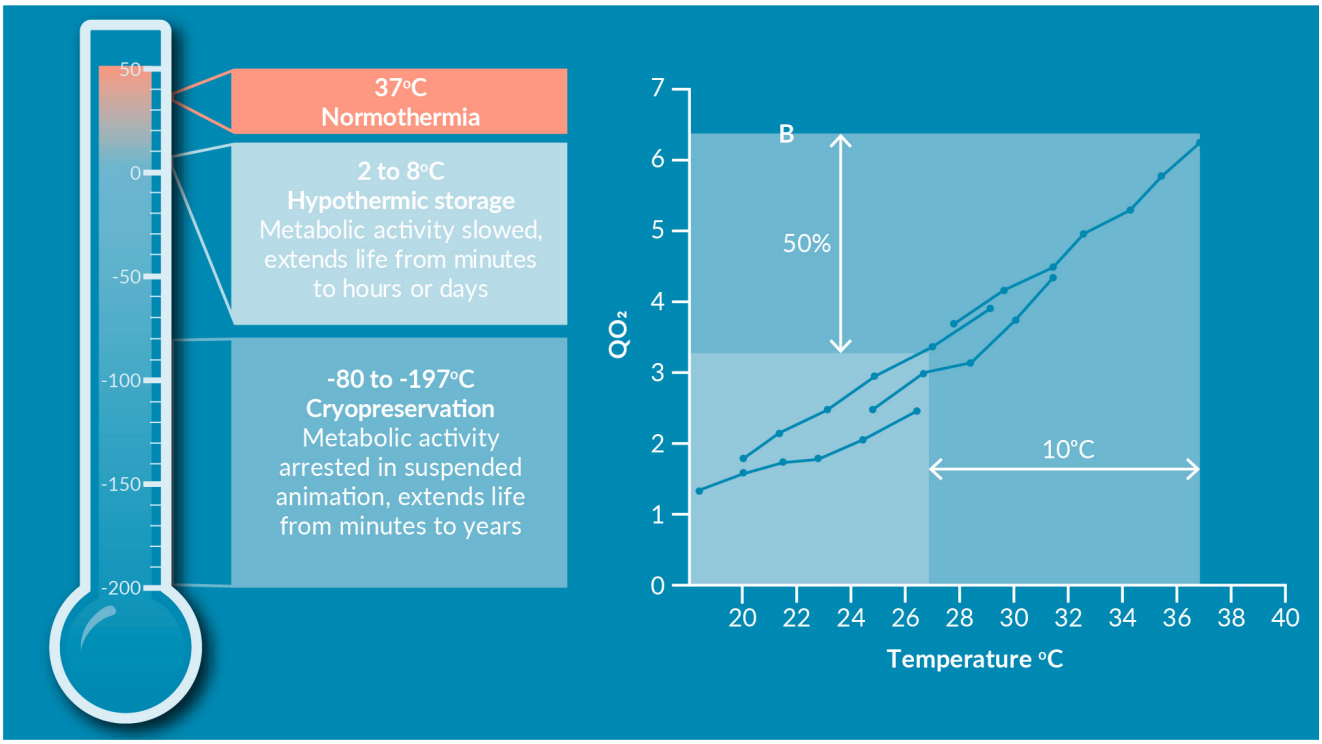
CGT manufacturing workflow.



Adapted from [8].

FIGURE 2

The relationship between temperature and cellular metabolic activity.



Graph modified from Fuhrman and Fuhrman 1959.

milieu. Changes in pH and salinity may irreversibly impact protein solubility and its functional structures, which are necessary for protein-protein interactions and trans-membrane positioning.

Temporal accumulation of these damages during hypothermic intervals and storage may eventually overflow beyond the tolerable limits for the cell, leading to irreversible activation of apoptosis, necrosis, and secondary necrosis cascades; at which point, the cell is lost. In addition, the actual onset of cell damage and cell death may not translate until post-preservation and re-warming, and may subsequently manifest as Delayed Onset Cell Death [5,10].

To alleviate some of these issues, an intracellular-like designed biopreservation media may be incorporated to replace traditional saline/culture media (or other formulations that mimic the normothermic isotonic ionic balance). By reducing the cross-membrane concentration gradient of ions during cold exposure, intracellular ionic balance and salinity would be less altered, even if membrane permeability is impacted. Biopreservation Critical Quality Attributes (BCQA) incorporate intracellular-like design, including impermeant (non-permeating) molecules such as large sugars, which exert membrane-stabilizing and osmotic-supporting effects, in order to mitigate cell swelling and membrane damage during storage. Free radical scavengers can decrease the burden of ROS. Also, buffers that are effective specifically at low temperatures, in contrast to traditional buffers for normothermic conditions, may be more effective at controlling toxic pH changes [5]. This intracellular-like approach to Biopreservation Best Practices is applicable to non-frozen hypothermic preservation and cryopreservation.

THE PHYSICS OF FREEZING

Another mode of cell and tissue biopreservation is cryopreservation. Hypothermia-induced

DOI: 10.18609/cgti.2020.150

acute stresses occur slowly and accumulate during the storage period. The accumulation of such adverse effects on cells usually trigger cell damage and cell death after hours to days in cold storage. On the other hand, acute cellular stresses during freezing conditions and cryopreservation occur within a relatively short period of freeze-thaw. For both modes of biopreservation, many cell damage and cell death effectors may only fully manifest over 24–72 hours post-preservation via Delayed Onset Cell Death [5,10]. To better understand the physical and chemical stresses during freezing conditions, consider a cell suspension in a simple salt solution such as physiological saline. In Figure 3, a typical phase diagram of a saline-like representative solution is shown. The phase diagram describes the state of the solution – liquid, solid, or both – at any given temperature and salt concentration.

The freezing process starts with cooling the solution to below its freezing point (Figure 3A). Once the first ice nuclei form at subzero temperatures, ice crystals grow until they reach an equilibrium with the remaining unfrozen fraction. As ice crystals form from pure water, the unfrozen fraction now contains a higher salt concentration and a lower freezing point. The cells remain in the channels of the unfrozen fraction [11,12].

As freezing continues by reducing the temperature, more water solidifies out of the solution in the form of ice, resulting in increased salinity, solute toxicity, and increasingly lower freezing temperature of the remaining unfrozen fraction (Figure 3B & C).

The cells in the unfrozen fraction are then exposed to increasing salinity (and solute toxicity) as the temperature plunges (Figure 3D). At temperatures in the range below -20°C, the salinity of the unfrozen fraction may be up to 10–20 times the normothermic initial salinity. Recall that cell membranes become more permeable at lower temperatures. This increased salinity, and solute toxicity, impacts the intracellular milieu during freezing. Therefore, the magnitude of freezing-related stresses due to physical effectors (ice formation), and biochemical effectors (salinity, solute toxicity, protein structural damages, intracellular signals, etc.) is not insignificant. Furthermore, the cells respond osmotically to increased extracellular solute concentration by shrinking in size due to water efflux. Cells that are sensitive to these mechanical and biochemical changes are more likely to experience cell injury and cell death during freezing, including as freezing continues toward the glass transition temperature (Tg) of the cell-solution mixture, and then as vitrification into a glassy state occurs, under appropriate conditions [11].

THE CELL RESPONSE TO FREEZING

Now consider how a cell is affected by this freezing process, in the context of manufacturing a cell-based product: A slow freezing rate will allow the cells to respond osmotically to the ever-increasing osmolality of the extracellular milieu by losing water and shrinking in size (Figure 4A). This process reduces the potential for intracellular ice formation; which is a major factor in damaging the cells beyond repair during cryopreservation [5,8,9].

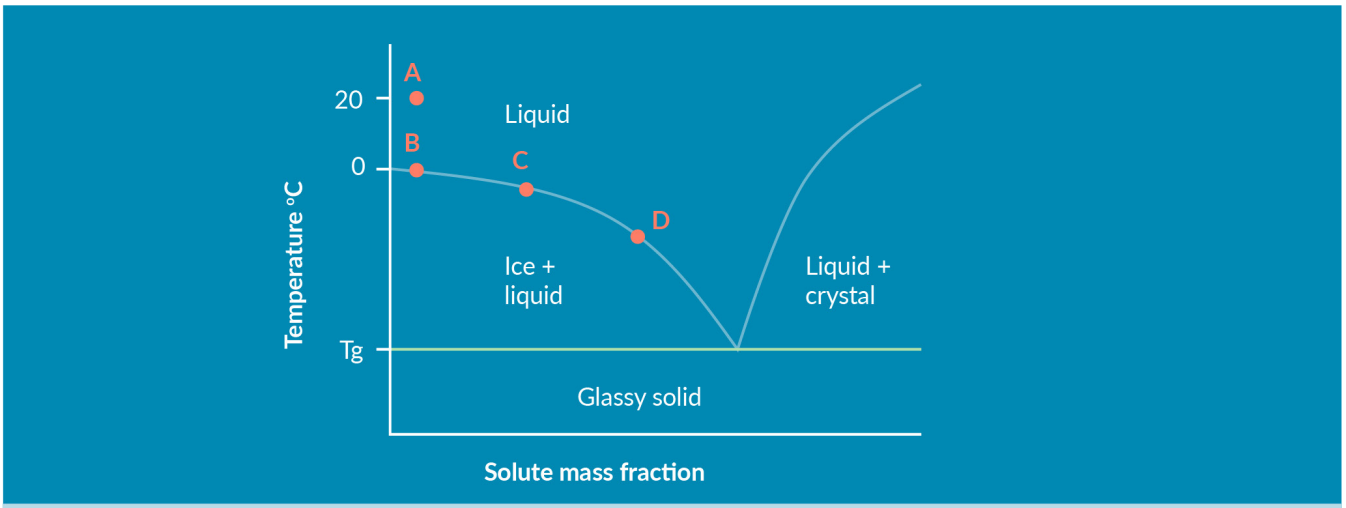
Osmotic shrinking, as a result of low temperatures and the cellular environment, is a dynamic process. As such, a fast freezing rate may not allow sufficient time for the cell to dehydrate enough water, and therefore increases the probability of intracellular ice formation (Figure 4B) [5,8,9].

Growth of intracellular ice can physically rupture membranes. In the case of fast freezing rates, the cell may be lysed if the amount of ice is excessive, or may be damaged beyond repair even with lesser amounts of intracellular ice (Figure 4B) [5,8,9].

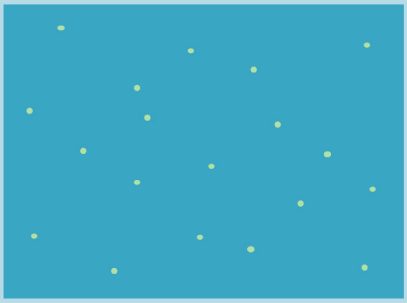
In general, freezing rates around -1°C/min or so are observed to allow water-membrane dynamics to dehydrate CGT-relevant cell types sufficiently to reduce intracellular ice formation (Figure 5A). However, the level of osmotically-induced volume shrinkage may reach as low as 30% of the original

FIGURE 3

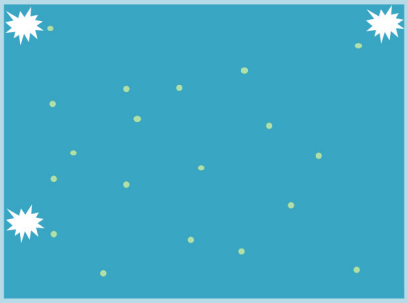
A typical phase diagram of a saline-like representative solution.



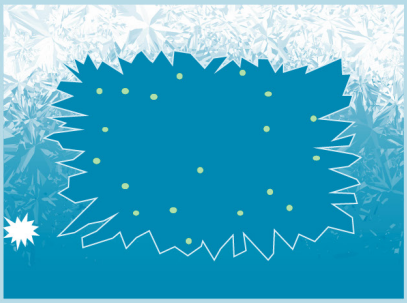
A. Room temperature
Salt solution, i.e., physiological saline at ambient room temperature



B. Freezing point T = -1.5°C
Freezing process starts with random nucleation of ice at subzero temperatures



C. Freezing point T = -10°C
A significant portion of water has turned into ice, leaving behind a 10x concentrated salt solution



D. Freezing point T = -23°C
At -23°C and below, further cooling result in formation of salt crystals, and the remaining liquid portion variably transitions into stages of a vitrified glassy state

cell volume. This may result in other forms of physical damage – including membrane folding and fusion, which is generally observed in the form of lower average cell volume, and an increase in the number of small non-cell vesicles post-thaw. The toxicity due to orders-of-magnitude increase in salinity, combined with mechanical cues from excessive osmotic shrinkage, induce adverse events in cells. These forms of cell damage and cell death include acute necrosis; and later Delayed Onset Cell Death (that becomes apparent as loss of viable recovery and function over hours to days post-thaw) [5].

DOI: 10.18609/cgti.2020.150

To reduce the osmotic shrinkage, and the toxicity due to increased solute concentration, cryoprotective agents (CPA) are added to the solution (membrane-permeable and/or non-permeating). One of the most well-known and most studied cryoprotective agents is dimethyl sulfoxide, or DMSO (Figure 5B) [5].

While referred to by some as an "anti-freeze" agent, DMSO offers protection against freezing in rather complex ways. In the unfrozen fraction, DMSO reduces salinity-induced toxicity and mechanical osmotic shrinkage by engaging water molecules and preventing ice crystal growth. As such, the cells are exposed to less salinity at any given temperature with the presence of DMSO. Furthermore, by permeating the cell, DMSO reduces the cell volumetric changes during freezing and minimizes intracellular ice growth [9]. This particular set of actions of DMSO may not be readily replicated by other non-permeating cryoprotective agents and sugars, or other permeating cryoprotective agents with similar efficacy.

WHY CRYOPRESERVE CELL-BASED PRODUCTS?

Clinical and commercial manufacturing models drive several critical aspects about

FIGURE 4

Cell responses to freezing.

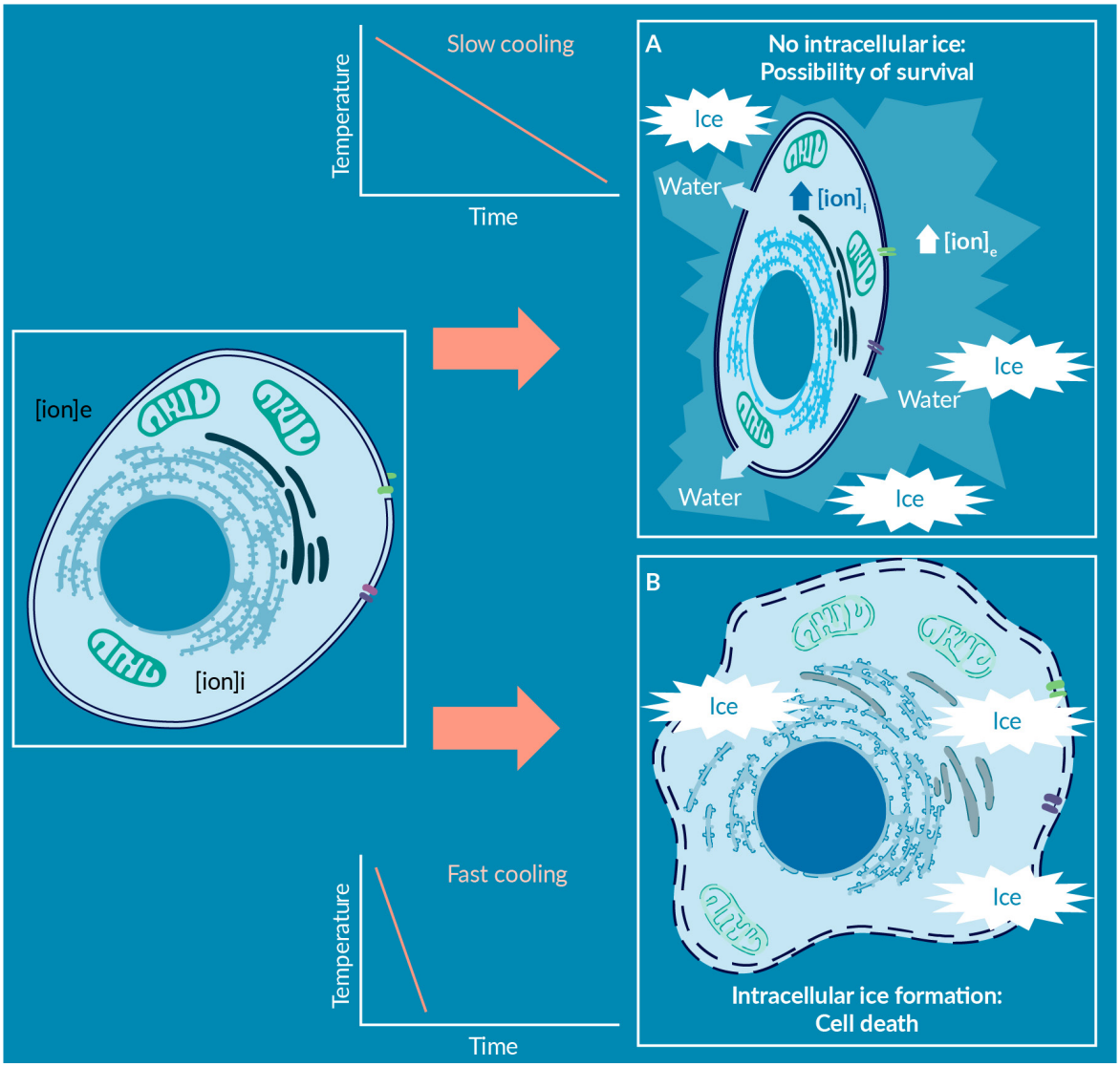
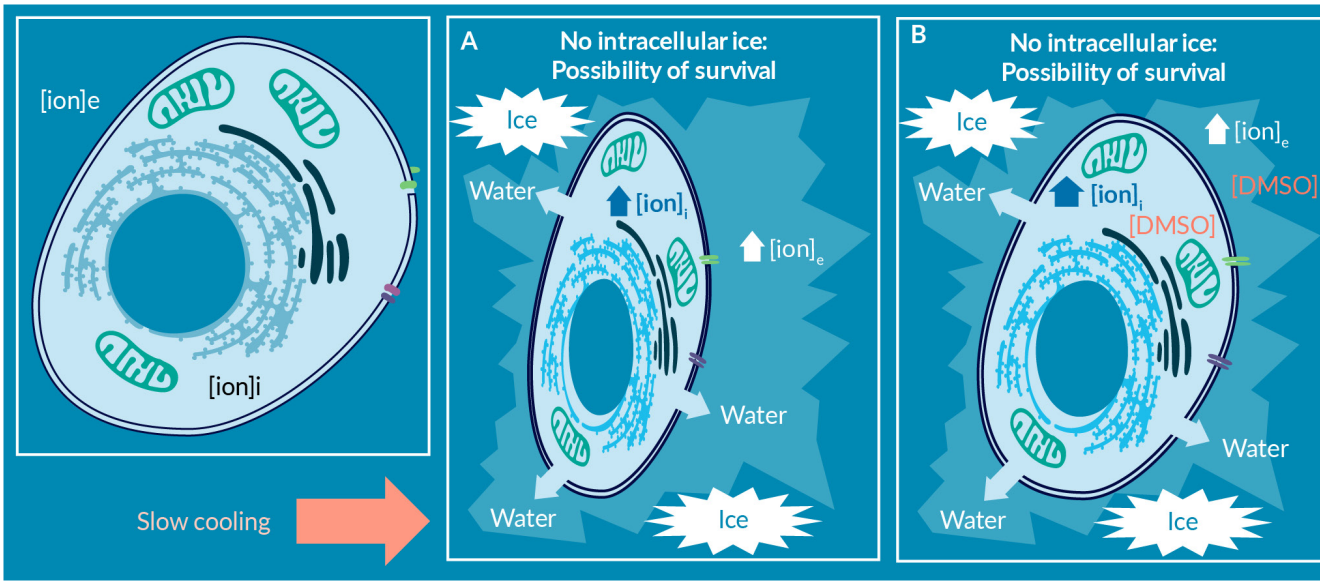


FIGURE 5

Addition of the cryoprotective agent, DMSO can offer protection against freezing.



the CGT process and workflow. While, in theory, "fresh" non-frozen materials may be preferred by some (if even possible/feasible) due to simplicity (no cryopreservation step, no LN2 dewar shipping step, no thawing, no documentation for cryo-related procedures, etc.), the spatial separation biologistics of source starting materials/manufacturing activities/patients, and the globalization of supply chain management, are ameliorated by the temporal time management benefits of cryopreservation.

Living cells age, differentiate, and/or degrade over time, even under normothermic conditions. A reduction in temperature at strategic points in the CGT workflow reduces the biological activity and metabolic demands of cells, and slows down degradation. As temperatures decrease, metabolic and enzymatic activity slows, and at or below a glass transition temperature (Tg) of approximately -120°C to -130°C, molecular motion in water-based systems is virtually arrested [9]. This vitrified state allows potential storage of the cell-based material for many years, and is a key temporal storage component of cell therapy manufacturing. An "investment" in cryopreservation buys time, provides flexibility, pays dividends through additional options, and is the most feasible current modality for long-term storage of CGT-related cell-based products.

