NOVEMBER 2023

Volume 9, Issue 10

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON

Cell therapy downstream processing & CMC

CELL & GENE THERAPY INSIGHTS

Volume 9, Issue 10

CONTENTS

SPOTLIGHT—CELL THERAPY DOWNSTREAM PROCESSING & CMC

Spotlight

Cell therapy downstream processing & CMC

EXPERT ROUNDTABLE: Current issues & future trends in cryopreservation for advanced therapies
Sean Werner, Jason Acker, and Allison Hubel

INNOVATOR INSIGHT: Empowering the future of cell therapy: leveraging partnerships to support advancement
Juan Patarroyo, Fiona Mack, Xavier de Mollerat du Jeu, and Josh Judkins

INTERVIEW: Addressing the risk presented by extractables and leachables in cell therapy manufacturing
Jason Creasey

INFOGRAPHIC: Exosome manufacturing
Thermo Fisher Scientific

INTERVIEW: Enabling patient access to CAR-T cell therapy in India
Shashwati Basak

INTERVIEW: Key considerations in expanding global manufacture of a commercialized CAR-T cell therapy
Sarah Snykers

Supply Chain Channel

VIEWPOINT: Navigating the final mile: how are hospitals providing capabilities for commercial cell therapy product delivery?
Alexey Bersenev

VIEWPOINT: Navigating the final mile: how are Australian hospitals delivering autologous cell and gene therapies?
Sharon Sagnella

Latest articles

COMMENTARY: Building rings with spheres: a cell therapy approach to incontinence
Martha Gilbert, Simona Čaputová, Delielena Poli, Manou Kooy, Richard M. Day, Georgia Sturt, and Josephine Parker

FAST FACTS: Cell culture in a chemically defined environment
Chengkang Zhang

FAST FACTS: Case study: media optimization control strategies
Alex Sargent

FAST FACTS: Reliable detection reagents in CAR-T/NK cell flow cytometry
Annika Graband

FAST FACTS: HEK293 suspension culture in stirred 3D-systems for gene therapy development
Jorge Escobar

WEBINAR DIGEST: Three distinct analytical ultracentrifugation methods for virus and viral vector characterizations
Susumu Uchiyama

CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING & CMC

SPOTLIGHT

INNOVATOR INSIGHT

Current issues & future trends in cryopreservation for advanced therapies

Jason Acker, Allison Hubel, and Sean Werner
(pictured from left to right)

Three industry experts discuss lessons learned in the cryopreservation sector over the last decade, as well as potential innovative solutions to current challenges within the space. The authors also explore the question of how industry and academia can come together to create these solutions.

Cell & Gene Therapy Insights 2023; 9(10), 1477–1491

DOI: 10.18609/cgti.2023.195

Q Can you summarize your past and current work in the area of advanced therapy cryopreservation?

JA: I am a Professor in Laboratory Medicine and Pathology at the University of Alberta. In that capacity, I lead a cryobiology research group that investigates basic freezing response of natural systems and their response to environmental stress as a prelude to understanding how to develop ways to mitigate issues in natural and engineered systems. I also consult with the industry to help ensure that the principles of cryobiology are properly translated.

AH: I am a faculty member in mechanical engineering at the University of Minnesota. My work has always involved the preservation of cells. In terms of contributions to the field, I have worked on the development of low-temperature Raman spectroscopy as a tool to understand freezing damage. I have also worked on the development of dimethyl sulfoxide (DMSO)-free methods of preserving cells—that technology is being commercialized and used in the cell and gene therapy space.

SW: I am not a cryobiologist by training—my background is in cell and cancer biology. However, since 2015, I have been working on the commercialization of cryopreservation vials and systems. I work with BioLife Solutions, and we have a range of products involved in the storage and processing of cell therapies for cryopreservation and cryostorage.

Q How are the cold chain logistics tools and services sectors continuing to evolve post-COVID-19, and what does this mean for the cell and gene therapy sector?

SW: A couple of key things have changed in the last few years. Pre-COVID-19, people used cold chain logistics, but the pandemic opened everyone's eyes to what was missing in terms of being able to store and globally ship vaccines at an ultracold temperature and at a really large scale. How do you ship that many vials around the world all at once? Post-pandemic, people are starting to look at what else is needed. We developed large repositories and companies evolved and developed methods to do storage at ultracold temperatures. Prior to the pandemic, these types of facilities really were not available at the scale they are now.

This progress has set the stage for us to be able to translate into accomplishing this at cryogenic temperatures. There are many challenges in that task. For example, it takes nine different cryogenic shipments around the world to complete a manufacturing line for an autologous therapy. However, there are now systems to make this process easier and allow us to use, for example, commercial airlines to carry these products whereas before that might not have been a possibility.