PROCESS DEVELOPMENT CONSIDERATIONS FOR CRYOPRESERVATION OF CELL-BASED THERAPIES

Given the physics of freezing, and its effects on cells discussed above, it is important to determine if cryopreservation is appropriate and achievable for each CGT process/product. As developers of CGT therapies designed for successful commercial viability have looked to achieve a functional cryopreserved product, it is of value to understand that optimal cryopreservation of cells is not simply a matter of lowering the temperature below freezing. Some may think that cryopreservation consists of just freeze and thaw. However, the steps within a cryopreservation (and thaw) optimized method consists of multiple steps, with each step within the overall method potentially as a point of Risk and point of potential Optimization (Figure 6). Cryopreservation is one of the most critical, and often underdeveloped, critical process parameters (CPP) of the manufacturing

DOI: 10.18609/cgti.2020.150

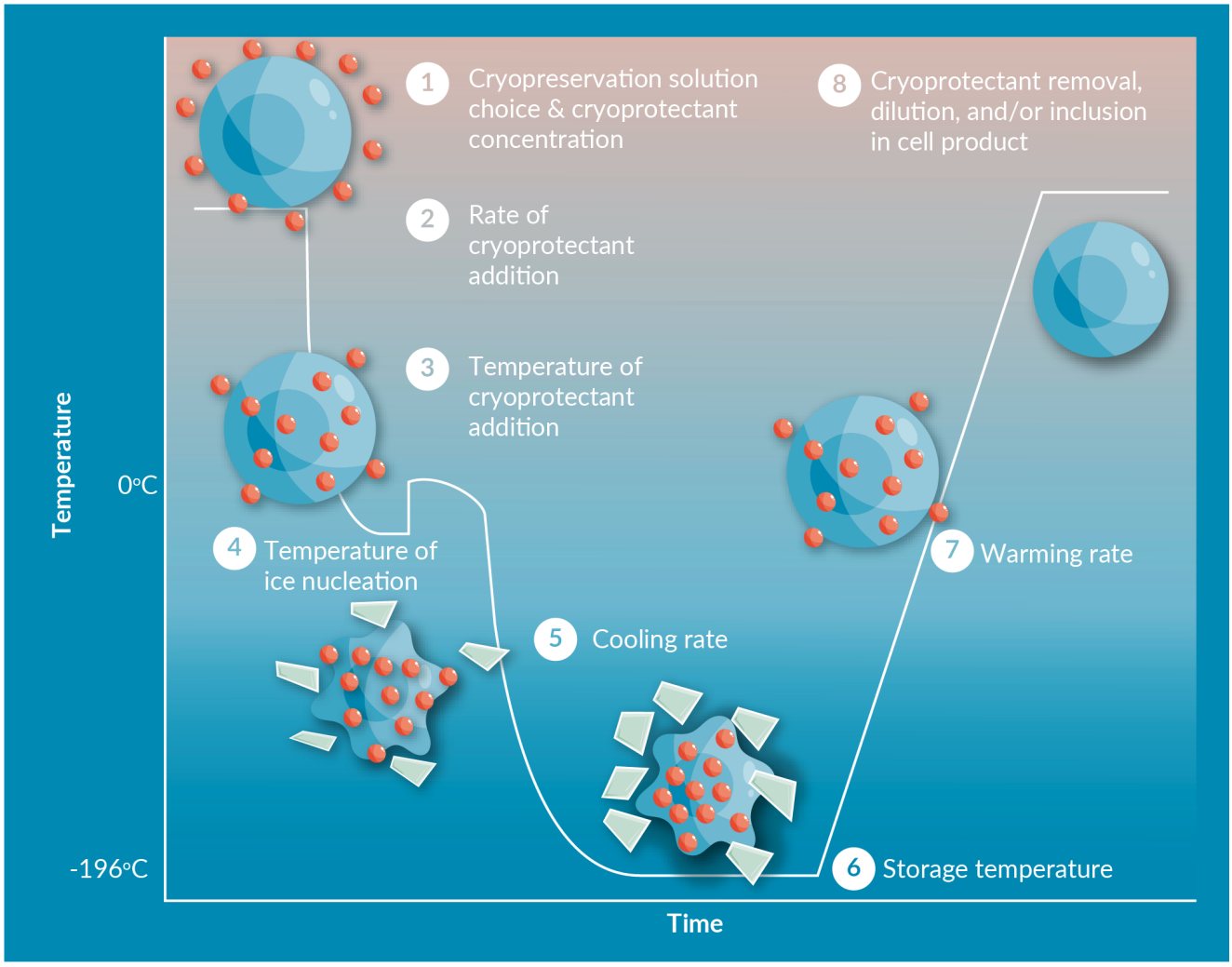
model. It may be helpful to look at the process in greater detail:

As illustrated in Figure 6, there are a number of steps within the cryopreservation method/protocol, that would be recommended to qualify/optimize from a Biopreservation Best Practices approach.

Consideration 1: Cryopreservation solution of choice. The traditional approach to the freeze media has been to formulate a home-brew cocktail of cryoprotectant (such as DMSO or glycerol), with serum (human or animal) or protein (albumin). These would be added to an isotonic (extracellular-like) vehicle solution such as culture media or saline-like solution, that had not been designed for low temperature biopreservation, but rather had been designed for normothermic ionic conditions. This formulation approach has been the traditional clinical center in-house home-brew cocktail, "grandfathered" into historical hematopoietic stem cell (HSC) transplant cryopreservation protocols [13], designed into some initial CGT cell therapies [14], and even incorporated into some guiding standards (USP <1044> Cryopreservation of Cells) [15]. In contrast, another more recent approach to the cryopreservation media has been to utilize a serum-free and protein-free intracellular-like formulation design, as discussed above [5,6,10]. This more recent methodology has been incorporated into many developing CGT, including ones that have obtained

FIGURE 6

The biopreservation best practices approach to cryopreservation.



Regulatory clearances and Marketing Authorisations [16–19].

Consideration 2: Rate of Cryoprotectant addition. Many research and clinical cryopreservation protocols proscribe slow/gradual/dropwise rates of addition of the cryoprotectant, in consideration to potential osmotic fluctuations and membrane permeability rates for the CPA. This consideration may, or may not, be impactful depending on the cell product/process. This consideration may also be less impactful with cryopreservation media that incorporate osmotic buffering components [20–22].

Consideration 3: Temperature of Cryoprotectant addition. Similar to the considerations related to the rate of CPA addition, some protocols proscribe a temperature for application of the freeze media. The choice of temperature may be related to facilitating more rapid permeability of the CPA, or related to reducing potential toxicity of the CPA [20–22].

Consideration 4: Temperature and consistency of ice nucleation. Some protocols may not speak to the point of ice nucleation within the cryopreservation procedure. Even with recognition of the ice nucleation, and related latent heat release, noted on freezing curves/graphs, there is often a passive approach to controlling ice nucleation within a method, let alone optimizing a method for consistent nucleation points from batch-to-batch of cell products. Lack of appropriate ice nucleation within a cryopreservation method may result in undercooling/supercooling of the sample, which may in turn be linked to deleterious intracellular ice formation and batch-to-batch variability. There are various approaches to the ice nucleation consideration [23], and even approaches for method consistency with passive freezing devices [24]. Programmable controlled rate freezers (CRF) are often utilized to provide consistent freezing rates and nucleation, however abnormal freezing curves and variable nucleation events may still occur and require troubleshooting [25].

Consideration 5: Cooling rate. Although most CGT cell products might find cooling/freezing rates of approximately -1°C/min (averaged, or focused on the initial stage around nucleation) to be adequate, if not optimal [8,9,11,26], it would be recommended (and often expected) to verify, and perhaps optimize, the freezing rates as appropriate for each manufactured cell product as an evidence-based Biopreservation Best Practice. Even with use of a programmable CRF, the stages within the CRF program may be optimized for various cell product parameters (cell type, cell volume, membrane permeability, cell concentration, product volume, product packaging, number of product units, etc.). CRF abnormal freezing curves may still occur and require troubleshooting [25].

Consideration 6: Storage temperature. Cryopreserved CGT products are generally stored in liquid nitrogen (LN2), to facilitate ultra-low cryogenic temperatures below their glass transition (Tg) temperature, and to enable many years of stability [27]. Alternatively, there may be potential for further consideration of shorter-term stability (weeks to months) at temperatures in the range of -80°C. The feasibility of varying storage temperatures (and the related pros and cons) may be worth exploring, and may be able to support short-term storage aligned with less burdensome storage/transport needs, with more robust cryopreservation methods and cold chain management [28,29].

Consideration 7: Warming/Thawing rate. In alignment with most CGT slow-freeze cryopreservation protocols, the most common thawing methods for those cryopreserved cell products involve fast-thaw methods with traditional 37°C waterbaths. At a superficial level, the process mirrors that of freezing: warming of the sample from cryogenic temperatures toward the solid-to-liquid phase transition, melting of ice to form liquid water, and rehydration of the cells. Similar to historical cryopreservation methods, this method of fast thawing has been largely adequate. The criticality of thawing rates is a noted point of discussion [26], and thaw methods (including rate of thawing) would be a worthwhile process parameter to investigate

DOI: 10.18609/cgti.2020.150

and verify for each cell product/process with an evidence-based approach to asses Risk and potential Optimization [8,9].

Consideration 8: Post-thaw wash, dilution, or direct application. There are a variety of approaches (and dogma) regarding the post-thaw status of the cryopreservation medium. One school of thought is that the cryoprotectant(s) must be removed post-thaw. The CPA removal might be via a single step wash/centrifugation, or via stepwise dilution and wash in consideration to osmotic fluctuations. There has also been development and application of various washing devices. Another approach would be to dilute post-thaw, but not wash/remove the CPA in entirety. And then there is the approach of avoiding wash or dilution with direct post-thaw application. Each of those approaches has potential benefits and drawbacks, that might range from extensive cell damage/loss (wash and removal methods) to potential (or perceived) cryoprotectant toxicity (direct application). Each approach also entails a different level of post-thaw manipulation, and potential variability at the point of post-thaw application [5,8,10,30].

BIOPRESERVATION BEST PRACTICES CONSIDERATIONS

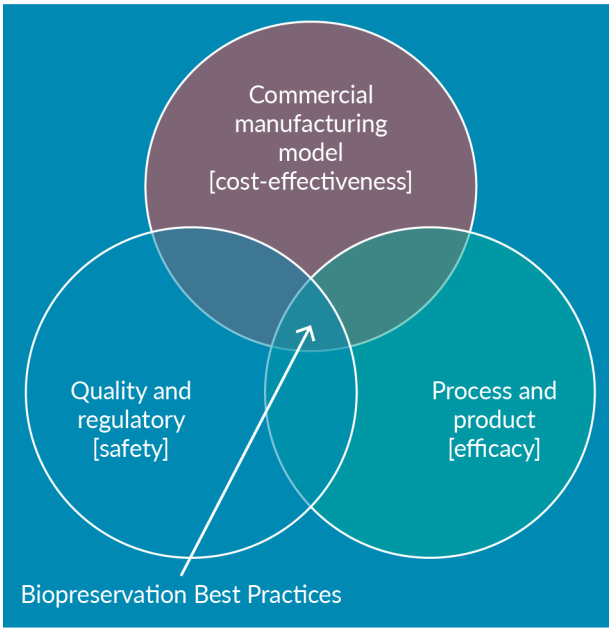
Most evidence-based best practices identify the process parameters, and investigate the characteristics that can impact the critical quality attributes of the product. Within the considerations of biopreservation, broader process best practices may overlap to more focused Biopreservation Best Practices that can serve as a guiding approach applicable to CGT manufacturing (Figure 7).

Often, the early-stage development of a product understandably focuses on the high-level product efficacy (recovery, viability, and perhaps some measure of functionality). Admittedly, if the feasibility of that aspect is not established, the other parameters may be moot considerations. The ability to manufacture the product tends to be an early translational focus, and as the product progresses along potential clinical or commercial development there is increasing scrutiny to Quality and/or Regulatory Risk considerations. Areas of overlap with focus on Biopreservation Best Practices may include:

1. Ability to integrate a biopreservation tool (media, equipment, method, etc.) into the CGT manufacturing process, including risk from process change.
2. Cost-effectiveness of those tools and technologies, such as pre-formulated biopreservation media or controlled rate freezer.
3. Efficacy of the tools, methods, and cell product.
4. Impact to Quality and Regulatory footprint, such as safety of biopreservation media and consideration to qualification for excipient application. Also, consideration to alignment with Good Manufacturing Practices (GMP).

FIGURE 7

Biopreservation Best Practices



Adapted from [9].

5. Qualification and validation of the tools, technologies, or methods.

6. Supplier reliability, risk, expertise, and qualification alignment. Also, supply chain security of the tools and technologies.

ADDITIONAL BIOPRESERVATION PROCESS PARAMETERS

As an extension of the number of critical steps within the cryopreservation process (Figure 6), there are Biopreservation Critical Process Parameters (BCPP) throughout the CGT manufacturing process, and including where biopreservation and stability might impact the quality attributes of the process/product (Figure 8).

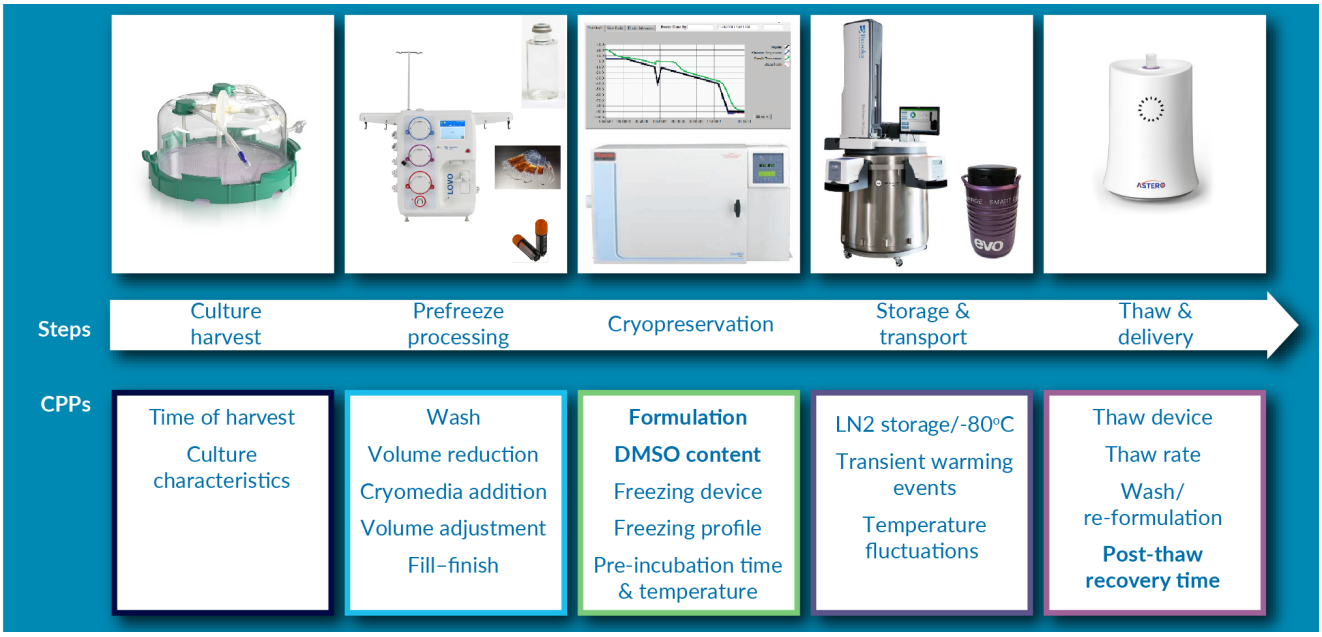
Cold chain management

Advances have been made in cold chain management systems, and monitoring of this critical part of the CGT workflow. Innovations in insulating materials have overcome shortcomings in insulated packaging performance. 'SMART' shippers with improved cloud-based data tracking and software technology have enhanced management of time-critical and temperature-sensitive products. Technology innovations have improved packaging, monitoring, logistics practices, data collection and data management; and incorporated them into unique, innovative, and self-contained systems [31–33].

SMART cold chain technologies such as Liquid Nitrogen (LN2) "dry vapor" SMART shippers and longer-range dry ice shippers are increasingly being utilized by late-stage clinical trial and commercialized therapy providers. The temperature monitoring and control, location tracking, chain of custody monitoring, and long temperature life of these shippers addresses a critical part of the supply chain biologistics [33]. With LN2 shippers, traditional LN2 dry vapor shippers experience reduced performance when not maintained upright, they may require palletization, and therefore may be restricted to wide-body aircraft and limited to large airport channels. New shipper technologies look to maintain temperature under some tilting, accommodate loading onto smaller

FIGURE 8

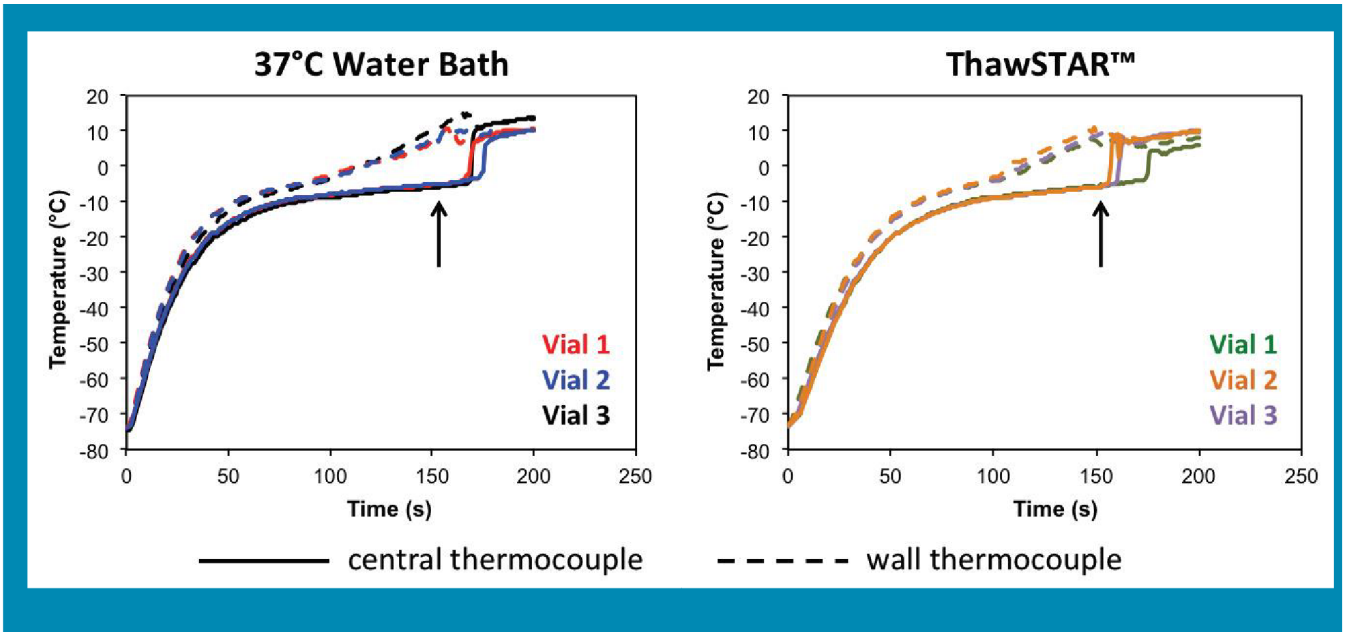
Biopreservation critical process parameters.



DOI: 10.18609/cgti.2020.150

FIGURE 9

Thermal profile of vials thawed in a water bath or ThawSTAR System.



Frozen vials were thawed in a 37°C waterbath (left panel) or in the ThawSTAR System (right panel). The temperature profiles recorded by both thermocouples were very similar for both the waterbath thaw and the ThawSTAR thaw. For the waterbath thaw, the vials were removed from the bath when a pea-sized ice chunk remained (arrow) and then gently tapped to melt the chunk. Similarly, ThawSTAR ejected the vial at the point where a pea-sized ice chunk remained (arrow). The final vial temperature is ~5–10°C.

regional aircraft that cannot support palletized cargo, and enable greater flexibility during transport [33].

Thawing

In order to transition from cryopreserved samples/product to application of the cells, the intermediate step is returning cell samples/products to the non-frozen state. Optimal thawing of these cells may be critical to successful downstream applications. Thawing rate and temperature may be parameters for potential optimization for cell size and volume, cell type, and cryopreservation media.

The most common and well-accepted method for rapidly thawing cryopreserved cell samples is partial submersion of the sample in a 37°C waterbath. There are several reasons for using this approach: waterbaths are relatively cheap and easily available, and they allow efficient heat transfer from the water to the sample due to the high heat capacity and thermal conductivity of liquid water. However, there are potential risks to using a waterbath for thawing, particularly in a clinical environment. These potential risks include:

1. Lack of scalability post-manufacturing.
2. User-to-user variability in subjectively determining thaw recognition times, final vial temperature, and ending point of ice.
3. Overthawing, or excessive warming, of samples.
4. No data management or chain-of-custody connectivity.
5. Contamination of sample contents.
6. Challenge in using a waterbath as part of a sterile process inside a biosafety cabinet or clean environment.

7. Restrictions in using waterbaths in GMP or clinical environments.

To overcome some of the limitations of using waterbaths for thawing, researchers and process engineers have explored other options such as dry bead baths or heat blocks [34,35]. Unfortunately, these solutions have inefficient thermal contact, resulting in reduced heat transfer, and may require 2–3 times longer (~7 minutes in a dry bead bath vs. ~2.5 minutes in a 37°C waterbath for a standard cryovial) to thaw samples. This slower rate of thaw may be negatively impactful to the cell product.

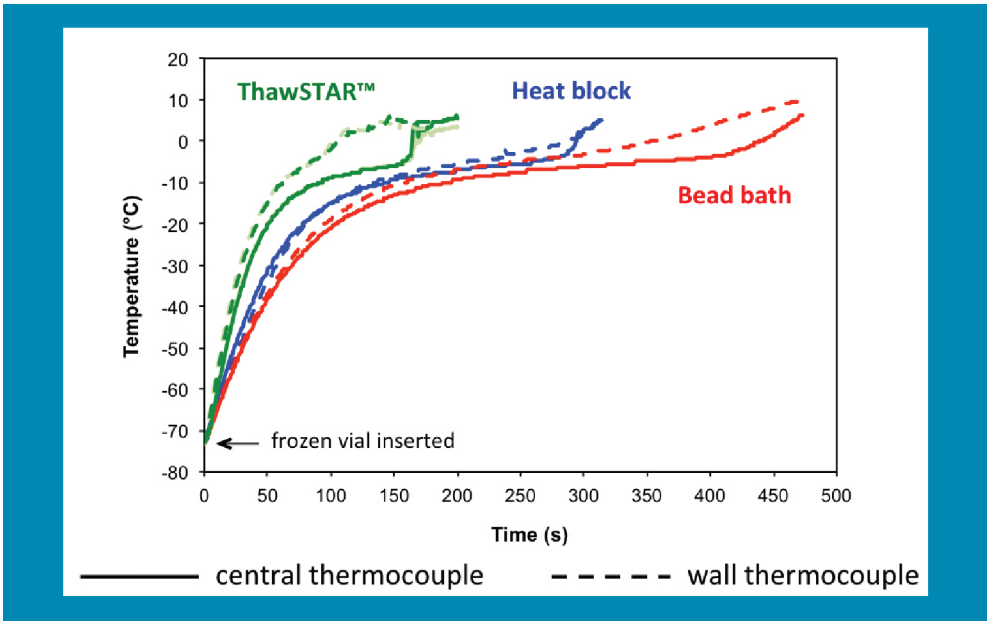
Innovations in water-free automated thawing technology have enabled sample thawing with similar thawing rates as waterbaths (Figure 9), more efficient thawing in comparison to other dry heat methods (Figure 10), cessation of active heating upon product transition from solid to liquid state, and physical separation of sample from heating interface upon thaw [36]. Equivalent post-thaw cell recovery and cell viability have also been demonstrated between newer water-free thawing technology and traditional waterbaths (Figure 11).

CONCLUSION

Cell and gene therapies are demonstrating clinical efficacy, and exhibiting early potential for commercial viability. The manufacturing and supply chain for cell and gene therapies would still benefit from substantial development and innovation, in order to model the robustness and efficiencies as experienced in the more mature fields of small molecule pharmaceuticals and large molecule biopharmaceuticals. Successful optimization of product development would benefit from a broad analysis of the product lifecycle and workflow. A methodical and diligent review of cell-based materials stability risk points (in essence, a Biopreservation Quality by Design, or BQbD), consideration to Biopreservation Critical Process Parameters (BCPP), and identification of Biopreservation Critical Quality Attributes (BCQA); would serve

FIGURE 10

Rapid vial thawing with ThawSTAR compared to dry bead bath or heat block.

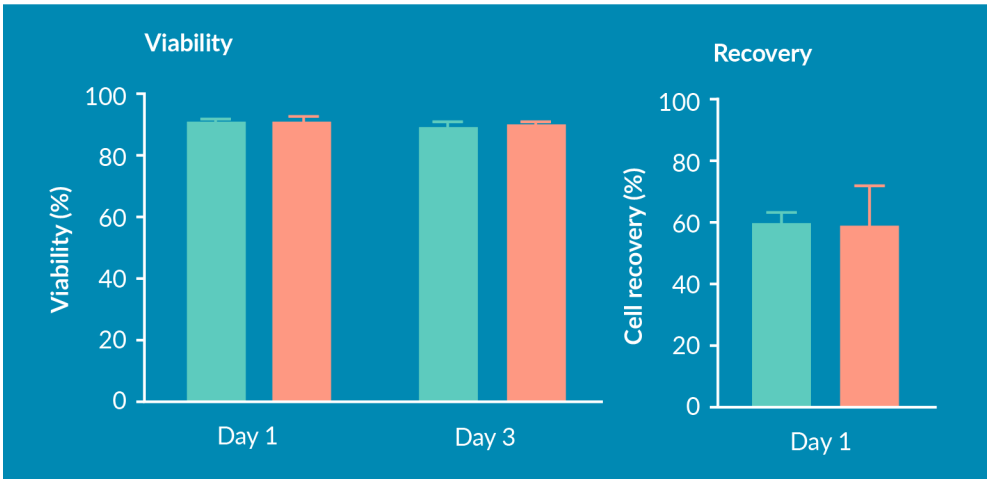


Frozen vials were thawed in either a ThawSTAR System (green traces), a 37°C bead bath (red traces), or an aluminum heat block equilibrated to 37°C (blue traces). The ThawSTAR System thaw time is 2-3X faster than these other dry thawing methods.

DOI: 10.18609/cgti.2020.150

FIGURE 11

Post-thaw cell recovery and cell viability with newer water-free thawing technology versus traditional waterbaths.



to identify stability gaps, increase system robustness, and optimize the overall CGT manufacturing and supply chain workflow. Optimizing the end-to-end Process utilizing Biopreservation Best Practices, and integrating the latest tools and technologies related to biopreservation media, controlled rate freezing and cryogenic storage, cold chain shipping management, and automated water-free thawing; would facilitate optimization of the CGT Product, and increase the probabilities for clinical and commercial success.

REFERENCES

1. Alliance for Regenerative Medicine Annual Report & Sector Year in Review: 2019.
2. Bersenev A, Kili S. Management of 'out of specification' commercial autologous CAR-T cell products. *Cell Gene Ther. Ins.* 2018; 4(11): 1051–8.
3. Chen LN, Collins-Johnson N, Sapp N, Pickett A, West K, Stroncek DF, Panch SR. How do I structure logistic processes in preparation for outsourcing of cellular therapy manufacturing? *Transfusion* 2019; 59: 2506–18.
4. Juliano L, Eastwood G, Berard T, Mathew AJ. The Importance of Collection, Processing and Biopreservation Best Practices in Determining CAR-T Starting Material Quality. *Cell Gene Ther. Ins.* 2018; 4(4): 327–36.
5. Hawkins BJ, Abazari A, Mathew AJ. Biopreservation Best Practices for regenerative medicine GMP manufacturing & focus on optimized biopreservation media. *Cell Gene Ther. Ins.* 2017; 3(5): 345–58.
6. Abazari A, Hawkins BJ, Clarke DM, Mathew AJ. Biopreservation Best Practices: A Cornerstone in the Supply Chain of Cell-based Therapies – MSC Model Case Study. *Cell Gene Ther. Ins.* 2017; 3(10): 853–71.
7. Clarke D, Smith D. Managing starting material stability to maximize manufacturing flexibility and downstream efficiency. *Cell Gene Ther. Ins.* 2019; 5(2): 303–14.
8. Abazari A. Process development considerations for cryopreservation of cellular therapies. *Cell Gene Ther. Ins.* 2019; 5(9): 1151–67.
9. Abazari A. Implementation of Biopreservation Best Practices to address a critical component of cell and gene therapy manufacturing: https://insights.bio/cell-and-gene-therapy-insights/implementation-of-biopreservation-best-practices-to-address-a-critical-component-of-cell-and-gene-therapy-manufacturing
10. Mathew AJ. Biopreservation Considerations for Regenerative Medicine GMP Manufacturing; 2018: http://ebook.liebertpub.com/biolife-solutions/biopreservation-considerations-for-regenerative-medicine-gmp-manufacturing
11. Mazur P. The role of intracellular freezing in the death of cells cooled at supraoptimal rates. *Cryobiology* 1977; 14: 251–72.
12. Chen HH, Clarke DM, Gao D. Direct concentration measurements of the unfrozen portion of solutions under freezing. *Cryobiology* 2010; 61: 161–5.

13. Berz D, McCormack EM, Winer ES *et al.* Cryopreservation of Hematopoietic Stem Cells. *Am. J. Hematol.* 2007; 82(6): 463–72.

14. KYMRIAH Prescribing Information, including Boxed WARNING, and Medication Guide: https://www.novartis.us/sites/www.novartis.us/files/kymriah.pdf

15. USP <1044> Cryopreservation of Cells. Sept 27, 2018: https://www.usp.org/sites/default/files/usp/document/our-work/biologics/resources/gc-1044-cryopreservation-of-cells.pdf

16. YESCARTA Product Information – European Medicines Agency: https://www.ema.europa.eu/en/documents/product-information/yescarta-epar-product-information_en.pdf

17. YESCARTA Product Monograph – Gilead Canada: http://www.gilead.ca/application/files/2715/8646/6805/Yescarta_English_PM_e214145-GS-002-Clean.pdf

18. Zynteglo Product Information – European Medicines Agency: https://www.ema.europa.eu/en/documents/product-information/zynteglo-epar-product-information_en.pdf

19. TECARTUS Package Insert – US FDA: https://www.fda.gov/media/140409/download

20. Nicoud IA, Clarke DM, Taber G, Stolowski KM, Roberge SE, Song MK, Mathew AJ, Reems J. Cryopreservation of umbilical cord blood with a novel freezing solution that mimics intracellular ionic composition. *Transfusion* 2012; 52(9): 2055–62.

21. Lawson A, Mukherjee IN, Sambanis A. Mathematical modeling of cryoprotectant addition and removal for the cryopreservation of engineered or natural tissues. *Cryobiology* 2012; 64(1): 1–11.

22. Best BP. Cryoprotectant Toxicity: Facts, Issues, and Questions. *Rejuvenation Res.* 2015; 18(5): 422–36.

23. Morris GJ, Acton A. Controlled ice nucleation in cryopreservation – A review. *Cryobiology* 2013; 66: 85–92.

24. BioLife Solutions Cryopreservation Protocol: https://www.biolifesolutions.com/wp-content/uploads/2018/01/6012_07-CryoStor-Product-Information-Sheet.pdf

25. Creer MH, Mathew AJ, Lemas MV. Practical Handbook of Cellular Therapy Cryopreservation. AABB Press 2015.

26. Baboo J, Kilbride P, Delahaye M *et al.* The Impact of Varying Cooling and Thawing Rates on the Quality of Cryopreserved Human Peripheral Blood T Cells. *Sci. Rep.* 2019; 9: 3417.

27. Meneghel J, Kilbride P, Morris GJ *et al.* Physical events occurring during the cryopreservation of immortalized human T cells. *PLoS ONE* 2019; 14(5).

28. Kofanova OA, Davis K, Glazer B *et al.* Viable Mononuclear Cell Stability Study for Implementation in a Proficiency Testing Program: Impact of Shipment Conditions. *Biopreserv. Biobank.* 2014; 12(3): 206–16.

29. Abazari A, Hawkins BJ, Fink J, O'Donnell K, Mathew AJ. Next Generation Technology, Procedures, and Products Facilitate Biopreservation Best Practices for Cellular Therapies. 2016: https://www.biolifesolutions.com/wp-content/uploads/2016/10/Biolife_Brooks_Whitepaper_OCT20_REL.pdf

30. Awan M, Buriak I, Fleck R *et al.* Dimethyl sulfoxide: a central player since the dawn of cryobiology, is efficacy balanced by toxicity? *Regen. Med.* 2020; 15(3): 1463–91.

31. O'Donnell K. Moving from passive to rescue design packaging: helping cells arrive alive with smart shippers. *Cell Gene Ther. Ins.* 2015; 1(2): 163–71.

32. O'Donnell K, Mathew AJ. Cell and Gene Therapies in Transit: Caution – Hazards Ahead. *Cell Gene Ther. Ins.* 2016: https://www.insights.bio/cell-and-gene-therapy-insights/journal/article/474/Cell-and-Gene-Therapies-in-Transit-Caution-Hazards-Ahead.

33. United Airlines Cargo, SAVSU. Transforming the future of medical shipments. 2019: https://ual.unitedcargo.com/Transforming-the-future-of-medical-shipments-SOCIAL-Page.

34. Röllig C, Babatz J, Wagner I *et al.* Thawing of cryopreserved mobilized peripheral blood--comparison between waterbath and dry warming device. *Cytotherapy* 2002; 4(6): 551–5.

35. Triana E, Ortega S, Azqueta C *et al.* Thawing of cryopreserved hematopoietic progenitor cells from apheresis with a new dry-warming device. *Transfusion* 2013; 53(1): 85–90.

36. ThawSTAR Automated Cell Thawing System: https://www.biolifesolutions.com/wp-content/uploads/2020/02/BioLife-ThawSTAR-Catalog.pdf

AFFILIATIONS

Todd CJ Berard
BioLife Solutions Inc.

Aby J Mathew
BioLife Solutions Inc.

DOI: 10.18609/cgti.2020.150

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Todd CJ Berard and Aby J Mathew are employees of BioLife Solutions, Inc. Todd CJ Berard is Chief Marketing Officer. Aby J Mathew is Executive Vice President & Chief Scientific Officer.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Draft submitted: *Aug 19 2020;* ***Revised manuscript received:*** *Oct 20 2020;* ***Publication date:*** *Nov 4 2020.*

BioLife's Complete Cell and Gene Therapy Workflow Solutions

Biopreservation media

CryoStor®
HypoThermosol®
BloodStor®

Water-free Automated Thawing

ThawSTAR®

Cold Chain Management

evo®

Cryogenic Freezers and Equipment

CBS®

Biological and Pharmaceutical Storage

SciSafe

To learn more about our biopreservation solutions, contact us at info@biolifesolutions.com

1.866.424.6543 | BioLifeSolutions.com

BIOLIFE SOLUTIONS™

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: BIOPRESERVATION &COLD CHAIN LOGISTICS

EXPERT INSIGHT

The supply chain: key considerations for biological starting material for ATMPs production

Elisabet Aguilar, PhD

Advanced therapy medicinal products (ATMPs) is a particularly complex, novel class of medicines, with some distinctive supply chain requirements that significantly impact the manufacturing process. A critical element in the supply chain is the provision of biological starting material. It is essential to have a detailed knowledge of the sample's properties and how these are affected by different factors such as time, temperature or transport medium to establish the distribution requirements. Additionally, it is indispensable to be fully aware of the legal framework where samples are regulated to comply with the requirements to guarantee their quality and safe use. The supply of starting material is a cornerstone for the establishment of a competent ATMP manufacturer, and its success relies on close cooperation with the procurement sites, a thorough knowledge of the starting material properties and regulations applying to it, together with an extended network of transport companies that could be involved in its supply.

Cell & Gene Therapy Insights 2020; 6(8), 1135–1140

DOI: 10.18609/cgti.2020.122

Advanced therapy medicinal products (ATMPs) are being consolidated as an innovative option to offer cutting-edge treatment for disease and injury, as highlighted by the recent rise of phase I-III clinical trials, its incorporation into health care systems, and the increase in commercialization [1,2].

The term ATMP is exclusive to the European Union and includes all of the cell, gene, and tissue therapies that are being developed. Regardless of location, the manufacture of this type of medicinal product is a complex, expensive, multiple-stage, and often extensive process, which not surprisingly presents one of the most challenging supply chains in the health field.

A distinctive aspect of this particular kind of medical product is that they are obtained, often completely but at least partially, from human biological material. From the point of view of the source of the material, ATMPs are classified as either autologous, when the donor is the same as the recipient, or allogeneic, which can be obtained from one or multiple donors. (Xenogeneic samples - i.e. from different species - are outside of the scope of this article). This distinction is relevant because it conditions the logistics of the whole process (procurement, manufacturing and distribution). The supply of biological material can be provided by four different establishments: *blood and transfusion centers*, which provide apheresis collection or whole peripheral blood samples; *hospitals* where the patient is treated and/or donor is procured, e.g.: skin, adipose tissue; medical centers belonging to a *transplant network*, which include a wider range of tissues - even those from deceased donors, e.g.: cornea, blood vessels, etc.; and *tissue and cell biobanks* where established cell lines are stocked and characterized, e.g. MSC, iPSC (Figure 1). The latter two establishments are exclusive for allogeneic therapies, while the procurement of samples for autologous therapies usually takes place at the same Center (Hospital/Blood center) where the patient will be treated.

The supply chain of biological starting materials has to comply with certain requirements to ensure the suitability and safety of the samples for ATMP production. The timeframe for living samples to reach the manufacturer is determined by viability, among other parameters, which will vary on shipment conditions (a critical limiting factor in any case). Furthermore, within the very same window, the time needed for processing by the manufacturer must be included. Frozen samples have significantly longer timeframes, providing them with greater flexibility for distribution but affecting viability. Additionally, samples must be temperature tracked during shipment to guarantee delivery in optimal condition and that quality is maintained according to the sample specifications.

Procurement of human tissues or cells requires compliance with a specific regulatory framework. In the EU, the legal framework is set out for tissues and cells under European Tissues and Cells Directive [3] and for blood and its components under European Blood Directive [4], with their respective commissions (see Table 1). These directives are transposed into each Member State law to implement the requirements established in EU legislation, with the possibility of applying stricter rules regarding quality and safety.

Biological material supply chain begins at the clinical site where altruistic donation is procured. The availability of the starting material, which is conditioned by clinical activity, is one of the most critical factors for the establishment of a successful ATMP manufacturer. However, the inherent properties of a living material - temperature sensitivity with a short lifespan; requiring exhaustive control during distribution - severely limits distribution and complicates supply chain.

Some important aspects to consider for successful starting material supply chain establishment could include the following (Figure 2):

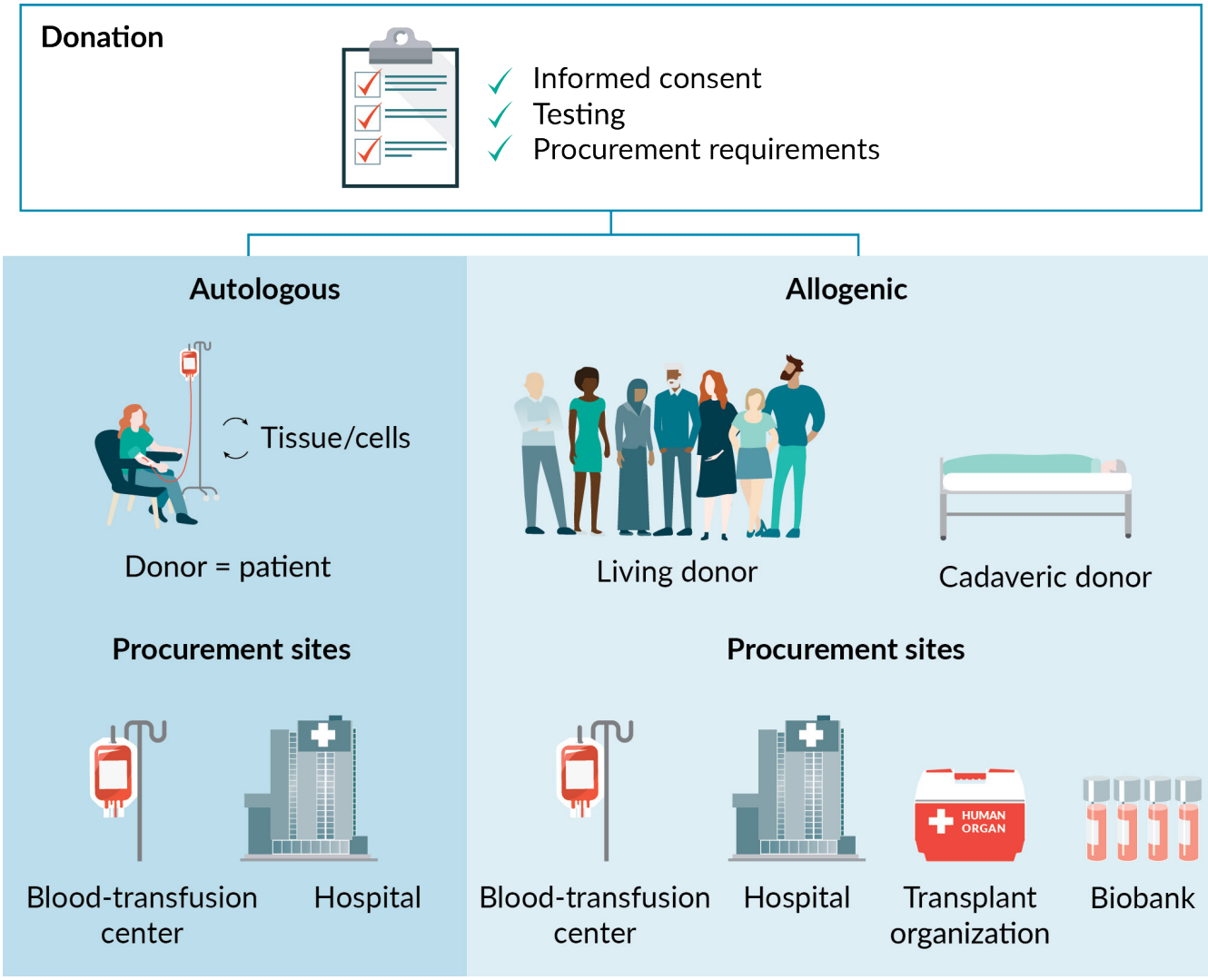
1. Regarding STARTING MATERIAL:

- **Knowledge of the starting material.** First of all, it is indispensable to have a thorough knowledge of the sample and

DOI: 10.18609/cgti.2020.122

FIGURE 1

Provenance of biological starting material.



how its viability is affected by processing, time, and temperature. In the case of fresh samples, it is necessary to compare different shipping conditions, analyze different transport media, temperatures, and/or packaging systems. On the basis of the results obtained, a time window in which the sample maintains an optimal quality to be used for ATMP manufacturing is established. It is convenient to further pursue these studies in order to define a time window as wide as possible, since this will determine the maximum transportation time from the place of collection to the manufacturer. Being aware of the time dependent viability curve would allow us to be objective in the decision-making process if a sample arrives out of specifications.

- **Sample efficiency.** In certain cases, samples are really scarce in quantity and/or volume, and are thus considered very valuable. In such cases, the cost of their distribution is assumed disregarding other aspects since no alternative exists - for instance, for autologous samples. In contrast, for some other types of samples that are easier to obtain (e.g. adipose tissue), it is advisable to precisely define their efficiency and cost-effectiveness. Considerations such as the donor's age and health history are relevant to evaluate the minimum quantity

TABLE 1

Legal framework in EU for procurement of human tissues or cells

Tissues & cells directives	
Directive 2004/23/EC	
Commission directive 2006/17/EC	Regarding certain technical requirements for the donation, procurement and testing of human tissues and cells [5]
Commission directive 2006/86/ EC	Concerning traceability requirements, notification of serious adverse reactions and events, additional technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells [6]
Commission directive 2015/565	Amending directive 2006/86/EC as regards certain technical requirements for the coding of human tissues and cells
Commission directive 2015/566	Implementing directive 2004/23/EC concerns the procedures for verifying the equivalent standards of quality and safety of imported tissues and cells
Blood directive	
Directive 2002/98/EC	
Commission directive 2004/33/ EC	On the technical requirements for blood and blood donation
Commission directive 2005/61/ EC	On the traceability requirements and notification responsibilities in case of serious adverse reactions and events
Commission directive 2005/62/ EC	That sets out community standards and specifications relating to the quality system for a blood bank

The Commission is currently carrying out the first formal evaluation of the EU blood and tissues and cells legislation
Information adapted from: [7] and [8].

of starting material that compensates the cost related to their distribution, avoiding waste and non-productive resources.