There are some logistical and storage facility solutions that are evolving, such as being able to store raw materials close to the cellular starting material collection point or the manufacturing facility, instead of always having to move things around on a just-in-time basis. There is a lot of work to do, but we are on the way.

AH: It is helpful to take a step back and say, "Why are we even talking about cold chain?" That is because the supply chain for cell and gene therapy is far more complicated

than for other types of medical therapies. We must keep cells viable and functional all the way along that supply chain. This realization has led to the development of some of the technology that Sean talked about.

JA: To build on Allison's comments, throughout the COVID-19 pandemic, we learned about the fragility of supply chains, which introduced variability that exacerbated a lot of the injury that was occurring in cryopreserved products. As a result, we were running into situations where products were not able to move through the supply chain successfully.

In some ways, the fact that we did get an interrupted supply chain during COVID-19 helped reinforce the importance of the supply chain phase when talking about an allogeneic or an autologous cell therapy product. This was an important and beneficial lesson from a very unfortunate situation that we all had to go through.

Q What have been some of the key issues around cryopreservation in the space over the past decade, and what have been some of the key related learnings for industry?

JA: Over the last decade, the industry has become a lot more aware of the basic cryobiology science that was done half a century or more, which focused on understanding the foundational elements of how to preserve, and then ultimately store a stable biological product. The problems that the cell therapy sector is facing today are problems that were recognized a while ago with respect to cryoprotectant toxicity: how do we add, remove, and select those cryoprotectants to mitigate that toxicity? How do we control ice, and how do we mitigate the amount of ice and the damage caused as a result of it? How do we understand the cell specificity for every one of our products with respect to what those optimal parameters are, so that we can maximize recovery? Each one of these elements factors into the process that ultimately becomes the cryopreservation methodology that a company would use, so this increased awareness of that long-established basic science has been helpful.

These learnings have been really well appreciated at the industry level because they are either dealing with a problem they cannot understand due to a lack of knowledge of the fundamentals, or they have encountered a problem that with well mapped out fundamentals can be resolved.

There is still a reliance on a standard 10% DMSO, 1 °C/min freezing rate approach in the commercial space, which was developed and validated for certain types of cells. But as we are starting to see much more sophisticated cell products being engineered with very specific properties, that approach is not going to work. You have to go back and rely on the science. That is where, again, the learnings from history are starting to be re-discovered. There has been some interesting dialogue within the industry around building scientific capacity in this field within a company or organization in order to bring more products through the design, development, and manufacturing stages.

AH: Another layer that has entered the space is the discovery of things like induced pluripotent stem cells (iPSCs), which are used as a source of starting material for cell therapies and regenerative medicine products. We are now taking a stem cell or a pluripotent cell and differentiating it into another cell type, creating completely different cells from those we can harvest from a patient's tissue or their peripheral blood.

"The problems that the cell therapy sector is facing today are problems that were recognized a while ago with respect to cryoprotectant toxicity: how do we add, remove, and select those cryoprotectants to mitigate that toxicity?"

We are expanding the potential for those cells to be used therapeutically, but it is not at all clear if, for example, an iPSC-derived natural killer (NK) cell is going to respond in the same way as a primary NK cell to current cryopreservation methods. So, we are creating more need for this fundamental cryopreservation knowledge because we are creating cell types that have different biology and different cryobiology. The field is getting to the point where it really needs our fundamental knowledge to move forward.

SW: One of the most important elements of a successful drug product is that you can demonstrate comparability: that you are making the same thing every time with the same ability to meet the quality specifications. For a cell therapy product, those specifications are sometimes less robust than we might like them to be. We are still trying to understand what the critical quality attributes of cell therapy products really are.

In the absence of being able to identify and test for all the attributes that are important, we have to be able to control the variability. Two of the really important steps in the overall cell manufacturing process where there can be a lot of variability are the cryopreservation process and then the thaw process on the clinical side. For us to be able to have really well developed, robust optimization of the cryopreservation process and the thawing process, the interim storage process must be well established. This is one of the ways that we can avoid running into problems with the therapy after the fact. In fact, we might not even know of the problems unless we go through the effort of optimizing those processes.

JA: One of the things we are realizing is a lot of the methods that were developed from a cryopreservation perspective, like the 10% DMSO, 1 °C/min freezing rate protocol, were developed for single autologous cell products where you only have to produce one dose. In that setting, you can have much greater control over the conditions under which that product is cryopreserved.

However, when you start to scale and move from one dose per batch to perhaps tens of thousands of doses per batch, the principles of cryopreservation become even more important because any problematic issues are scaled up, too. For example, the impact of cryoprotectant toxicity can be minimized when exposing one bag of a product. But if you have to expose 10,000 bags, by the time you have gone through the fill-finish and labelling process, that becomes a lot more significant.