2. Regarding PROCUREMENT SITE:

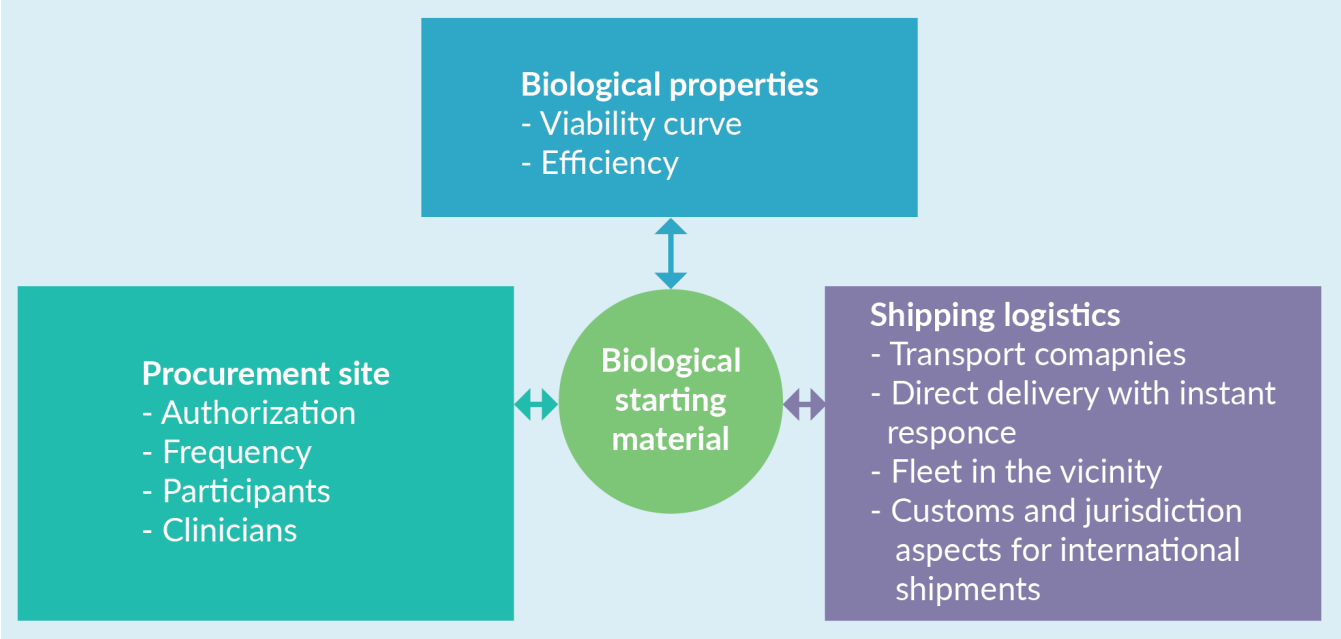
- **Procurement authorization.** The ATMP manufacturer must establish an agreement with the procurement site covering roles and responsibilities according to regulatory requirements. It is highly recommended to contract centers that already have the procurement authorization for the type of cell and tissue in use, since *de novo* authorization is a lengthy process that requires the incorporation of new SOPs into a hospital system. Having an agreement with more than one authorized procurement center would reduce the risk of lack of starting material for the manufacturing process.

- Procurement activity history. For allogeneic samples, it is useful to know the frequency with which the establishment acquires the biological material of interest: namely, whether it is a habitual practice yielding a high availability of samples, or on the contrary, if it is a scarce type of tissue/cell the extraction and availability of which is limited. This information would enable the manufacturer to plan their activity, improving the management of resources. In the case that the biological material does not come from a usual or frequent practice, the manufacture of ATMPs could be limited

DOI: 10.18609/cgti.2020.122

FIGURE 2

Summary of the points mentioned through the document related to the biological starting material supply chain



and in some cases even stopped, due to lack of starting material; in those cases, as mentioned above, it would be advisable to operate with more than one procurement site.

- **Personnel involved.** It is important to know the medical staff involved in the procurement of the starting material. Regardless of whether the samples are autologous or allogeneic, if the staff is limited, not committed enough, and/or if highly specialized clinical expertise is required, there may be occasions when the material is lost due to personnel issues. The inclusion and participation of the medical team in the supply chain in some way favors cooperation and facilitates the development of this type of activity. The clinical staff must be sufficiently and actively engaged in the procedure, since their involvement has a direct impact on the manufacturer's production.

- **Shipping logistics.** Biological living starting material has a short timeframe for delivery and is often temperature sensitive. The different procurement sites, such as biobanks, usually coordinated amongst themselves and have internal circuits for shipping samples. Ideally, it would be useful for the ATMP manufacturer to integrate the collection of the starting material into such circuits, if feasible with respect to the individual sample specifications. If this is not feasible, direct shipment to the manufacturer would be required. Time requirements considerably limit the distribution of the biological samples and consequently, the manufacturer will need to be located relatively close to the place of procurement. In addition, international shipments must take into account the management of customs clearance and related procedures in the respective states.

Progress in the development of ATMPs needs to go hand in hand with the development and improvement of supply chain logistics at the different stages, with particular emphasis on the supply of starting materials. With the increase in volume and widespread use of this type of medicine, this emerging niche market will be developed and expanded

to accommodate logistics compatible with the characteristics of living material, while offering competitive prices. It is essential to know the available companies that offer direct delivery service with instant response, and at the same time and very importantly, that have a fleet in the vicinity of the procurement sites to shorten time from procurement to arrival at the manufacturer.

Successful supply of starting material is essentially based on a narrow partnership with the procurement sites, a thorough knowledge of their operation and the rules that regulate them, a fluid, two-way communication between the partners involved, and broad knowledge concerning the network of transport companies that could be involved in the supply chain.

REFERENCES

1. Hanna E, Rémuzat C, Auquier P, Toumi M; Advanced therapy medicinal products: current and future perspectives. Jour.l of Mark. Access & Health Policy. 2016, 4(1), 31036.
2. Cuende N Rasko JEJ, Koh MCB, Dominici M, Ikonomou L; Cell, tissue and gene products with marketing authorization in 2018 worldwide. *Cytotherapy*. 2018, 20(11), 1401–1413.
3. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.
4. Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components.
5. Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.
6. Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.
7. European Commission, tissues and cells: https://ec.europa.eu/health/blood_tissues_organs/tissues_en
8. European Commission, blood: https://ec.europa.eu/health/blood_tissues_organs/blood_en

Affiliation

Elisabet Aguilar, PhD

Andalusian Network for Design and Translation on Advanced Therapies

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author declares that they have no conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Aug 18 2020;* ***Revised manuscript received:*** *Sep 01 2020;* ***Publication date:*** *Sep 22 2020.*

DOI: 10.18609/cgti.2020.122

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: BIOPRESERVATION & COLD CHAIN LOGISTICS

EXPERT INSIGHT

Utilization of risk-based approach to characterize starting material for autologous CAR-T manufacturing

Jean Stanton

Chimeric antigen (CAR) T cell therapy is a cell-based, personalized cancer immunotherapy. It takes advantage of the cytotoxic potential of T lymphocytes to kill tumor cells in an antigen dependent manner. This therapy has proven to be an effective therapy against certain hematologic malignancies. As with other biologic products, efforts to optimize the manufacturing process can help ensure the safety and efficacy of the product. Cell based products are far more complex than any other active pharmaceutical ingredients, therefore it is even more critical that sponsors understand the impact of the starting material (apheresis cells) for the manufacturing process. The industry is learning about health authority expectations in the last few years, with the commercialization of two CAR-T products, Kymriah™ and Yescarta™. Demonstrating an understanding of the starting material, including its impact on manufacturing and the means to control its impact where needed, is one way sponsors can respond to the expectations. In this article, the reader will find the application of a risk-based approach to identify both the key attributes of starting material that can impact CAR-T product manufacturing and the means to control for those attributes.

Cell & Gene Therapy Insights 2020; 6(8), 1067–1078

DOI: 10.18609/cgti.2020.117

Chimeric antigen receptor (CAR) T-cells are genetically engineered T cells, modified to bind to cell surface antigens expressed by cancer cells, signaling and co-signaling the

cytotoxic function of these cells. Researchers developed the first-generation CAR-T cells almost thirty years ago [1]. While technically innovative, these early CARs did not persist in the body, and therefore were not clinically effective. Over time, second generation CARs were developed and in 2017 became the first autologous CAR-T cell therapy approved by the United States Food and Drug Administration (FDA) with Kymriah® (Novartis). Since that time a second autologous CAR-T therapy has been approved by the FDA, with Yescarta® (Gilead) and other countries have granted approval including European Union, Japan, Australia and Canada. Results continue to be favorable, so much so that there are more than 250 clinical trials for autologous CAR-T therapies currently open [2].

Apheresis collection of T cells from patients represent a new scenario for collection centers and practitioners. Historically, peripheral blood mononuclear cells (PBMC), specifically lymphocytes, have been found in two settings:

1. Healthy donors to collect lymphocytes for infusions after stem cell transplant; and
2. Leukemic patients with high blast concentrations.

And while autologous stem cells collections in patients with malignancies have been characterized, much less has been published on the characterization PBMCs collections [3]. Much can be found in the literature regarding optimizations including both CAR-T manufacturing and vector manufacturing [3–5]. Many have also written about the starting material and its variability however the lion's share has been focused on hematopoietic stem cells (HPCs) and donor lymphocyte infusions (DLI) [6–8]. And though this information the principles and protocols of apheresis is not easily transferrable to PBMC collection for CAR-T manufacturing. Whereas patients or donors of HPCs or DLI often have very high total white cell counts due to medication or the disease process itself and the target cells are large, immature lymphocytes; eligible patients for CAR-T typically have low total white cell counts and the target cells are mature lymphocytes. These cells are smaller and more dense, creating technical challenges for apheresis. Ultimately, there is a need in the industry to better characterize PBMC or lymphocyte collection in those populations of patients with leukemia, who need these cells for their survival.

Regulatory expectations to control process variability during drug manufacturing is not new. The risks resulting from process variability has been acknowledged for drugs and biologics. As a result, controls to address process variability has become a regulatory requirement world-wide. Cell and gene therapy products are no different. The potential risks to patients may be different but the expectation is the same, developers must provide to the regulatory agency a manufacturing control strategy that both addresses process variability, efficacy and patient safety [9].

Sound science and a risk-based approach to product development can increase the assurance of product quality for a cell and gene therapy product, just as it does for other biologics. By appropriately characterizing the risks, a manufacturer can design a robust strategy to control risks and ensure the quality of the product. A practical application of such an approach has been published by the Parenteral Drug Association (PDA) [10]. It is worth noting that despite published guidance, practical application of quality metrics is an evolving science as understanding of what constitutes risk changes. Methodologies described in this PDA document can assist a developer in the optimization of the quality of the starting material for an autologous CAR-T product. To illustrate the utility of the risk-based approach, an example will be provided in this article. The aim of this article is to stimulate industry discussion around practical tools that developers can utilize as an aid toward addressing the variability of starting material for autologous CAR-T therapies.

There is no one risk assessment tool that works for all products, there are many which can be used by a developer. Examples of the

DOI: 10.18609/cgti.2020.117

tools which can be found in the literature include: Failure Mode Effect Analysis (FMEA), Criticality Analysis (CA), Process Capability Assessment and Hazard Analysis, to name a few. The PDA has published several reports which offer more details on the application of such tools [10–13]. Two tools have been selected to evaluate risk for the example provided in this article, Criticality Assessment and Process Capability (also referred to as Material Risk Assessment). CA is a simple method used to aggregate data, prioritize risk and organize decision making. Process Capability Assessment takes the qualitative results from the CA and evaluates the ability to control and detect the variability of the material [14].

Prior to evaluating the starting material of a product, a developer must first determine the critical quality attributes of the final CAR T product for infusion. Once a product's CQA have been identified, the next step is to determine the strength of the link between the apheresis starting material and CQAs for that product. CQAs are ascertained by understanding the desired characteristics of a new product. Common characteristics for cell-based products are; origin of cells, cell source, mode of action, therapeutic indication, dosage, storage conditions, shelf life and target population. Once the characteristics or attributes of a product have been determined, they serve as the basis for a product's quality attribute. Quality attributes are those attributes of a product that are likely or certain to influence the safety and efficacy of a product. It is important to highlight; the process used to identify and assess quality attributes is iterative. Throughout development, new quality attributes may be defined (immunophenotypically memory T cells) or removed (replication competent lentivirus) as science evolves.

Once a quality attribute is identified, it's criticality can be assessed. The assessment can be a qualitative or a quantitative process. The assignment of criticality is based on impact to patient safety and the level of confidence in understanding of the attribute. Once the criticality of a product's attributes is determined, they can be used to evaluate the severity of the risk or the strength of the link between the CAR-T product's critical quality attribute (CQA) and the starting material used for product manufacturing [15]. Establishing the criticality of the material attributes for autologous CAR-T apheresis starting material aids in the prioritization of additional assessments needed to manage variability of that starting material.

To demonstrate the practical application of risk-based tools, an autologous CD19 CAR-T product will be used. The end to end process flow is pictured below (Figure 1). It should be acknowledged that genetically modified cell-based products are far more complex than any other API. However, in order to present the use of the risk-based approach to optimize a control strategy, the example used will not address every attribute of a CAR-T product that is critical. The aim of the example is to demonstrate the actual tools and how they can be used by taking the reader through the process but limiting the product and material attributes to those that relate to the starting material.

FIGURE 1

Process flow diagram for autologous CD19 CAR-T product.

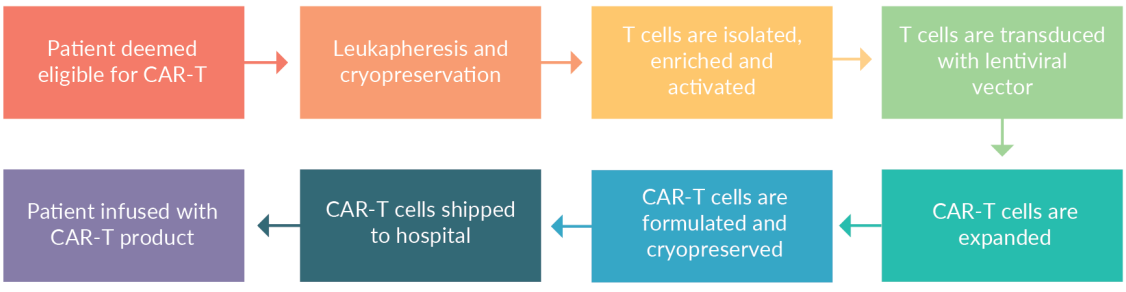


TABLE 1

Examples of quality attributes for autologous CD19 CAR-T products.

Category of attribute	Attribute
Safety	Microbial contamination (bacterial, viral, etc.) Endotoxin Replication competent retrovirus
Purity (impurity)	Percentage T-cell Percentage viability Viral vector proteins Residual CD19 B cells Other cell types
Potency	Percent transduction CAR expression Cytokine response to CD19 expressed cells Proliferation response to CD19 expressed cells Killing of CD19 cells
Content	Number of CAR$^+$ T-cells/kg body weight Total number viable cells

For the autologous CD19 CAR-T example, several quality attributes have been identified in the table below (Table 1; note: the list in Table 1 is not complete).

Each quality attribute is assessed for severity of harm to the patient and the uncertainty score provides the level of confidence in assessing the criticality of the attribute. Descriptions used to define severity and uncertainty for the autologous CD19 CAR-T example are shown below (Tables 2 & 3).

The criticality assessment results for the autologous CD19 CAR-T product attributes are provided in Table 4 [16–18].

For autologous CAR-T products, there has been much presented in the literature on the variability between patients due to disease progression, co-morbidities, treatment history, underlying prognostic factors and physical condition of the patient. Conversely, optimal pre-apheresis parameters both for maximizing overall collection efficiency and obtaining the optimal product composition are not clearly defined [19]. This means that garnering a better understanding of these variables on the final CAR-T product is a critical part of ensuring the product's safety and efficacy. CAR-T developers must be able to:

- Identify critical material attributes (CMA) of the apheresis material which can potentially impact CQAs of the product'
- Develop a knowledge base, either from the literature or in-house data, of those specific characteristics of the patients; and
- Identify the means to control those aspects which have a strong link to a product's CQAs.

For the autologous CD19 CAR-T product example, once the CQAs have been identified the next steps would be:

- Determine the strength of the link between the apheresis starting material and the CQAs of the CAR-T product. This can be done utilizing the CA tool;
- Conduct a Process Capability Assessment (PCA). This is another risk-based tool to evaluate the controls already in place for the apheresis material and whether they are adequate to deliver the product quality attributes identified.

The criteria used to determine linkage between starting material and CQA and the

TABLE 2

Criteria for impact (severity) assessment rating.

Rating	Impact
Negligible to low	Marginal patient impact; no potential for decreased safety; attribute is not expected to impact safety or efficacy
Medium	Small potential for patient impact that does not change the overall risk/benefit profile for the product; attribute may have a manageable adverse effect, but significant patient impact is improbable
High	Significant to catastrophic patient impact, changing the risk/benefit profile of the product

DOI: 10.18609/cgti.2020.117

TABLE 3

Criteria for uncertainty scoring.

Uncertainty	Prior knowledge
Low	Extensive literature available on this attribute; in-house data (*in vitro*, nonclinical or clinical) available, which provides a small level of confidence about the impact on efficacy or safety or the validity of the assessment
Medium	Attribute well understood based on scientific rationale; in-house data (*in vitro*, nonclinical, or clinical) available, which provides a moderate level of confidence about the impact on efficacy or safety or the validity of the assessment
High	Limited scientific understanding of this attribute; no clinical experience; limited in-house data, which provides a high level of confidence about the impact on efficacy or safety or the validity of the assessment

results of criticality assessment for the autologous CD19 CAR-T product starting material are provided in Tables 5 & 6 [16,20–29].

Two published case studies, evaluating criticality of material attributes in product development, recommend that for those material attributes deemed 'potentially critical', should be managed as they would a CQA until there is in-house data to support modifying the criticality of the attribute as critical or non-critical [30,31]. As stated earlier in this article the second tool, PCA, take those attributes of the starting material deemed 'critical or potentially critical' and evaluates a process's ability to control and detect the variability of the material. The criteria for scoring and results of the PCA for the autologous CD19 CAR-T product is found in Tables 7–9.

Health authorities expect that when a product contains human cells or tissues, relevant donor and sourcing material regulations apply. For apheresis, that means controlling the ingress routes from the external environment immediately prior to the collection and throughout the entire apheresis process. This includes using sterile consumables (apheresis kit), cleaning/maintenance of the apheresis machines, utilizing experienced nursing staff to collect, training on aseptic technique, and creating adequate welds when disconnecting the apheresis bag from the rest of the collection kit/tubing. Collection sites ought to validate their process for collections, verifying the controls are effective against sources of contamination from the external environment. A site should also monitor contamination events at their institution and make improvements as necessary.

All human sourced tissue or cells should be treated as if they have the potential to transmit disease, autologous apheresis starting material included. In the USA, the FDA does not require autologous cell donors be tested for adventitious agents with approved donor test kits [21] yet some centers chose to add the additional layer of control and test for the adventitious agent. Other regulatory agencies, such as the European Medicines Agency (EMA) do require donor testing [32]. Having said that, there are ways on reducing the risk of cross-contamination by a potential donor. Prior to collections, the patient can be evaluated by a physician, looking for active infection. The patient's chart can also be reviewed for active infection or behavioral risks. There are also tests available to confirm clinical infections. Another means to reduce the risk of cross-contamination occurs throughout (prior to, during and after) apheresis. Often referred to as 'Chain of Identity' the steps include:

1. Confirming the patient's identity;
2. Utilizing unique patient;
3. Utilizing unique donation identifiers;
4. Reconfirming the two unique identifiers prior to generating product labels;
5. Applying labels to product bags prior to their use;
6. Add secondary verification steps after labeling apheresis bag.

TABLE 4

Results for criticality analysis of autologous final CD19 CAR-T for infusion example.

Attribute	Severity	Uncertainty	Result	Rationale
Safety				
Sterility	High	Low	CQA	Sterility is a general safety requirement for all parenteral dosage forms to assure that cell products are free of microbial contamination
Adventitious agents	High	Low	CQA	Test performed on lentiviral vector. Wide range of viral contaminants being measured
Mycoplasma	High	Low	CQA	Mycoplasma can cause serious contamination in cell cultures, which may affect phenotypical characteristics and normal growth of the cells; some species can be pathogenic. (Note: the severity is only of high severity if it truly reflects the presence of mycoplasma and not a false positive)
Endotoxin	High	Low	CQA	Endotoxins (mainly lipopolysaccharides from gram negative bacteria) are highly pyrogenic substances that cause dose dependent fever and shock
Replication competent retrovirus	High	Low	CQA	The use of the lentiviral vector as a tool for gene transfer to cells presents a relatively low, theoretical safety risk to patient. The possibility of RCL generations can adversely impact patient safety
Purity				
Percentage T-cells	High	Low	CQA	The overall purity of T cells in the CAR-T products may be potentially impacted by presences of unwanted cell populations. The higher presence of unwanted T cells in the products offers more potential that unwanted cells may have been genetically modified
Percentage viability	High	Low	CQA	For *ex vivo* genetically modified cells, it is recommended to have at least 70% viability
Viral vector proteins	Med	Low	CQA	Residual protein can be immunogenic leading to allergic reactions, act as an adjuvant stimulating an immune response
Residual CD19 B cells	High	Low	CQA	The CAR gene unintentionally introduced into a single leukemic B cells. The target antigen is masked and goes unrecognized by the CAR product
Subpopulation T cell	Med	Med	CQA	Subpopulation of T cells may persist and expand differently in the patient. High proliferative capacity is required for anti-tumor efficacy [16]
Other white cells	Med	Med	CQA	Monocytes have been shown to inhibit T-cell activation and expansion [17]
Potency				
Transduction efficiently	High	Low	CQA	The active ingredient of the product is a CAR positive viable T cell. Transduction efficiency is used to calculate the final cell dose. It is essential to control this transduction step to ensure manufacturing consistency
CAR expression	High	Low	CQA	In-house cell base binding study demonstrated specific finding to human $CD19^+$ cells but not to non-human cells
Proliferation response to CD19 expressed cells	High	Low	CQA	Elevated levels of IL-6 measured in the blood. CAR Ts from these patients also tended to carry IL-6 receptors. Together, these characteristics support the early stages of T cell memory, involving cell proliferation and enhanced survival [18]
Killing of CD19 cells	High	Low	CQA	A series of *in vitro* studies was conducted to assessment the activity of the CAR positive cells on CD19 positive tumor cells
Content				
Total number viable CAR-T cells	High	Low	CQA	The transduced viable T cells are the active ingredient of the final product administered to the patient

DOI: 10.18609/cgti.2020.117

TABLE 5

Criteria for scoring the link between starting apheresis material and CQA for final autologous CD19 CAR-T product for infusion example.

Impact	Criteria	Criticality of material
High	Small to moderate change of this raw material attribute has a significant impact on a CQA and/or uncertainty is high	Critical
Medium	Large change of this raw material attribute or a small change in raw material attribute in combination with other factors has a significant impact on a CQA	Potentially critical
Low/negligible	The raw material attribute has no impact on CQAs, and uncertainty is low	Non-critical

An additional control, to reduce this risk further, may be to have the patient tested prior to collection, using methods approved by a regulatory body for donor testing. These tests are much more sensitive than clinical test methods used in clinical laboratories found within a hospital.

Obtaining enough T cells for manufacturing is challenging when the donors are leukemic patients who have gone through treatments, prior to becoming CAR-T patients. There are several ways that developers can control for having adequate numbers in their apheresis material. It is important to

TABLE 6

Results of criticality assessment for the starting material used in the manufacture of final autologous CD19 CAR-T product for infusion example.

CQA	Criticality of apheresis material	Justification
Sterility	Critical	Farrington reported that full aseptic precautions are necessary to prevent contamination of peripheral blood at collection. Cases reported where contamination events occurred when aseptic practice was not followed [20]
Adventitious agents	Critical	Infectious disease testing in the USA of a patient/donor of autologous material is not required [21]. Most other countries require some form of donor testing. As the product will be infused only to the patient, there are other risks of potential cross contamination between products at the manufacturing site and operator exposure
% CAR-T cells in final product	Critical	The correlation between pre-collection peripheral $CD3^+$ T-cells and manufacturing success has been reported. Some developers have set ALC thresholds for collection. In some cases, additional $CD3^+$ T cells minimum prior to collection was established [22,23]
Viability (%)	Potentially critical	Sorensen reported post collection, T-cell viability immediately starts to diminish. At ambient, viability reduction is much quicker. Preserving cells at 2–8C increase may maintain viability up to 24 hours [24]
Residual $CD19^+$ cells	Critical	Ruella reported a case when a leukemic B cells was unintentionally transduced, conferring resistance to CD19 CAR-T cells, leading to patient relapse [25]
T cells sub-populations	Critical	Singh et al. reported patients with T-cell populations enriched from early lineage cells expanded better *in vitro* [16]
Off target WBCs (NK, Mono)	Critical	Bryn reported how monocytes can inhibit T-cell activation [26] Stroncek reported the potential impact of monocytes on T-cell transduction, finding that CAR-T cells product deemed manufacturing failures had up to twice the number of monocytes [27] Allen reported the association between high proportions of NK cells and lower $CD3^+$ cell collection yields as likely due to the inverse relationship between NK $CD3^+$ cells [28]
Non-WBC contaminants (RBCs, PLTs)	Potentially critical	Fesnak reported red cells and platelets make accurate lymphocyte enumeration difficult and/or confounds flow cytometry. Other components such as plasma and platelets can induce clumping. Assmus reported red cells have been shown to interfere with the clinical efficacy of some types of therapeutic cells [29]

TABLE 7

Criteria for occurrence scoring.

Rank	Rating	Criteria
10	Frequently	Material attribute variability is frequently expected (i.e., more frequently than 1 in 10 batches of the material). Material is complex and likely to degrade or introduce a degradant to the process. Failure of the material attribute is likely to happen frequently
7	Fairly frequently	Material attribute variability is expected fairly frequently (i.e., probability of 1 in 10 to 1 in100 batches of the material). Material is somewhat complex and somewhat likely to degrade or introduce a degradant to the process. Failure of the material attribute is likely to happen fairly frequently
4	Fairly infrequently	Material attribute variability is expected fairly infrequently (i.e., probability of 1 in 100 batches of the material). Material is fairly simple in nature and unlikely to degrade or introduce a degradant to the process. Failure of the material attribute is likely to happen fairly infrequently
1	Infrequently	Material is consistent and simple. Failure due to the material attribute is likely to happen infrequently (i.e., almost never)

understand the types of treatment the patient has been subject to and when their last treatment was completed as this can compromise the number and function of T cells [19]. Determining the pre-collection $CD3^+$ count can help predict if the collection will be adequate. This number is also needed to determine the number of blood volumes to be processed and how long the procedure will last. Understanding if and how a collection sites monitors their collection efficiency can be useful towards being able to predict how many blood volumes will need to be process. Collection efficiency (CE) is the proficiency of a collection site's equipment, procedure and operators to collect the cells utilizing apheresis machines. CE can be a useful tool toward assuring target cell counts are reached. Cantwell reported that CE could be used to predict the number of blood volumes required to achieve a specific target cell count of hematopoietic stem cells [33]. It would be useful to conduct similar research for mononuclear cell collections in leukemic patients. Another control may be to draw samples mid-way through a procedure to see if they are on track to collect the original target cell number. Finally, at the manufacturing site, there are several means that sites chose to ensure adequate number of T cells. One example is testing, be it in-process testing to monitor steps throughout processing or release testing for $CD3^+$ cells (or T cells). Additional controls to reduce risk even further can include steps such as understanding patient co-morbidities or hematologic lab values. Patients with certain disease states, like multiple myeloma, can have elevated serum proteins that reduce the collection efficiency of an apheresis machine. Plasmapheresis of these patients prior to mononuclear cell collection has be shown to reduce protein level and improve the chances of collecting an adequate number of lymphocytes [34].

Manufacturing sites can also include steps to enrich the product for lymphocytes, the simplest way being density gradient separation. Sites can also include steps utilizing magnetic bead to select positively for the cells desired [35]. This step can also be used for the example above to reduce the risk of having inadequate numbers of T-cells in the

TABLE 8

Criteria for detection scoring.

Rank	Rating	Criteria
10	Impossible	Failure of the material attribute is not detected at all or not until the product reaches the patient
7	Moderate	Failure of the material attribute can be detected by batch release procedures
4	Highly likely	Failure of the material attribute is likely to be detected prior to the final unit operation
1	Almost certain	Failure of the material attribute is likely to be detected prior to its use in the process. Appropriate controls are in place to maintain stability of the material attribute after testing and prior to its use in the process

DOI: 10.18609/cgti.2020.117

TABLE 9

PCA results for final autologous CD19 CAR-T product for infusion example.

CQA	Severity	Controls in place		Occurrence	Detection	Risk rating
Sterility	10	Pre-apheresis	Physical evaluation of patient for infections	1	4	40
			Medical record review for infections			
		Peri-apheresis	Validated collections process utilizing aseptic technique. Sterile consumables			
Adventitious agents	10	Pre-apheresis	Physical evaluation of patient for infections	1	4	40
			Medical record review for infections			
			Clinical laboratory testing for active infections (HIV, HBV, HCV)			
		Pre- and post-apheresis	CoC/CoI steps to identify the patient, pre-label collections, and utilize unique patient/donation identifiers			
% T cells in final product	10	Pre-apheresis	Prior therapies – time of last chemotherapy should be confirmed	7	1	70
			Pre-counts lymphocytes			
		Peri-apheresis	Collection efficiency specific to the institution, apheresis machine, software and operator			
			Draw sample midway through procedure to assess number $CD3^+$ T cells			
		Post-apheresis	Test apheresis material for CD4 and $CD8^+$ cells			
			Test final product CD4 and $CD8^+$ cells			
Residual tumor cells	10	Pre-apheresis	Patient treatment plan and eligibility of patient based on last treatment date	1	7	70
			Residual tumor cells can be reduced by collecting patient cells while the patient is in remission.			
		Post-apheresis	Positive selection of CD4 and CD8 cells isolated prior to expansion			
			Test final CAR-T product for phenotype $CD19^+$ and NK cells			
			Use of magnetic beads to positively select CD4/CD8 T cells			
Sub-populations T cells	10	Pre-apheresis	Absolute lymph count (ALC) > 0.3×10^9/L	4	4	160
		Peri-apheresis	Target 2×10^6 cells			
		Post-apheresis	Use of magnetic beads to positively select CD4/CD8 T cells			
			In-process testing for number of CD4/CD8 cells			
			Test final product for $CD4^+$ and $CD8^+$ cells			
Off target WBC	10	Post-apheresis	Elutriation step to remove unwanted monocytes and plasma cells	4	1	40
Off target non-WBC	5	Pre-apheresis	ALC count >500 microliter	4	1	20
		Post-apheresis	Preculture processing on MNC to remove red cells and platelets			
			Use of DNase remove unwanted plasma components and platelets			

final product. Finally, DNase can be used to remove unwanted plasma components that may lead to clotting or clumping.

Some have determined that calculating ALC for a patient can help determine both processing volume and time spent on an apheresis machine. Reducing the volume and time can aid in the reduction of red cells and platelets that end up in the collection bag [19]. Others have reported the used of pre-culture processing of the mononuclear cells can help provide a relatively pure lymphocyte population [5].

As stated at the beginning of this article, the intent was to provide the reader with a practical application of risk-based tools to address the variability of starting material for autologous CAR-T therapies that can be used in discussion with regulators. Previous documents published have focused on the use of such tools for the manufacturing process. The overall goal of this article was to provide an example of those same tools used to control variability for starting material used in CAR-T manufacturing. It is felt that the tools described are flexible enough to provide developers with a means toward developing and effective control strategy for the starting material. It is also understood that the risk based approached described is an iterative process. Throughout clinical development with an increase in information and characterization of the process and product, the tools should be revisited periodically to see if there needs to be any modification to the control strategy. Although many challenges lie ahead for the industrialization of CAR-T manufacturing, there is reason for optimism. Decades of experience with risk-based tools in the industry have provided the foundation for the use of risk-based approaches towards the optimization of the CAR-T manufacturing and the apheresis processes used to collect the starting material.

REFERENCES

1. Styczynski J. A Brief History of CAR-T: From Laboratory to Bedside. *Acta Hematologica Polonica*, 2020; 51(1): 2–5.
2. National Library of Medicine, Bethesda, MD, USA: www.ClinicalTrials.gov
3. Molloy M, Conry C, West K. *Chimeric Antigen Receptor T-Cell Therapies for Cancer*. Elsevier, Inc., 2020.
4. Tyagarajan S, Spencer T, Smith J. Optimizing CAR-T Cell Manufacturing Processes during Pivotal Clinical Trials. *Mol. Ther. Meth. Clin. Dev*. 2020; 16: 136–44.
5. Fesnak A, Lin C, Siegel D *et al*. CAR-T therapies from the transfusion medicine perspective. *Trans. Med. Rev.* 2016; 30: 139–45.
6. Allen E, Stroncek D, Ren J *et al*. Autologous lymphapheresis for the production of chimeric antigen receptor T cells. *Hemapheresis* 2017; 57: 1133–41.
7. Ceppi F, Rivers J, Pinto N *et al*. Lymphocyte apheresis for chimeric antigen receptor T-cell manufacturing in children and young adults with leukemia and neuroblastoma. *Trans. Cell. Eng.* 2018; 58: 1414–20.
8. Tuazon S, Li A, Gooley T *et al*. Factors affecting lymphocyte collection efficiency for the manufacture of chimeric antigen receptor T cells in adults with B-cell malignancies. *Cell. Ther*. 2019; 59: 1773–80.
9. Yonatan Y, Timmins NE, Zandstra PW. Quality cell therapy manufacturing by design. *Nat. Biotechnol.* 2016; 34: 393–400.
10. Parenteral Drug Association, 2018. Technical Report No. 81 Cell-Based Therapy Control Strategy.
11. Parenteral Drug Association, 2014. Technical Report No. 44 Quality Risk Management for Aseptic Processes.
12. Parenteral Drug Association, 2012. Technical Report No. 54 Quality Risk Management for the Design, Qualification and Operation and Manufacturing Systems.
13. Parenteral Drug Association, 2013. Technical Report No. 60 Process Validation: A Lifecycle Approach.
14. Garcia T, Cook G, Nosal R. PLQI Key Topics- Criticality, Design Space and Control Strategy. *J. Pharma. Innov*. 2008; 3: 60–8.
15. Rathore A. Roadmap for the implementation of quality by design (QbD) for biotechnology products. *Trends Biotechnol.* 2009; 27: 546–53.
16. Singh N, Perazzelli J, Grupp SA *et al*. Early Memory Phenotypes Drive T Cell

DOI: 10.18609/cgti.2020.117

Proliferation in Patients with Pediatric Malignancies. *Sci. Trans. Med.* 2016; 8: 52–63.

17. Stroncek D, Lee D, Ren J *et al.* Myeloid Cells in Peripheral Blood Mononuclear Cell (PBMC) Concentrates Inhibit the Expansion of Chimeric Antigen Receptor T Cells. *Blood* 2015; 128: 383–95.

18. Yeager A. T Cell Proliferation Linked to CAR-T Responses. *The Scientist* 2019; 366: 64–9.

19. Paroder M, Le N, Pham N *et al.* (2020) Important aspects of T-cell collection by apheresis for manufacturing chimeric antigen receptor T cells. *Adv. Cell Gene Ther.* 2020; 1: 1.

20. Farrington M, Matthews I, Marcus R *et al.* Contamination of bone marrow transplants from peripheral blood. *BMJ* 1994; 309: 958.

21. Human Cells, Tissues, and Cellular and Tissue-Based Products. 21 C.F.R. 1271 (2003).

22. Ceppi F, Rivers J, Annesley C *et al.* Lymphocyte apheresis for chimeric antigen receptor T-cell manufacturing in children and young adults with leukemia and neuroblastoma. *Transfusion* 2018; 58: 1414–20.

23. McGuirk J, Waller EK, Qayed M *et al.* Building blocks for institutional preparation of CTL019 delivery. *Cytotherapy* 2019; 19: 1015–24.

24. Sorensen J, Jarisch A, Smorta C *et al.* Pediatric apheresis with a novel apheresis device with electronic interface control. *Transfusion* 2013; 53: 761–5.

25. Ruella M. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nature Med.* 2018; 24: 1499–503.

26. Bryn T, Yaqub S, Mahic M *et al.* LPS-activated monocytes suppress T-cell immune responses and induce FOXP3+ T-cells through a COX2-PE2-dependant mechanism. *Interntl Immunol.* 2007; 200: 20235–245.

27. Stroncek DF, Ren J, Lee DW *et al.* (2016) Myeloid cells in peripheral blood mononuclear cells concentrates inhibit the expansion of chimeric antigen receptor T cells. *Cytotherapy* 2016; 18: 893–901.

28. Allen E. Autologous lymphapheresis for the production of chimeric antigen receptor T cells. *Transfusion* 2017; 27: 1133–41.

29. Assmus B, Tonn T, Seeger FH *et al.* Red blood cell contamination of final cell product impairs efficacy of autologous bone marrow mononuclear cell therapy. *J. Am. Coll. Cardiol.* 2010; 55: 1385–94.

30. CMC Biotech Working Group, CASS/ ISPE. (2009). A-Mab; A Case Study in Bioprocess Development. CASSS. [Online] 2009.

31. CMC-Vaccines Working Group, Parenteral Drug Association. A-VAX: Applying Quality by Design to Vaccines. Bethesda, MD: Parenteral Drug Association, 2012. p. 383, Case Study

32. Commission Directive Implementing Directive 2004/23/EC if the European Parliament and of the Council as regards certain technical requirements for donation, procurement and testing of human tissues and cells. 2006/ 17/EC (2006).

33. Cantwell L, Blunk B. Validation of an Apheresis Collection Prediction Algorithm for Hematopoietic Progenitor Cells Using the Spectra Optia Cmnc Protocol. *Transfusion* 2019; 25: 635.

34. Wahlin A, Grubb A, Holm J *et al.* (1988) Effects of Plasmapheresis on the Plasma Concentration of Proteins Used to Monitor the Disease Process in Multiple Myeloma. *J. Int. Med.* 1988; 23: 263–7.

35. Stroncek D. Exploring the sources of Variability Related to the Clinical Translation of Regenerative Engineering Products: Proceedings of a Workshop. National Academies of Sciences, Engineering and Medicine: Health and Medicine Division: Forum on Regenerative Medicine, 2019.

AFFILIATION

Jean Stanton
Janssen Pharmaceuticals

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author declares that they have no conflicts of interest.*

Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Jul 11 2020;* ***Revised manuscript received:*** *Aug 14 2020;* ***Publication date:*** *Sep 8 2020.*

DOI: 10.18609/cgti.2020.117

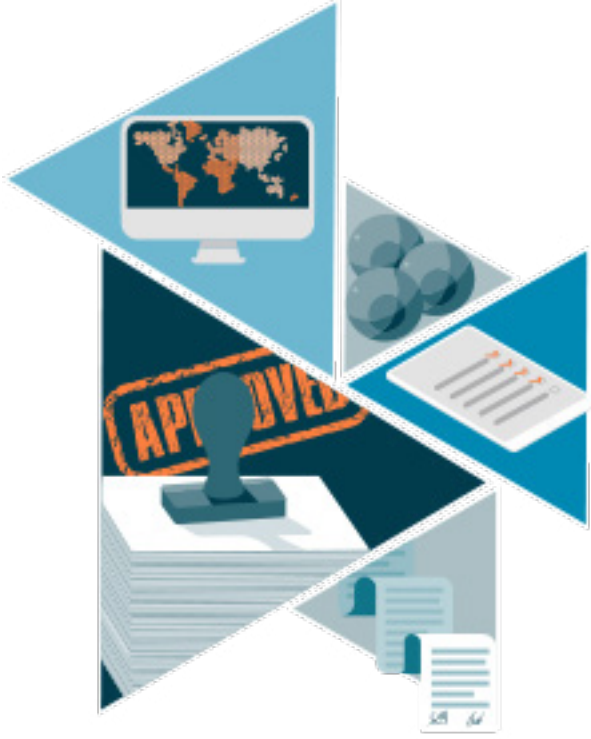
CELL & GENE THERAPY INSIGHTS

September 2020

Regulatory Insights

CELL & GENE THERAPY INSIGHTS

Regulatory Trends



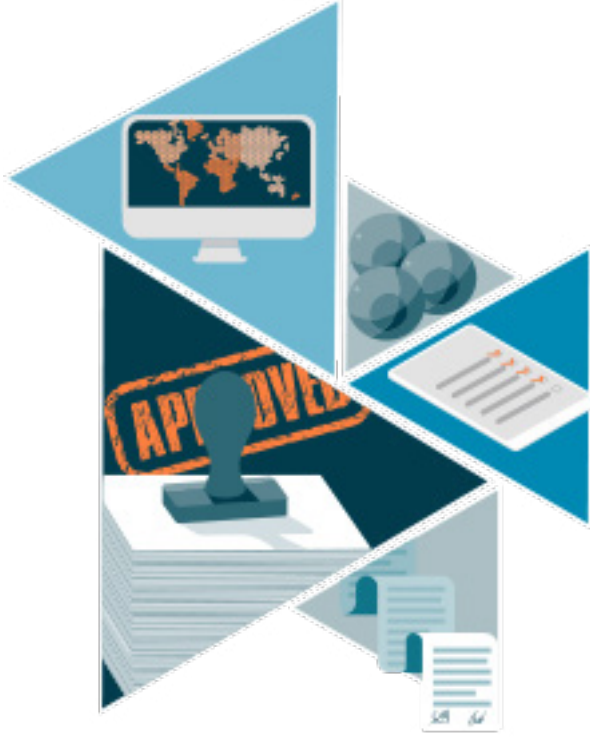
September 2020
Volume 6, Issue 8

REGULATORY PERSPECTIVE

INTERVIEW

REGULATORY PERSPECTIVE

CELL & GENE THERAPY INSIGHTS



Regulatory Insights

REGULATORY PERSPECTIVE

Advanced therapy regulation in the UK: what might the future hold post-Brexit?

Patrick J Ginty

The development of Advanced Therapy Medicinal Products (ATMPs) in the European Union (EU) is subject to a unique 'patchwork' of associated but distinct European regulations and directives. However, the UK's exit from the EU has provided an opportunity to evaluate all medicines legislation that currently applies in the UK, including those governing the regulation of ATMPs. Despite the challenges and uncertainties associated with the UK's exit from the EU, it is important to acknowledge that this change can create new opportunities from which the UK can benefit, whilst staying aligned to the EU. The UK can take the opportunity to orient its legislation and associated procedures towards the unique requirements of ATMPs throughout their life-cycle; from the procurement of cell and tissue-based starting materials, the Gene Modified Organism (GMO) legislation through to manufacture, licensure and adoption. This paper discusses where, if at all, the UK might diverge or expand from the EU regulatory framework for ATMPs and what challenges and advantages this might bring.

Cell & Gene Therapy Insights 2020; 6(8), 1171–1178

DOI: 10.18609/cgti.2020.126

The publication of the EU Regulation for Advanced Therapy Medicinal Products (ATMPs) (EC/1394/2007) was a landmark moment in the history of cell, gene and tissue-based therapies in Europe, as it provided a pathway to market for a sub-set of medicinal products

that provide a unique set of scientific and regulatory challenges. As part of this Regulation, it was mandated that all ATMPs be granted marketing authorisation (MA) via the centralised procedure by the European Medicines Agency (EMA) and ultimately the European Commission (EC). It also mandated that ATMP approval would be subject to receiving a positive opinion from not only the Committee for Medicinal Products for Human Use (CHMP) but also from the newly formed Committee for Advanced Therapies (CAT). Since the ATMP Regulation came into force in 2008, the EU regulatory framework has developed and evolved with the aim of providing robust scientific guidance for developers, whilst aiming to increase a more harmonised approach across the EU member states responsible for regulating both clinical trials and Good Manufacturing Practices (GMP). For example, the current Clinical Trial Directive (CTD) (2001/20/EC) will be replaced by the delayed Clinical Trial Regulation (CTR) (EU/536/2014) in December 2021 with the aim to provide greater clinical trial harmonisation, including a new European-wide submission portal and consolidated assessment and approval system. Up until Brexit, the UK, as a member of the EU, had been a prominent voice in all medicines regulation across Europe, especially in the field of ATMPs where it had been at the forefront of shaping legislation and guidance, as well as leading a large proportion of EMA assessment procedures.

However, after the conclusion of the Brexit transition period, which is currently due to end on December 31st, 2020, and notwithstanding an unlikely extension, the UK is set to become a "third country" in relation to the EU. After this period, ATMPs will require a separate marketing authorisation in both the EU and the UK, and the UK will be excluded from the centralised system for clinical trials. Clearly this would also be the case in the situation of a no-deal Brexit. It is important to keep in mind that these changes and many others will apply to all medicines of which, despite the rapid growth of the field, ATMPs are a small percentage when compared to the more traditional small and large molecule drugs. That said, the development of ATMPs in the EU is subject to a unique 'patchwork' of associated but distinct European regulations and directives, many of which do not apply to more conventional pharmaceuticals, even those of biological origin. So, whilst for many medicines, aiming for a regulatory 'status-quo' after Brexit would effectively ensure the stability of the pharmaceutical sector, this does not account for some of the nuances of ATMP regulation. Similarly, a "status quo" would not take into account that there could be additional measures which, if implemented, could provide the sort of advantages offered by operating within a single sovereign regulatory framework.

The new medicines and medical devices bill, when granted Royal Assent in the UK, will enable the existing regulatory frameworks to be updated after the end of the transition period. This will result in the transition of the majority of existing EU requirements into UK law, but also permit the regulatory framework to be amended and supplemented. Importantly, the amendment and supplementation of the existing regulatory framework does not necessarily translate into "reinventing the wheel". Existing standards for the safety, efficacy and quality of medicines are built on a number of global harmonisation attempts e.g. the International Conference on Harmonisation (ICH) and the Pharmaceutical Inspection Co-operation Scheme (PIC/S), and there is little perceived advantage in diverging from those fundamental global regulatory pillars, or more specifically from the scientific approach adopted by the European Medicines Agency (EMA) and the National Competent Authorities (NCAs) in the EU. Equally, it should be remembered that the UK's Medicines and Healthcare Products Regulatory Agency (MHRA) has been an active member of the European framework and has been heavily involved in driving those policies implemented by the European Commission and EMA. Therefore, fundamental elements of clinical trial authorisation (CTA)

DOI: 10.18609/cgti.2020.126

or MA applications are unlikely to diverge from the EU, even if the procedures required for authorisation may do e.g. due to the implementation of the new EU Clinical Trial Regulation.

Clearly, there are a number of regulatory pathways and procedures that may change, whether it be through simplification of a procedure or, the addition of "accelerated" pathways to licensure. The MHRA is known for being an innovative regulatory agency, with successful implementation of procedures and processes to both foster innovation and speed products to market. Examples of this include the Innovation Office (IO) regulatory advice service and the Early Access to Medicines Scheme (EAMS). Some areas certainly lend themselves to change more readily than others, if not only because it could be the simplification of an assessment procedure that does not impact the scientific integrity, or the level of regulatory robustness applied. Ultimately, the MHRA is likely to follow a path of "evolution" and not "revolution" with regard to aligning with global standards for quality, safety and efficacy. So, what could be different?

REGULATION OF TISSUES & CELLS AS STARTING MATERIALS

With the obvious exception of those developers of in-vivo gene therapies, the regulatory requirements for the donation, procurement and testing of tissue- and cell-based starting materials for onward manufacture into ATMPs, represents the first link in the chain and an area of potential scrutiny for the UK post-Brexit. However, divergence from the European Tissues and Cells (2004/23/EC) or Blood (2002/98/EC) Directives would seem unlikely given the desire to maintain harmonised standards, especially those for donation and testing, which can already create issues for materials transferred between the EU and US. In the UK starting materials are regulated by the Human Tissues Authority (HTA) based on both the UK Human Tissue Act, the Human Tissue Act (Scotland) and the Tissue Quality and Safety Regulations (TQSR), whilst the regulation of blood as an starting material is based on the UK Blood Safety and Quality Regulations (BSQR) and within the remit of the MHRA. However, there is certainly scope for some streamlining of the interactions between regulatory authorities in the UK. To this end, the HTA and MHRA have already agreed to allow collection of blood as a starting material for an ATMP to be performed under either an HTA or Blood Establishment licence in the UK. The pragmatism of both the UK regulatory bodies involved bodes well for the future, irrespective of the nature of the Brexit deal.

CLINICAL TRIALS

One area that might lend itself to change are the EU requirements and procedures related to Genetically Modified Organisms (GMOs), which have long been viewed as a bottleneck to the conduct of clinical trials involving gene therapy medicinal products and other medicines consisting of gene-modified cells. This is often not due to technical issues, but more as a result of the divergence in approach and protracted "approval" processes across different EU member states. The GMO legislation in Europe consists of two key directives 1) Directive 2001/18/EC on the deliberate release (DR) into the environment of genetically modified organisms and 2) Directive 2009/41/EC on the contained use (CU) of genetically modified micro-organisms. Although the fundamental concepts of both Directives are based upon an assessment of risk related to the GMO aspects and then any steps taken to protect humans and the environment, the implementation of each Directive varies enormously throughout Europe. The UK follows the CU requirements during clinical trials, as regulated by the Health and Safety Executive (HSE), with the burden of responsibility largely falling on the clinical trial site (as a contained use facility) along-side the sponsor, to comply with the requirements

for the containment of GMOs. Elsewhere in Europe, member states implement either the CU or DR requirements (or in some cases both), with little or no harmonisation with respect to the documentation requirements, procedures or timelines.

The new CTR in Europe provides a more harmonised approach to clinical trial approvals in multiple member states but it does not consider the divergent procedures and timelines for GMOs. Therefore, a single UK regulatory submission that considers the approval of the GMO aspects, in addition to the CTA and perhaps also ethical approval via the Health Research Authority (HRA), would be highly desirable in terms of planning and conducting clinical trials. The UK is already one of the go-to places in the world for clinical trials involving ATMPs, accounting for 12% of global clinical trials [1]. Therefore, further simplification of the approval process using a "single front door" and thus shorter timelines could strengthen this offering, albeit for an approval in a single country and not multiple countries. This would not necessarily exclude the HSE but could allow a single coordinated review, with a single decision that reflects the very low risk associated with the vast majority of ATMPs, especially those that are cell-based. Another possibility would be to follow the approach taken in the US, whereby sponsors can claim a categorical exclusion from the requirement to submit an environmental assessment as part of the IND, such is the low risk.

Another area of clinical research that will likely come under scrutiny will be the hospital exemption (HE) clause, which was introduced as part of the ATMP regulation to allow individual patients to have access to products on a non-routine basis and at the request of the treating physician. The idea being that patients can have access to innovative medicines that do not yet have a marketing authorisation, provided the conditions within the legislation are met. As HE is regulated nationally, it has been subject to very different interpretations throughout Europe and has been used as a pathway to treat patients in EU member states where there is already an existing authorised product for the same indication. The industry has been concerned for some time about HE being a disincentive to take the route of a centralised marketing application, as mandated in the ATMP regulation [2]. In addition to HE, the UK has the "Specials" route which also allows the supply of unlicensed medicines but with the provision that there is not a licenced equivalent product already available, making it distinct from the HE clause[3]. Therefore, it will be interesting to see if HE remains part of the legislation in the UK post-Brexit with respect to ensuring that there as a strong incentive for companies to ensure the safety and efficacy of new therapies through the submission of a national MAA and balancing this with the desire to foster innovation.

GOOD MANUFACTURING PRACTICES

Good Manufacturing Practice (GMPs) are also an interesting area for exploration. Logically, GMP should be as harmonised as is possible, as any great divergence may prevent future addition of ATMPs to the scope of mutual recognition agreements for recognising regulatory inspections and QP certification. Such levels of mutual recognition are not yet in place between the EU and any other territory for ATMPs, with the exception of Switzerland, but given the UK's prominent role in the development of EU legislation and guidance for medicinal product GMP, there is certainly an opportunity for reciprocal flexibility to be introduced. This would significantly ease the burden of the requirement to QP certify between the UK and EU for clinical trials, but perhaps more importantly, pave the way for the prevention of repeated batch testing for marketed products manufactured in the UK, as currently mandated for products manufactured in third countries. Repeat testing of ATMPs in many cases may not be feasible due to limitations on material available for QC, short shelf lives, increased costs, etc.,

DOI: 10.18609/cgti.2020.126

and although exemptions from repeat testing can be permitted, achieving a suitable level of harmonisation is not going to be straightforward. It should be noted that EU GMP requirements for ATMPs are now subject to a standalone guideline in Europe (EudraLex Volume IV, Part IV) that in itself diverges from previous incarnations of EU GMP and PIC/S guidelines and has not been without some scepticism from both PIC/S and the industry [4,5,6]. Recent developments from PIC/S have indicated that although their own guidance regarding GMP for ATMPs is evolving, it will not be standalone and global harmonisation remains a moving target for these innovative medicines [7]. The evolving guidelines also hint at the development of more innovative methods of applying GMP in order to prevent innovation from being stifled. For example, some cell- or tissue-based ATMPs, do not lend themselves to long-term storage or transport and may require a scale-out approach. Therefore, there is a question over whether conventional GMP requirements may possibly encumber clinical development and commercialisation. For example, a short shelf life product, which is autologous and does not have economies of scale for manufacture, may require a different manufacturing and supply model, whereby one or more manufacturing steps occur closer to the patient [8]. If the product is intended to treat 100s if not 1000s of patients a year, this could be further restricted by the need to manufacture at multiple licenced manufacturing sites with full QP oversight and certification, not to mention the increased inspection burden placed upon the regulatory authorities. It is clear there will be little appetite for the UK to diverge on the requirements for some form of authorisation to be in place for every manufacturing site, nor remove the need for QP certification but there may be scope to adapt the licencing model and introduce some flexibilities into the way that QP certification and oversight is conducted, in order to facilitate innovative manufacturing models. In other words, divergence only occurs where there is a requirement to diverge from, so additional legislation that permits regulatory flexibilities for the manufacturing of innovative products like ATMPs is certainly an attractive approach. However, any changes or flexibilities to GMP cannot be considered in isolation, as the requirement for the scale-out of manufacturing models also presents more data-driven (e.g. product comparability) and quality management related challenges, which require further investigation [9].

LICENSING PATHWAYS & REIMBURSEMENT

The licencing pathways for medicines, including ATMPs, is also a potential area for further incentives and flexibilities to be introduced. Perhaps the biggest challenge for the UK will be maintaining its role as a high priority market once not part of the EU. Based upon numbers alone, a UK-only marketing authorisation will not be comparable with that of the US, EU or Japan, though closer to the latter. The risk is that the UK will fall down the pecking order in terms of access to medicines and that the MHRA could have a diminished role in the assessment process, as it seems unlikely that the UK would or could, for example, justify a full assessment of licence application for a product already approved in the EU. However, this refers to the standard 210-day assessment, as required for any ATMP going through the decentralised procedure and does not take into account accelerated or novel licensing pathways. The EMA can currently grant accelerated assessment to products, thus reducing the 210-day assessment period to 150 days, with eligibility being based upon a justification that the medicinal product is expected to be of major public health interest. So, could the UK have a more rapid assessment than 210 days as standard as an incentive, with a further reduction in assessment time for game-changing medicines? This would surely depend upon the product in question and the assessment procedure. US FDA has been using rolling review procedures for some time [10] to allow parts of a dossier

to be reviewed as and when they become available, something that was being considered by the UK in the event of a no deal Brexit, and may be considered again regardless of the outcome of the political negotiations [11].

Accelerated assessment can also be made more accessible by development tools such as the PRIority MEdicines (PRIME) scheme. PRIME designation is broadly equivalent to both breakthrough designation (BTD) and Regenerative Medicine Advanced Therapy (RMAT) designation in the US, providing early dialogue with the EMA for enhanced interaction and optimisation of development plans for medicines which are promising candidates for targeting unmet medical need. Unlike RMAT, PRIME is not specific to cell, tissue and gene therapies, and this could be a key differentiator, as although a high percentage of ATMPs are granted PRIME status (around 50% of all successful applications are ATMPs), they tend to be largely in the field of oncology, and largely for rare indications [12]. There is an opportunity for the UK to build on the Early Access to Medicines Scheme (EAMS) whereby the PIM (Promising Innovative Medicine) designation currently allows patients to gain access to a medicine that does not yet have a MA, but offers benefit in an area of unmet clinical need. EAMS is not seen as a pathway to commercialisation and would have a different context in the presence of a national licensing pathway for ATMPs. However, an equivalent to PRIME designation could provide access to a comparable conditional marketing authorisation that gives full agency support and scientific advice to accelerate the route to market for ATMPs that target unmet clinical need e.g. approval based on limited clinical data. The UK could look to Japan where they have introduced their own conditional marketing authorisation for regenerative medicine products, basing the initial approval upon initial signals of efficacy followed by the requirement to collect further clinical data post-approval. The Sakigake designation, broadly equivalent to PRIME and BTD / RMAT, has been introduced as a mechanism to further accelerate this pathway, so there are obvious comparisons to be drawn [13]. The true long-term success of the Japanese approval pathway is yet to be determined but the other aspect to it which may come into play, is the complex and challenging requirement to gain reimbursement for an approved ATMP. As previously stated, ATMPs are approved centrally by the EMA but there is no realistic option for centralised reimbursement, which must be negotiated country by country, making pricing and reimbursement a national concern.

Therefore, as with the Japanese system, there is certainly an opportunity for the UK to examine the interrelationship between licensure, reimbursement and market access. The UK was the first EU market that considered the appropriateness of its assessment frameworks for ATMPs and what changes are needed to address the challenges these therapies present in terms of both health technology assessment and adoption [14]. As a result, the UK provides the most progressive assessment frameworks that help capture the greater benefits of ATMPs while accounting for common limitations (such as deviation from mainstream supporting evidence datasets).

The UK's Accelerated Access Review (2016) reported that an accelerated access pathway (AAP) for strategically important, transformative products should align and coordinate regulatory, reimbursement, evaluation and diffusion processes to bring these transformative products to patients more quickly [15]. The pathway aims to bring forward reimbursed access by up to four years in some cases and is expected to provide the opportunity to collaborate with the NHS to collect both real-world and clinical trial data to evaluate product outcomes and pathway changes and use this data elsewhere.

One thing that has become clear, is that regulatory speed alone is insufficient. If the authorised product is not adopted, it may well be the end of the innovator company or at least result in withdrawal of the product from the market. Therefore, it is this linkage between those in the healthcare ecosystem that will likely make the most significant

DOI: 10.18609/cgti.2020.126

change to the UK. A system where innovators are engaged with all involved in the regulation and uptake of their products from the earliest stages of development will likely make a transformational difference in the UK.

LOOKING BEYOND EUROPE

There are also procedures and mechanisms which may be adopted or adapted from other regulatory frameworks, which again do not change the fundamental legislation in place but provide additional flexibilities for sharing data with regulatory agencies. One such example is the drug master file system, that is much utilised by the US FDA but has no direct equivalent in Europe. The existing use of plasma master files (PMFs), vaccine master files (VMFs) and active substance master files (ASMFs) does create precedent for the use of a master file system, though none are directly applied to the development or manufacture of ATMPs.

According to Annex 5 in the Guideline on Active Substance Master File Procedure CHMP/QWP/227/02 Rev 4 [16], the ASMF procedure cannot be used for biological active substances because "The characterisation and determination of biological active substances' quality requires not only a combination of physico-chemical and biological testing, but also extensive knowledge of the production process and its control". Therefore, any ATMP-focussed master file system implemented in the UK would have no reciprocal system in the EU but may be a possible method of incentivising those companies that hold master files in the US, to make a similar submission to the UK authorities to permit cross-reference to the data e.g. starting materials, raw materials, etc. as part of a CTA or MA. By cross-referencing the master file number, the sponsor does not have to provide all the quality information relating to the material in question within their filing. However, the sponsor will remain responsible for the use of that material within their manufacturing process, despite not necessarily being aware of the content of the material in question and therefore its quality e.g. if shared confidentially between the master file applicant and the MHRA. Therefore, it is not without complexity.

It may also be feasible for the UK blood and tissue establishments to become certified according to the United States' Clinical Laboratory Improvement Amendments (CLIA). This would be an advantage relative to the rest of the EU for developers procuring starting materials in the UK and transferring these to USA and therefore having to comply with 21 CFR Part 1271.

In conclusion, successfully delivering on these perceived advantages following a European exit hinges on a complex balance between maintaining alignment with the EU's regulatory framework for medicines and to the extent possible, adapting UK legislation and procedures towards a streamlined and efficient process for the development and commercialisation of ATMPs with the aim of timely patient access. Only time will tell if the UK can see a regulatory advantage to remain competitive on a global scale but there is clearly scope for some changes that would benefit both ATMP developers and patients alike.

REFERENCES

1. Catapul, Press release: UK accounts for over 12% of global cell and gene therapy clinical trials https://ct.catapult.org.uk/news-media/general-news/press-release-uk-accounts-over-12-global-cell-and-gene-therapy-clinical
2. Alliance, Regenerative Medicine, https://alliancerm.org/sites/default/files/ARM_position_on_HE_final.pdf
3. MHRA, The supply of unlicensed medicinal products ("specials") https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/373505/The_supply_of_unlicensed_medicinal_products__specials_.pdf
4. PIC/S https://picscheme.org/en/news/pic-s-reaffirms-its-stance-on-proposed-

eu-atmp-gmp-guidelines-and-highlights-gaps-relating-to-patient-safety

5. Biopharma, https://www.biopharma-reporter.com/Article/2016/09/30/Cell-gene-therapy-GMP-draft-guidance-criticised-by-advocacy-group

6. NSF, Pharma Biotech, https://www.nsf.org/newsroom_pdf/pb_review_eu_atmp_gmp_guidance.pdf

7. RAPS, Regulatory Focus™ https://www.raps.org/news-and-articles/news-articles/2019/10/pics-opens-consultations-on-manufacturing-atmps

8. Harrison RP, Rafiq QA, Medcalf N. Centralised versus decentralised manufacturing and the delivery of healthcare products: A United Kingdom exemplar. *Cytotherapy*. 2018; 20, 179–186.

9. Hourd P, Ginty PJ, Chandra, A , Williams DJ. Manufacturing models permitting roll out/scale out of clinically led autologous cell therapies: regulatory and scientific challenges for comparability. *Cytotherapy*. 2014; 16, 1033–1047

10. FDA, U.S. Food & Drug, https://www.fda.gov/patients/fast-track-breakthrough-therapy-accelerated-approval-priority-review/fast-track

11. Guidance note on new assessment routes in a no-deal Brexit https://www.gov.uk/guidance/guidance-note-on-new-assessment-routes-in-a-no-deal-scenario#guidance-on-the-rolling-review-route

12. EMA, PRIME: priority medicineshttps://www.ema.europa.eu/en/human-regulatory/research-development/prime-priority-medicines

13. Strategy of SAKIGAKE as a Package https://www.mhlw.go.jp/english/policy/health-medical/pharmaceuticals/dl/140729–01–02.pdf

14. Exploring the assessment and appraisal of regenerative medicines and cell therapy products https://www.nice.org.uk/Media/Default/About/what-we-do/Science%20policy%20and%20research/Regenerative-medicine-study-march-2016.pdf

15. Accelerated Access Review: final report https://www.gov.uk/government/publications/accelerated-access-review-final-report

16. EMA, Guideline on Active Substance Master File Procedure https://www.ema.europa.eu/en/documents/report/final-guideline-active-substance-master-file-procedure-revision-4_en.pdf

AFFILIATION

Patrick J Ginty
Head of Regulatory Affairs,
Cell and Gene Therapy Catapult,
London, UK

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *The author would like to thank both Mr Daniel Rabbie and Dr Jacqueline Barry for their important input and support in the creation of this paper.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

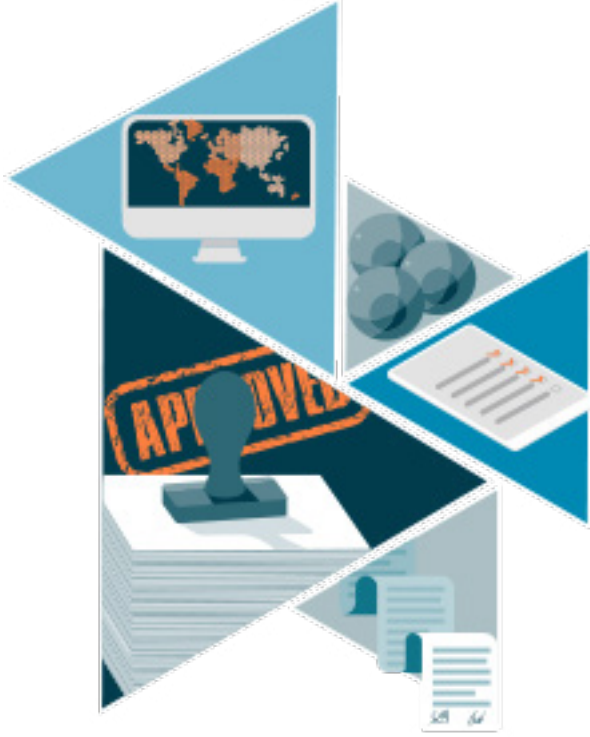
ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Revised manuscript received: *Sep 2 2020; Publication date: Sep 18 2020.*

DOI: 10.18609/cgti.2020.126

CELL & GENE THERAPY INSIGHTS



Regulatory Insights

-

INTERVIEW

EMA CAT perspective: the European Union regulatory landscape for ATMPs

DR MARTINA SCHÜSSLER-LENZ In January 2020, was elected on her second mandate as the Chair of the Committee for Advanced Therapies (CAT) of the European Medicines Agency's (EMA). The CAT is the committee responsible for evaluating the quality, safety and efficacy of marketing authorisations of cell and gene therapies (Advanced Therapy Medicinal Products, ATMPs). Dr. Martina Schüssler-Lenz received her medical degree at Mainz University Hospital, Germany. Following this, she was trained in Haematology/Medical Oncology and completed an Internal Medicine Residency and Fellowship at Berlin Moabit Hospital and at Mainz University Hospital. She worked as a research and clinical fellow at Memorial Sloan Kettering Cancer Center and at the Instituto Municipal de Investigacion Medica in Barcelona, Spain, before she joined pharmaceutical industry for clinical drug development projects in haemato-oncology. Since 2005 she is working as M.D. at the Paul-Ehrlich Institute in the Section Advanced Therapies and Tissue Preparations, where she has reviewed Advanced Therapies in clinical trials and marketing authorizations, and has provided advice to ATMP development and life cycle management. She is committed to facilitate the development of Advanced Therapies and has a specific focus in providing guidance to academic developers of ATMPs. Dr. Schüssler-Lenz has been a member of the EMA Committee for Advanced Therapies for many years before she was elected as its chairperson in February 2017 and re-elected for a second term in January 2020.

Cell & Gene Therapy Insights 2020; 6(8), 1127–1134

DOI: 10.18609/cgti.2020.121

Q Are there any particular lingering and/or underestimated regulatory challenges that you see troubling ATMP developers today?

MS: It's good to see that within the 11 years of the Committee for Advanced Therapies (CAT) being in place, the development of Advanced Therapies has gained momentum. We have approved 15 ATMPs in the European Union (EU), 10 products are on the market, and many more are coming. If we look at the five recently approved products, they are all gene therapies. For that reason, I'll focus mainly on this recent experience with gene therapies including genetically modified cells.

There are several recurring issues that we see in the marketing authorization dossiers. The first of these is the lack of validated commercial manufacturing processes. We acknowledge the need to optimize the manufacturing process of ATMPs throughout their development, but this process should be accomplished before the confirmatory trials starts. What we see often is that main safety and efficacy data are generated with a pre-commercial product process that is not well defined and controlled. If changes are introduced at late stages of the clinical development we ask for a suitable comparability program. The data provided are often limited and this causes not only a lot of discussions in the CAT but also delays the review process. We have published some guidance for ATMP developers. One is a Question and Answer document on comparability considerations for ATMPs. The other one is the Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells with detailed guidance on quality aspects. It was good to see the interest and feedback to this guideline during external consultation. We have now reviewed all comments and the final version will soon be published on the EMA homepage.

The second recurring issue in the review of marketing authorizations is the often limited clinical database. Let me take the gene therapies for hemophilia A or B as one example. They may provide long-term expression of factor VIII or factor IX after single-dose treatment. The submitted clinical data should allow us to judge how long the effect lasts and how long patients do not need to use factor replacement. The duration of efficacy is therefore a key element in the benefit-risk assessment of gene therapies.

> "There are several recurring issues that we see in the marketing authorization dossiers. The first of these is the lack of validated commercial manufacturing processes."

Another recurring issue is that ATMP developers neglect planning for post-authorization evidence generation.

The question is, why do we have these recurring issues? Is there anything that we can do on our side to help ATMP developers? Regulatory and scientific support is one way to help, and it is good to see that many gene therapies receive increased support in the EMA Priority Medicines Scheme. Is the European regulatory system in general fully set up to meet the needs of these developers? Are there structural or other issues that

DOI: 10.18609/cgti.2020.121

hamper ATMP development and create a barrier to a full and complete dossier at marketing authorization?

One thing we hear in discussions with academic and industry ATMP developers alike is that we have limited production capacity for ATMPs in the EU – both a limited production capacity for viral vectors and for ATMPs in general. The competition for production facilities makes it difficult for academic developers in particular to gain access to appropriately manufactured viral vectors. This is a big issue as we are aware of how important academic developers are, and the key role they play in driving innovation.

Q What changes need to be made to tackle these challenges?

MS: We see an urgent need to increase the EU production capacity for ATMPs. Autologous cell-based ATMPs, for example CAR T cells, are shipped from the patient to the production site and back to the patient. Currently, many CAR T cell products are manufactured in the US, and we see that sites are built up in China for supply to EU patients. Production of autologous cell-based ATMPs outside of the EU results not only in challenges related to supply chains, but may reduce the chance for EU patients to receive a high quality product in due time, before the patient`s disease has progressed and can no longer be treated. It is good to see that gaps in EU based production of medicines have been identified in the SARS-CoV-2 pandemic, and patient access to innovative medicines is now addressed on EU level with EC`s new pharmaceutical strategy.

Looking at pre-authorization data generation, I mentioned the limited clinical databases at marketing authorization. In addition to this, we observe that EU patients are under-represented versus patients from other regions. The reason for this is quite obvious. Research and development of most ATMPs are initiated in the United States and moved over to the EU towards late-stage clinical trial settings.

We are well aware that our EU clinical trial authorization system is not yet harmonized in practical terms. The clinical trials regulation came out in 2014, but it has not yet been fully implemented. We have different timelines in the authorization process and different requirements for genetically modified organisms (GMOs). I think this is recognized as a competitive disadvantage in the European Union. We understand that ATMP developers may avoid countries with clinical trial approval processes that are too long and GMO procedures that are too complex.

I think that a lot of work on this has been done at our level. For example, the CAT and our colleagues from National Competent Authorities have supported the European Commission with a good practice document on the assessment of GMOs in the context of clinical trials. Clearly, more needs to be done. We hope that with the full implementation of the clinical trials regulation, most country-specific differences will disappear, and we also hope this will provide a better situation to do clinical trials with ATMPs in the EU.

This addresses the pre-authorization phase, but there is also room for improvement with regards to post-authorization data generation and patient access to ATMPs. We would like to see a well worked out strategy for post-authorization data generation in the marketing

authorization dossier. If companies detail their plans for safety and efficacy follow-up this reduces uncertainties and supports a positive benefit-risk assessment. Not only that, it could also support patient access to ATMPs. Timely patient access to ATMPs is high on our agenda. We are concerned that EU patients in the 27 member states do not have equal access to ATMPs, and we need to reflect on what we as regulators can contribute to reduce hurdles.

This will certainly require activities on different levels. One aspect is HTA and reimbursement bodies' preparedness for ATMPs. ATMP characteristics create challenges in generating evidence of sufficient quality for HTA bodies. Also, the payment models used for medicines in general may not be suitable for ATMPs. EU health technology assessment and reimbursement bodies look more and more at post-authorization data generation, and whether good quality real world data can close gaps. We are now working with developers, more so than we have done before, to make them aware that early planning of post-authorization measures could also help downstream decision-makers.

We also discuss whether better communication with registry holders, pharmaceutical companies, and physicians' and patients' associations regarding the EU registry landscape would be beneficial. An EMA initiative with a cross-committee task force on registries has been ongoing since 2015 and there have been positive effects. But if we look at how it worked out for the two CAR T cell products, Yescarta™ and Kymriah™ after we approved them in 2018, it was an important learning curve for me and my colleagues in the Committee. It took quite some time until the EBMT (European Society for Blood and Marrow Transplantation) registry was set up to receive the CAR T cell treated patients. EMA colleagues work on specific guidance documents for registry-based studies. More needs to be done by all parties and it is recognized that we are not quite there yet.

Q What has been the impact of the COVID-19 pandemic on the European Union's regulatory community - firstly, on a purely operational basis? How are you endeavoring to minimize its effect on the development, manufacture, and distribution to patients of ATMPs?

MS: Preparations have been made on national and EMA levels to prioritize and accelerate procedures. This includes procedures for clinical trial authorization, for advising companies that develop treatments or vaccines and for authorization review. We also have to take into account that all of these meetings are now virtual. Our CAT meetings and all interactions are virtual. This has worked quite well - the EMA system and systems on the national level are now set up to effectively foster the exchange of information on a virtual basis.

Although I miss the personal exchange with my colleagues the general impact on the regulatory work capacity has been limited. So far for ATMPs we have had no delay on submissions for marketing authorizations. We do expect that there will be some delays, based on slower recruitment into clinical trials and for other reasons. One issue I see is that for marketing authorizations with ATMPs, we need to perform Good Clinical Practice (GCP) and Good Manufacturing Practice (GMP) inspections. Taking into account that most ATMPs are currently

DOI: 10.18609/cgti.2020.121

produced in the United States, and that the main clinical trial sites are often in the United States, we have an issue with on-site GCP and GMP inspections. We have now implemented virtual inspections as far as is possible but of course, that has its limitations. The barriers to travel may potentially impact on the timely continuation of the marketing authorization process, particularly given the fact that that most of our ATMPs are currently in accelerated regulatory pathways. We will have to wait and see whether this inability to do GMP and GCP inspections abroad will impact timelines moving forward.

"It is exciting to see that ATMPs such as CAR T cell therapies have added an additional treatment modality."

Q As an oncologist, what particularly excites you when you look across the ATMP field today, and what role do you envisage ATMPs playing in future in the overall cancer therapy armamentarium?

MS: We are all increasingly aware that in the cancer field we need a multiplicity of treatment modalities. It is exciting to see that ATMPs such as CAR T cell therapies have added an additional treatment modality. I have followed cancer immunotherapies with interest since my time at Memorial Sloan Kettering Cancer Center back in the 1980s. Up until the end of the 1990s, cancer immunotherapies did not play an important role. It is great to see how much this has changed as the field has moved towards the immunological approach.

CAR T cells as a form of adaptive immunotherapy have shown to be efficacious in certain hematological malignancies, while other types of cancer immunotherapies, such as mRNA-based immunotherapies, are still in clinical development. We have yet to see how much they can add to the armamentarium in hematological oncology.

When I envisage the future of ATMPs in this space, I consider the complexity of production and treatment when using autologous CAR T cell products. I predict an important role for off-the-shelf ATMPs. Whether they are derived from allogeneic cells, or involving recombinant AAV vectors or genome editing technology, off-the-shelf ATMPs are evolving fast. I think a big step will be to reduce complexity in manufacturing. This is something that is coming, and it will further increase the role of ATMPs in this field.

Q What is on CAT's agenda for the near-to-mid-term?

MS: The CAT has been very active in providing new guidance and revising existing guidance, and this will continue. As mentioned above, we have looked at the guidance to developers for the pre-authorization phase. The guidelines on investigational ATMPs are also new - they have been in the external consultation phase and will now be finalized. We have revised the guidelines on genetically modified cells and have added a chapter on the clinical

guidance for CAR T cells. And we have drafted or issued answer documents for ATMPs that are out of specification, which has been important for those same types of products.

There have been some delays in getting the final guidelines out due to Brexit and the Brexit Continuity Plan. After the Brexit Continuity Plan, we now work under the Covid19 Continuity Plan. We are working hard to finalize these guidelines and get this support to ATMP developers - we hope that we can continue and achieve our goals as planned.

One of my own interests has always been to support academic developers. In this area, we have had CAT regulatory sessions at the European Society of Gene and Cell Therapy (ESGCT) conference for the last 2 years. I think this has been very well received - it puts us in direct contact with ATMP developers at an international conference, which is something that we would like to continue to pursue. We will see how the upcoming annual ESGCT meeting is run, and how we can continue to foster and support academic developers as well as industry.

Q Are there any plans afoot for evolution in terms of evolving the CAT's organization or processes?

MS: We are continuously reviewing which internal processes and interactions with other committees can be further improved. Our setup is a multidisciplinary committee with quality, clinical, and non-clinical experts, and we also have healthcare professionals and patients represented. Our tasks are diverse, but we have excellent expertise across the committee. The committee is also stable without much fluctuation, and that's thanks to the national competent authorities that delegate their experts to our committee. We have brought CAT up to speed to be prepared for the increase in marketing authorizations – we have seven under evaluation just now, and we count on ten marketing authorization procedures per year moving forward. Our stable composition and expertise will give us the strength we need to handle that workload.

In terms of processes, it has been my focus to increase the exchange of information between CAT and other committees. We have formalized the interaction so that committee members from the Committee for Medicinal Products for Human Use (CHMP) and the Pharmacovigilance Risk Assessment Committee (PRAC) link into our discussions, and vice versa. This exchange of information is important if you have a system like we have in the European Union, where you have different committees with different responsibilities. I'm a strong believer in good communication between colleagues from different committees so that we are all on the same page, and we have no surprises when it comes to benefit-risk assessments at the end.

Q What specific areas of regulation should be the focus of harmonization efforts moving forward, for you - both within the European Union itself, and on a global basis?

MS: Harmonization efforts related to ATMPs have been leveraged to the ICH (International Council on Harmonization) level now with one gene therapy

DOI: 10.18609/cgti.2020.121

project, and we will see how that develops further. Below the ICH level, we have exchanges between regulators from different regions.

> "Our focus is on the link between pre- and post-authorization, and the involvement of all stakeholders..."

We have better harmonization in some areas than others, and sometimes our stakeholders do feel that more harmonization efforts are needed for ATMPs. But when we look at specific ATMP-related regulatory topics such as similarity of orphan ATMPs, for example, while the FDA may call it "sameness" instead, the fundamental content and the way that the FDA and the EU think about the topic is along the same lines. I find that quite reassuring. Harmonization efforts are still required on certain levels, but between the regions that deal with ATMPs most frequently, I would say that we have achieved quite good overall harmonization.

On the other hand, ATMPs are still excluded from the mutual recognition agreement for Good Manufacturing Practice and batch release. This is often a source of concern for developers coming to the EU and a recurrent topic in our discussions with ATMP developers. We need to increase our efforts to resolve this.

Q How do you see cooperation and collaboration between HTAs and regulatory bodies continuing to develop to help ensure approved ATMPs can thrive on the market, and benefit patients on an ongoing basis?

MS: This is an area of priority for us because everything we do is about patient access. The better we plan for the exchanges with HTAs and reimbursement bodies, and get them acquainted with the specificities of ATMPs, the better it is for everyone.

Of course, we must recognize our different tasks and responsibilities; benefit-risk assessment is our duty, and cost-benefit and reimbursement is not. But we certainly do what we can on our end to promote exchange of information with HTAs and reimbursement bodies: we have webinars on approved ATMPs, we have parallel scientific advice, and we advise developers to ensure early interaction. And as I mentioned previously, post-authorization data generation is something that HTAs and reimbursement bodies increasingly look into in the area of ATMPs.

The EU has recently published its plans for a new strategy to improve and accelerate patient access to safe and affordable medicines. I hope this mid-term initiative is also going to feed into the needs that we have for ATMPs and patient access. As already mentioned, developers should look at available disease registries, because HTAs and reimbursement bodies will also look at these resources and how they can be used for data generation. Really, one feeds into the other: our common task is to foster patient access.

Q Finally, can you sum up your own chief priorities and goals for your second 3-year term as Chair of the CAT? What do you aim to achieve over this period?

MS: We want high-quality, safe and efficacious ATMPs to reach patients. ATMPs hold the promise to offer potential new treatments to patients with unmet medical needs, and we are in a special area where we look at innovative treatments that in many cases are developed for patients who have little or no other options.

We have discussed the need to address issues that represent barriers for developers and for patient access. Our focus is on the link between pre- and post-authorization, and the involvement of all stakeholders in continuing to support developers through guidelines, interaction, and workshops. I would add to that the importance of involving patient representatives and patient needs in our decision process. That's something that we want to look at more closely: to see how CAT can better foster exchanges with patient organizations so that all parties are involved that need to be involved.

I hope that after these difficult times where we had to focus on Brexit continuity and Covid-19 continuity, we will get back to a more personal exchange with all of our stakeholders. This is obviously not completely in our hands, but hopefully by next year. The personal exchange with the different parties involved in ATMP development is so important.

AFFILIATION

Dr Martina Schüssler-Lenz
Chair, European Medicines Agency Committee for Advanced Therapies (CAT),
Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author declares that they have no conflicts of interest.*

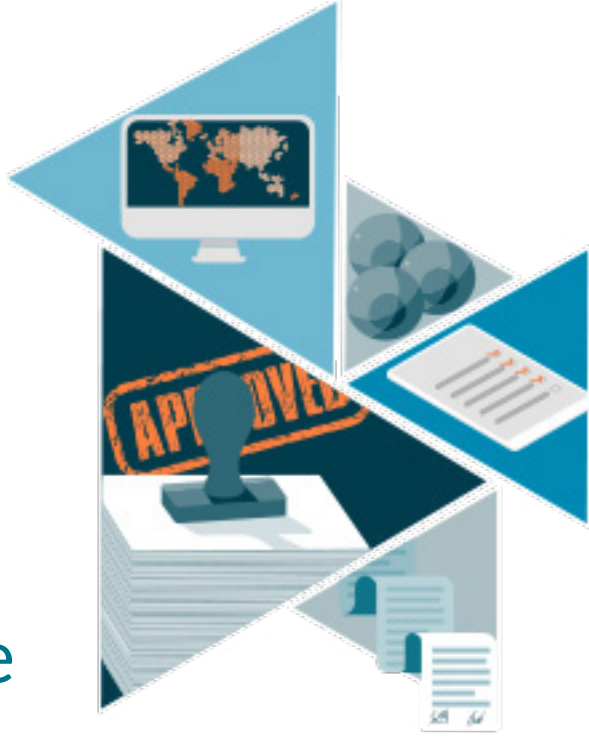
Funding declaration: *The author received no financial support for the research, authorship and/or publication of this article.*

Article source: *Invited;*

Interview conducted: *Jun 29 2020;* ***Revised manuscript received:*** *Sep 2 2020;* **Publication date**: *Sep 22 2020.*

DOI: 10.18609/cgti.2020.121

CELL & GENE THERAPY INSIGHTS



Regulatory Insights

REGULATORY PERSPECTIVE

Regulatory perspective on ATMPs: device combinations

Ilona Reischl & Stefan Strasser

The medicinal product and medical devices fields in Europe are subject to different legislations that were historically seen as largely independent from each other. Innovative drug-device combinations challenge this view and demand stronger interfaces between these two fields. The new Medical Device Regulation provides a new framework for drug–device combinations, but with necessary guidance under development, significant uncertainty remains. Additional questions arise when two innovative fields intersect, as in the case of advanced therapy medicinal product (ATMP) combinations with medical devices. With rapidly progressing scientific knowledge and potentially groundbreaking new therapeutic approaches, it is vital to provide a clear regulatory path for developers to facilitate the development of the new safe and efficacious medicinal products for patients in need.

Cell & Gene Therapy Insights 2020; 6(8), 933–940

DOI: 10.18609/cgti.2020.103

INTRODUCTION & DEFINITIONS

From a legal perspective, oversight over medicinal products and medical devices in the European Union (EU) is strictly separated. Medicinal products are regulated by Directive 2001/83/EC or Regulation (EC) 726/2004. For Advanced Therapy Medicinal Products the lex specialis additionally to be considered is Regulation (EC) 1394/2007 (ATMP Regulation). Medical devices are

currently regulated by the Directives 90/385/EEC for Active Implantable Medical Devices and 93/42/EEC for Medical Devices according to risk class. Through these legislations, legal oversight is separated by placing medicinal products with integral medical devices under the medicines framework and medical devices with ancillary medicinal products under the medical device framework. The determining factor is the primary mode of action of the combination. This principal separation will remain, but a more refined outline of interfaces between legislations is included in the Regulation (EU) 2017/745 on medical devices (MDR) which was set to come into force on 26 May 2020, but will be delayed for one year until 26 May 2021 due to the pandemic.

The development stage for medicinal products is covered by the dedicated Regulation (EU) 536/2014 (also called the Clinical Trials Regulation or CTR), the MDR addresses both the framework for the clinical investigation of medical devices and their market access. The CTR is also awaiting its practical coming into force until the go-live of its central database and workflow-tool, the Clinical Trial Information System (CTIS).

Considering that new legislation will be practically implemented for medicines and medical devices in the coming years, there is a need to revisit the mentioned interfaces. This is all the more relevant as innovative products are challenging the traditional borders between medicines and medical devices. A clear path to market is required to facilitate the novel developments. A recent publication by EFPIA has outlined remaining uncertainties [1] on drug device combinations in general, however, in the following, we focus on the regulatory path to market for combinations of ATMPs and medical devices.

As a first step, the legal definitions need to be understood. Support for the classification of ATMPs is provided through the classification procedure by the Committee of Advanced Therapies (CAT) that is available for free [2]. Products are classified based on legal definitions, which include that of a 'combined ATMP' provided in the ATMP Regulation in Article 2 d):

d) 'Combined advanced therapy medicinal product' means an advanced therapy medicinal product that fulfils the following conditions:

- It must incorporate, as an integral part of the product, one or more medical devices within the meaning of Article 1(2)(a) of Directive 93/42/EEC or one or more active implantable medical devices within the meaning of Article 1(2)(c) of Directive 90/385/EEC, and
- Its cellular or tissue part must contain viable cells or tissues,

OR

- Its cellular or tissue part containing non-viable cells or tissues must be liable to act upon the human body with action that can be considered as primary to that of the devices referred to.

It is important to emphasize that the text should not be interpreted to imply that only ATMPs containing cells or tissues can be classified as combined ATMPs. All ATMPs containing an integral medical device are to be considered as 'combined ATMPs'. CAT classification examples for combined ATMPs can be found on the EMA webpage [2]: autologous adipose-derived stem cells obtained from a stromal vascular fraction seeded on a collagen matrix scaffold (2017); allogenic adipose-derived stem cells (ADSC) differentiated *in vitro* towards the cardiovascular lineage and combined with carrier and implanting device (2017); viable chondrocytes cultured within a 3D hydrogel (2017).

This brings us to the second important definition, that of an 'integral medical device'. This definition is provided in Article 1 (3) of the currently applicable Directive 93/42/EEC on medical devices:

3. Where a device is intended to administer a medicinal product within the meaning of

DOI: 10.18609/cgti.2020.103

Article 1 of Directive 2001/83/EC (1), that device shall be governed by this Directive, without prejudice to the provisions of Directive 2001/83/EC with regard to the medicinal product.

If, however, such a device is placed on the market in such a way that the device and the medicinal product form a single integral product which is intended exclusively for use in the given combination and which is not reusable, that single product shall be governed by Directive 2001/83/EC. The relevant essential requirements of Annex I to this Directive shall apply as far as safety and performance-related device features are concerned.

In the MDR this definition is provided in Article 1 (9). While minor changes were made to the text, the general meaning and interpretation remains the same:

9. Any device which is intended to administer a medicinal product as defined in point 2 of Article 1 of Directive 2001/83/EC shall be governed by this Regulation, without prejudice to the provisions of that Directive and of Regulation (EC) No 726/2004 with regard to the medicinal product.

   However, if the device intended to administer a medicinal product and the medicinal product are placed on the market in such a way that they form a single integral product which is intended exclusively for use in the given combination and which is not reusable, that single integral product shall be governed by Directive 2001/83/EC or Regulation (EC) No 726/2004, as applicable. In that case, the relevant general safety and performance requirements set out in Annex I to this Regulation shall apply as far as the safety and performance of the device part of the single integral product are concerned.

To summarize:

- Only an ATMP with an integral device part is classified as combined ATMP
- The safety and performance requirements of the medical device regulation legislation apply to integral devices

As further background, the medical device legislation stipulates that medical devices may be placed on the market and/or put into service only in three situations:

- They have been CE marked for a declared intended use based a declaration of conformity following a procedure linked to their classification;
- They are the object of a clinical investigation or
- They constitute a custom-made product.

DEVELOPMENT: CLINICAL TRIAL STAGE

As outlined above, the two principal situations are possible – that of a combined ATMP (i.e. ATMP and integral device part) and that of an ATMP associated with a non-integral medical device.

Combined ATMPs

A clinical trial with a combined ATMP is processed according to the medicines legislation exclusively. Most countries have extended timelines for the assessment of clinical trials with ATMPs and future timelines are outlined in the CTR. In order to assess the safety and performance requirements of the device part, sufficient information needs to be provided in the dossier by the sponsor of the clinical trial. From an assessment perspective, adequate expertise is required on the part of competent authorities and ethics committees. In practice, this translates to the need for communication between experts. The interaction will be significantly facilitated if each side has a minimum understanding of the other's terminology and requirements.

Of note, where the ATMP is a genetically modified organism (GMO), an additional legal interface, that of the GMO legislation needs to be addressed. Much effort has recently been invested in harmonizing member states' requirements. Detailed information on this topic is provided on the webpage of the European Commission [3].

ATMP with non-integral medical device(s)

The requirements of the medical device legislation fully apply to non-integral medical devices associated with ATMPs. The use of the non-integral device in a clinical trial of the ATMP would legally only be permitted if the device has a been legally introduced to the market, e.g. if the device carries a valid CE mark and its utilization is covered by the declared intended use (the exceptions defined in the medical device legislation are unlikely in the setting of a systematic investigation). Use of a device (that does not fall under the exceptions) outside the intended use or use of a device that does not have a valid CE mark mandates the clinical investigation setting ('Clinical investigation' is the equivalent technical term in the medical device framework to 'clinical trial' in the medicines setting). Consequently, the project should be considered a combined trial according to both legislations, i.e. requiring approval as a clinical trial and as a clinical investigation. Where drug and device are co-developed the CE mark is usually not yet available and the likelihood of the need for a combined trial is therefore high.

Another setting that mandates a combined trial is where information on the performance or safety of a CE marked device is systematically collected in a clinical trial.

Finally, a study might be considered a combined trial according to both legislations based on its study design: When a combined ATMP is compared to the device component on its own, e.g. matrix plus cells versus matrix alone, where the matrix fulfills the definition of a medical device.

Clinical trials according to both legislations pose multiple challenges. In this respect, it is important to note that while all clinical trials follow the same procedure (validation, assessment, approval) the procedural requirements for clinical investigations are risk-proportionate, requiring either an approval or notification. Different combinations of procedures could therefore arise, adding a further layer of complexity. Centralized information on how combined trials are managed by National Regulatory Agencies needs to be improved, particularly as clinical trial units (for medicinal products and medical devices) are not necessarily located in the same National authority and the degree of interaction varies.

In addition, the legal timelines for the assessment of a clinical trial versus a clinical investigation are currently not aligned. The differences will be removed with the practical application of the CTR and MDR. The MDR further makes specific reference to the need for "interoperability of the medical device electronic system with the EU database for clinical trials on medicinal products for human use (MDR Art. 73 2.) as concerns combined clinical investigations of devices with a clinical trial under that Regulation". Considering the overall complexity of developing the independent databases, establishing interoperability is not a minor task.

Safety reporting requirements according to both legislations further create complexities that probably require further guidance.

Documentation requirements are outlined in section 4.3 of the 'Guidelines on Good Clinical Practice specific to Advanced Therapy Medicinal Products' [4]. In short, the investigational medicinal product dossier and the protocol should include summary information on the characteristics, performance and intended use of the device; and provide information on the regulatory status and compliance with the EU medical device legislation. The cover letter should highlight the medical device aspect. The Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials, which is currently

DOI: 10.18609/cgti.2020.103

in draft, will provide further information on the required scientific content (Table 1) [5].

MARKETING AUTHORIZATION

The following regulatory requirements apply when ATMPs with integral or non-integral parts are submitted for MAA.

Combined ATMP, i.e. integral device setting

The widely discussed article 117 MDR amends Directive 2001/83/EC to introduce a general requirement for a Notified Body opinion in situations where the device component of a drug device combination product falls under the second subparagraphs of article 1(8) or article 1(9) of the MDR. These two subparagraphs, in short, reflect situations where:

- The medicinal product incorporates a medical device or
- The medical device is integral to the medicinal product and used for its administration.

Dedicated guidance is currently being developed [6]. The 'Guideline on the quality requirements for drug-device combinations' (EMA/CHMP/QWP/BWP/259165/201) has progressed past the public consultation phase. In addition the 'Questions & Answers on Implementation of the Medical Devices and *In Vitro* Diagnostic Medical Devices Regulations ((EU) 2017/745 and (EU) 2017/746)' (EMA/37991/2019) provides further information.

Both documents clarify that ATMPs are not subject to the requirements of article 117 MDR. Article 9 of the ATMP Regulation applies for combined ATMPs. The associated procedure for interaction with Notified Bodies is outlined in the 'Procedural advice on the consultation of Notified Bodies in accordance with Article 9 of Regulation (EC) No. 1394/2007' (EMA/354785/2010). As part of the general topic on the Implementation of the Medical Device and *In vitro* Diagnostics Regulation this document will be evaluated for a need for actualization/revision. While principles will remain the same, it seems likely that changes of details will be needed e.g. on the section on the identification of a Notified Body (NB), to reflect the changing landscape due to MDR and IVDR implementation. Further, it is acknowledged, that the procedure so far has not been practically put to the test.

The principle for the evaluation of combined ATMPs is that any available result of the evaluation of the medical device component by a NB shall be included in the MAA and shall be recognized, reflecting the principally distinct oversight of the legislations. Where no NB assessment is available at MAA, EMA/CAT may seek an opinion on the conformity of the device part with the essential requirements of the relevant Medical Device

TABLE 1

ATMP device combinations in clinical development.

	+ Integral device	+ Non-integral device	
		ATMP	Device
Legislation	Medicines/CT legislation ATMP regulation Device legislation/safety performance requirements	Medicines/CT legislation ATMP regulation	Medical device legislation
Procedure	Clinical trial legislation	Clinical trial/combined trial – depending on setting	
Guidance	GL on GCP for ATMPs Investigational ATMP Guideline (draft)	Investigational ATMP Guideline (draft)	Device related guidance

legislation from a designated NB. In contrast to the framework for article 117 MDR that foresees a direct interaction between EMA and the respective NB, any procedural interaction between the EMA/CAT and the NB is trilateral, including the Applicant for MAA of the combined ATMP.

However, the need for NB interaction can arise even in situations where a NB opinion has been provided for example when the combination with the ATMP is assumed to have an effect on the technical, clinical and biological characteristics of the device, or when the intended use of the device component is changed compared to it being used separately. Overall, questions to the NB appear more likely in the situation where no NB opinion is available, but the complexity of the device component is a factor to be considered. A maximum of three interactions with NBs are foreseen during the MAA procedure with decreasing likelihood of occurrence, as outlined in the procedural guideline: The first round may be initiated at/after the presubmission meeting, when the need is identified. The second round of interaction will be launched when an additional need for clarification is identified at day 80. Exceptionally, a third round of interaction is possible after day 120, in case additional questions are identified.

In preparing the MAA, developers can leverage the concept of the 'Risk-based approach', which has been explicitly introduced for ATMPs in Annex 1, part IV of Directive 2001/83/EC and would also cover the device part of a combined ATMP. At the center of this risk-based approach is the product-specific determination and justification of the extent of quality, non-clinical and clinical data to be included in the MAA in accordance with scientific guidelines.

As the combination with a medical device therefore has the potential to add both procedural complexity and an additional layer to evaluation an early interaction with EMA, e.g. prior to the pre-submission meeting is highly advisable.

In terms of placement of medical device related information, the procedural guideline defines section 3.2.R of the CTD. The required content related on the medical device component(s) is detailed in ATMP-specific guidelines.

ATMPs associated with non-integral devices:

Considering the nature of ATMPs combination with an integral rather than a non-integral device might be more frequent. To recap, 'non-integral' implies a device that is not part of a single integral ATMP and not intended exclusively for use in the given combination. Currently, it appears more likely that any associated device would be specific to the ATMP it is associated with. Nevertheless, in the setting of a non-integral device, all medical device legislation requirements apply.

Non-integral medical devices co-packaged with medicinal products are not exempt from legal requirements and therefore would require a CE mark to be legally introduced to the market. This is referenced in section 3 of the Q&A on the Implementation of the MDR and IVDR. The Guideline on the quality requirements for drug-device combinations, once finalized will provide support on dossier structure and content. This guideline will provide additional information to the ATMP-specific guidelines.

The ATMP Regulation foresees one additional medical device-related provision: The expectation that information on medical devices used during surgical procedures for application, implantation or administration that may have an impact on the efficacy or safety of the ATMP is expected in module 5, as per Annex I, Part IV, Section 5.2.1 Dir2001/83/EC (Table 2).

OUTLOOK & ADDITIONAL CONSIDERATIONS

Legislations are developed to ensure the safety and efficacy of the products for the

DOI: 10.18609/cgti.2020.103

TABLE 2

ATMP device combinations at MAA.

	+ Ingegral device	+ Non-integral device	
		ATMP	Device
Legislation	Medicines legislation/ ATMP regulation safety/performance requirements	Medicines legislation ATMP regulation	Medical device Legislation
Procedure	Centralized MAA	Centralized MAA	Conformity assessment (not part of MAA)
Guidance	ATMP specific guidance	ATMP specific guidance	ISO and device related guidance Drug device GL

patients who need them. Scientific progress and innovation continuously advance and tend to challenge the established legal framework. Combination of medicines with devices have evolved beyond the classical prefilled syringe, which increases complexity for development and review. While the requirements of each applicable legislation need to be fulfilled, interface issues need to be addressed and the more legislations apply to a given product the more interfaces arise. Sufficient flexibility and clarity of procedures and regulatory requirements is needed to facilitate product development and to foster innovation, while ensuring patient safety and transparency.

While it is relatively easy to separate legal oversight, the strict separation of drug and device related information for scientific assessment is not sufficient and cannot address the needs for evaluation of a combined product. The same data might be evaluated with different focus and perspectives. Beyond legislations and procedures, the content of dossiers of drug device combinations for approval of clinical trials and marketing authorization application needs to be sufficiently detailed to enable evaluation.

Development of ATMPs has an immanent complexity and depending on the nature of the product might require expertise in the tissue and cells legislation for the procurement of starting materials and the GMO legislation in case of genetically modified viral vectors. The need for medical device expertise is added in the case of combined ATMPs or ATMPs associated with devices. Considering the varied nature of ATMPs under development, the risk-based approach specific to the ATMP legislation is the essential tool to determine product-specific data requirements.

The review of complex ATMPs is equally demanding for regulatory agencies as the development of multidisciplinary expertise and/or the establishment of closely interacting expert teams with different backgrounds is challenging. In our experience, a fruitful discussion requires an understanding of legal framework, terminology, concepts and procedures from the respective other fields. The closer interaction of medicines and medical device experts has been very rewarding, however, it is clear that the initially required input in building the required expertise is disproportionate to the currently still small number of ATMPs with dual aspects under development.

In conclusion, while beneficial steps have been taken, more guidance and clarification is needed. This is not easily achieved in the situation we currently find ourselves in, where regulatory resources are already under pressure due to the fallout of Brexit and the pandemic. Nevertheless, the need for action has been prominently recognized in the EMA strategic reflection paper 'Regulatory Science to 2025', which contains as a core recommendation the need to 'Create an integrated evaluation pathway for the assessment of medical devices, *in vitro* diagnostics and borderline products' [7].

REFERENCES

1. "EFPIA", December 2019: https://www.efpia.eu/media/413418/reflection-paper-on-regulatory-uncertainties-for-co-packaged-and-cross-labelled-drug-device-combinations-under-mdr.pdf
2. "EMA" https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/advanced-therapies/advanced-therapy-classification
3. "European Commission": https://ec.europa.eu/health/human-use/advanced-therapies/gmo_investiganional_de.
4. "European Commission": https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/atmp_guidelines_en.pdf
5. "EMA": https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-non-clinical-clinical-requirements-investigational-advanced-therapy_en.pdf
6. "EMA": https://www.ema.europa.eu/en/quality-requirements-drug-device-combinations
7. "EMA Regulatory Science to 2025", 31 03 2020: https://www.ema.europa.eu/en/news/advancing-regulatory-science-eu-new-strategy-adopted.

AFFILIATIONS

Ilona Reischl
Author for correspondence
Austrian Agency for Health and Food Safety – Austrian Medicines and Medical Devices Agency (AGES-MEA), Vienna, Austria
ilona.reischl@ages.at

Stefan Strasser
Austrian Agency for Health and Food Safety – Austrian Medicines and Medical Devices Agency (AGES-MEA), Vienna, Austria

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Revised manuscript received: *Jul 23 2020;* ***Publication date:*** *Aug 13 2020.*

DOI: 10.18609/cgti.2020.103

CELL & GENE THERAPY INSIGHTS

EXPERT INSIGHT

Using serum-free media to streamline and optimize CAR T-cell manufacturing workflows

Chengkang (CK) Zhang & Amber Jones

CAR T-cell therapies have led to breakthroughs in treating hematological malignancies, but the complexity and cost involved in their production limits their availability to patients. One challenge in CAR T-cell manufacturing outside of efficacy of your CAR-T product is achieving consistent expansion of T cells available after gene transfer. Optimizing the media used to support T-cell proliferation is, therefore, a key consideration—but it has proven difficult to develop media that enable consistent expansion while meeting regulatory quality requirements. However, recent advances in cell culture strategies have improved productivity and performance in CAR T-cell workflows using serum-free media, enabling the development of large-scale regulatory-compliant processes capable of producing billions of T cells within a short timeframe. This article will discuss how serum-free media can streamline CAR T-cell manufacturing workflows, highlighting how this can reduce time-to-market and make these treatments more widely available to patients.

Cell & Gene Therapy Insights 2020; 6(8), 1121–1126

DOI: 10.18609/cgti.2020.121

INTRODUCTION

CAR (chimeric antigen receptor) T-cell therapy plays a central role in the development of innovative therapeutic approaches to battle various cancers and non-cancer targets. Therapies utilizing CAR T cells have led to breakthroughs in treating hematological malignancies, and research is now exploring how to extend their use to treat solid tumors. In such therapies, T cells are extracted from a patient via apheresis and process-specific cell selection and genetically

engineered *ex vivo* to express the CAR, which is an artificial protein receptor capable of binding to specific cancer cell antigens and activating T cell functions. The genetically engineered cells are then expanded using a cell culture medium and returned to the patient for treatment.

Choosing an appropriate cell expansion medium is key to ensuring the health and performance of the relatively small populations of cells present at the beginning of the CAR T-cell manufacturing process, and after gene transfer. Traditionally, serum-containing media are widely used in such processes, but these come with a range of challenges. Serum can vary in quality between batches, and may contain toxic substances and impurities that not only require additional time and investment to remove, but can put the final product—the cells needed for patients—at risk. Moreover, as the industry becomes increasingly competitive, with numerous new CAR T-cell products under development, high-quality serum is becoming more difficult and expensive to obtain.

Due to these complexities and costs, the availability of CAR T-cell therapies to cancer patients is currently limited, despite their significant promise. Serum-free media offer ways to overcome these challenges, providing more reliable and effective ways to produce valuable CAR T cells.

THE LIMITATIONS OF SERUM-BASED MEDIA

The cell therapy industry is heavily reliant on serum derived from either animal or human donors, with demand for serum increasing as more cell therapy products enter clinical phases. However, serum is a complex mixture of a large number of constituents, and the quality of serum varies inherently between individual donors, causing the quality of subsequent products to differ between batches. This fluctuating quality can lead to inconsistency—and runs the risk of compromising CAR T cells produced for oncology therapeutics.

Even serum sourced from healthy human or animal donors may contain pathogens. Serum is usually sterilized by filtration through multiple 0.2 µ or 0.1 µ filters after collection, because heat-treatment may adversely affect its growth-promoting properties. This filter-sterilization procedure is, however, unreliable because small viruses, viral fragments or prions may pass through the filters. As a result, stringent quality control tests must be performed to ensure that serum is free from adventitious agents.

Serum that passes the sterility quality tests may contain undefined cytokines, hormones and growth factors, whose presence may influence the phenotype of the expanded T-cell product. Growth factors such as transforming growth factor beta (TGF-β) cannot be filtered out, and are present in sera at various concentrations in different lots, contributing to lot-to-lot variability. TGF-β induces the generation of regulatory T (Treg) cells that may suppress CD8 T-cell activity, which can diminish the efficacy of the final CAR T-cell product. Hormones such as glucocorticoids that are known for their anti-inflammatory and immunosuppressive actions may be present within sera, again impacting the final CAR T cells produced.

As a further consideration, prior to CAR T-cell infusion, cancer patients are typically pre-treated with lympho-depleting chemotherapy to ensure that endogenous T cells do not suppress the proliferation of infused CAR T cells [1]. Therefore, to safeguard patient health, there is an even greater pressure and need to successfully produce a high percentage of viable CAR T cells—and to ensure that this is done consistently.

To ensure high levels of sterility and an absence of unwanted substances or impurities, scientists must qualify all the raw materials and components they use in the serum-based expansion media. However, the need to check the quality of each serum lot before it can be used increases the overall cost of the CAR

DOI: 10.18609/cgti.2020.121

T-cell expansion process, and can delay CAR T-cell manufacture if there is a shortage of high-quality serum. With increasing numbers of new CAR T-cell therapies under development, competition for good quality serum is becoming steeper and more prevalent—and this may eventually increase the cost of CAR T-cell products.

SERUM-FREE MEDIA: OPTIMIZING CAR T-CELL EXPANSION

The use of serum-free media offers a potential way to address the challenges, limitations and costs associated with serum-containing media—including concerns around fluctuating quality, the need for extensive supplementation and the complexity of regulatory requirements. Serum-free media bring reliability, flexibility and versatility to the CAR T-cell expansion process, facilitating large-scale and regulatory compliant processes that are capable of producing billions of T cells in a short timeframe [2].

Improved quality & consistency

Serum-free media may use proteins (such as serum albumin) that are purified from plasma, and the additional processes involved in purifying these proteins help to eliminate some of the undesirable contaminants mentioned above. Using serum proteins derived from human plasma instead of materials derived from cattle also minimizes the risk of including transmissible bovine spongiform encephalopathy in the end products.

To eliminate inconsistency and ensure that vein-to-vein time is achieved within expectations, scientists require greater control. This control can be provided by serum-free media, which eliminate much of the variability that may compromise CAR T-cell production. Lower levels of variability also reduce the need for lot-by-lot qualification, which in turn helps the final product reach the market faster.

Streamline the supplementation process

Many available media are offered in the form of a liquid base that requires the addition of one, two, three or more components—such as serum, critical amino acids and other supplements—before use to stimulate cell growth and maintain high cell viability [3]. However, these supplements are often stored separately and under different conditions to the base media, and have different shelf lives, making their storage and maintenance a challenge. Some supplements must be stored in freezing conditions and thawed before use, adding time, inefficiency and complexity to the overall cell culturing process.

Serum-free media are 'complete' and ready to use, reducing the risk of contamination by eliminating the need for additional supplementation, which streamlines the overall cell expansion process.

This high-performance serum-free media is applicable not only to T-cell therapies, but also many other cell types, such as natural killer (NK) cells and dendritic cells. Due to their high versatility, serum-free media form highly suitable bases for a wide range of therapeutic applications (either with or without cytokine supplementation, depending on the specifics of the process).

Current Good Manufacturing Practice (cGMP) release criteria

Regulatory compliance and cGMP processes underpin the successful production of CAR T-cell therapies, and are, therefore, essential in ensuring effective therapeutic treatment reaches those in need. To protect patients, release criteria are strict. For example, the criteria for commercially manufactured Tisagenlecleucel (CTL019; Kymriah®), a medication used to treat B-cell acute lymphoblastic leukemia, which usually demand at least 80% viability before the therapy can be used to treat patients. Achieving high levels of T-cell viability, though, requires consistency and

high quality: even small changes in processes and raw materials, such as the composition of the growth media, can introduce inconsistency that puts the quality of the CAR T-cell product at risk.

By bringing the control and definition needed to eliminate inconsistencies and variability, serum-free media facilitate the production of CAR T cells that meet rigorous cGMP criteria. These media are a safe, high-quality alternative to serum-based media for effective CAR T-cell expansion in-line with regulatory requirements. They enable effective scale-up and are available in versatile formats—bottles or bags with plug-and-play connectors—that can be tailored to meet the specific needs of various cell production platforms.

TRANSLATION INSIGHT

Serum-free media made of non-animal origin materials hold great promise—and as the cell therapy industry moves away from serum and its galaxy of undefined, inconsistent components, more therapies will move towards serum-free media expansion. Media that do not rely upon blood-derived supplements offer increased regulatory compliance and process control, and will bring many more specific choices and opportunities regarding quality and performance in the development of novel cell therapies.

A notable area for future advancement regards chemically defined media. The introduction of recombinant versions of proteins and growth factors that are derived from blood will further eliminate the need for undefined, animal origin constituents from cell culture media and bring even-more consistent performance. Not only will this facilitate compliance with regulatory quality requirements, it will also enable the development of therapies that will present less risk to patients, while removing the variability associated with human sourced components.

While moving to this higher quality level of chemically defined media, manufacturers of expansion media should always ensure that performance remains top priority to safeguard cell viability. Overall, this will contribute to reaching the ultimate goal of cell-based therapeutics—ensuring patient safety and product efficacy—in a timely, efficient and reliable way.

REFERENCES

1. Srivastava S, Riddell SR. CAR T Cell Therapy: Challenges to Bench-to-Bedside Efficacy. *J. Immunol.* 2018; 200(2): 459–68.
2. Ou J, Si Y, Tang Y *et al.* Novel bio manufacturing platform for large-scale and high-quality human T cells production. *J. Biol. Eng.* 2019; 13: 34.
3. Yao T, Asayama, Y. Animal-cell culture media: History, characteristics, and current issues. *Reprod. Med. Biol.* 2017; 16(2): 99–117.

AFFILIATIONS

Chengkang (CK) Zhang
R&D Senior Scientist Media Development, Lonza Walkersville, Inc.

Amber Jones
Global Product Manager, Lonza Walkersville, Inc.

DOI: 10.18609/cgti.2020.121

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors declare that they have no conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *date*; ***Revised manuscript received:*** *date*; ***Publication date:*** *date*.

Lonza

Pharma & Biotech

TheraPEAK™ X-VIVO™ Hematopoietic Cell Culture Media

The next step towards scaling up your cell therapy.

TheraPEAK™ X-VIVO™ Media are scalable, high-performing media that can be used from discovery to commercialization. Currently used in FDA approved cell therapies, TheraPEAK™ X-VIVO™ Media are highly cited and can be used for a variety of hematopoietic cell types.

Visit: www.lonza.com/therapeak-x-vivo
USA: +1 800 638 8174 (toll-free)
Europe: +1 800 521 0390 (toll-free)
International: +1 301 898 7025
Email: scientific.support@lonza.com