As a result, we are seeing that those standard preservation processes that have been historically used well in autologous products are not translating well to the allogeneic world. Most in the cryobiology community would say, "That's obvious, but now how do we solve the problem?" That is where the industry is now working with cryobiologists who have some historical context and can help come up with innovative ways to try to address these problems.

Q What is the current state-of-the-art in cell and gene therapy cryopreservation and associated transportation and storage technology—for instance, as used with the commercialized CAR-T cell therapy products and their cellular starting materials?

JA: The industry has evolved in the sense that the materials used for the containers are a little more robust. They are chemically defined, qualified materials now, so we have better materials that we are using in the cryopreservation process. However, the cryotechnology that is used, unfortunately, still falls into that 10% DMSO, 1 °C/min standard way of approaching the cryopreservation process.

For a CAR-T product or indeed, any T cell product, that standard approach works adequately well—again, there are nuances around the cryobiology of T cells that would suggest that you could use other methods that would be even more effective. However, when you start to look at other products like an NK cell or a heavily engineered CAR-T or CAR-NK cell, that approach is not going to work. We are seeing the cracks now in the state of the art of the cryopreservation process because it is just not allowing the industry to scale. It is not giving the post-thaw recovery or the stability throughout the supply chain that the industry is asking for.

It is necessary to go back and look at the fundamental approach from a process design standpoint, and look at those areas from the cryopreservation process itself in order to further refine, optimize, or completely redevelop using different approaches that have been known in the industry for decades, and which will allow us to overcome some of those challenges. Many of the conversations that are currently going on are about taking the next generation of ideas and moving them into the industrial context.

Unfortunately, recognizing the challenges that many companies are facing with respect to cryopreservation is generally the last step in the process. As a result, the cryobiologists are inheriting a whole bunch of process decisions that are very difficult to change, which makes implementing something different even more problematic.

AH: Let's say that we are wickedly successful—that there are dozens of cell therapies for dozens of diseases that have been approved by the regulatory agencies around the world, and that are now being used in the clinic. Then we go to the cell therapy ward where these patients are being infused, and we have nurses or cell therapy technicians thawing different bags to go to different patients for different diseases.

The result of this success would be a mess because, according to interviews with nurses, each of these different cell therapy companies has a different method of thawing, infusing, documentation, and shelf-life. One of the technologies that needs to be developed is one that handles those products once they leave the hands of the cell therapy developer and go to the clinical site.

The other layer of this is that people need to be communicating with each other and with cryobiologists so that we can develop best practices for thawing and post-thaw handling of cell therapy products that make it implementable in a clinical context, especially if we are talking about a dozen different patients with a dozen different cell therapies for different disorders all happening simultaneously. That is something that people are really just becoming aware of that could be an emerging area of importance down the road.

SW: I believe we are already at that mess, to be honest. A few well-known hospitals have talked about this as being one of the big problems they are facing. You have got to have unique, dedicated pieces of equipment and unique, dedicated processes for each therapy. The clinical study is one thing where you have the data to report, but then once you move on from the clinical study, you have to trust that your clinicians, nurses or practitioners are going to be following those processes and procedures.

There is already a great deal of difficulty in simply trying to maintain things as they are currently. We have largely moved on from the concept that we cannot freeze or adjust these cells. Using fresh cells does not really work from a logistics perspective. You would have to move your products around the world in three days, which clearly was never going to work. Cryopreservation has given us the opportunity to take into account things like the messiness of scheduling a patient to come in for a visit. We now do not have to throw out a US$500,000 product because it sat out overnight before a patient could get to the point of care. Cryopreservation allows us to address that issue, but now we have to figure out how we do this at a global scale.

Q What are some of the key historical and ongoing issues and challenges with the containers utilized for advanced therapy cryostorage and transportation?

AH: There are some tensions when we talk about containers for cryopreservation. The first tension is heat transfer. To freeze something, you have to remove heat, so the container must enable efficient heat transfer. That is one of the reasons why bags in presses have been a common paradigm for freezing large volumes. The other tension is the question of using the container in an automated setting, now that we are moving to the scale-up paradigm. The third layer of tension has to do with materials because we must have materials that are usable at cryogenic temperatures. The container issue has been a source of tension due to the need to balance these three specific considerations.

JA: To lend a little historical context, some of the first cell products that were cryopreserved were red blood cells and stem cells. Red blood cells always need to be stored at –80 °C. The blood bag technology was used because it can maintain container closure at –65 to –80 °C temperatures. Bag breakage was a problem, though, early in the history of red blood cell cryopreservation, until we learned how to pack them and get them into the right protective box so that they do not get juggled and broken as part of the shipping process. That process was never going to work for lower temperatures. The early blood bags that were used for cord blood or stem cells, for example, were actually those same container systems. There were significant challenges in storing those at liquid nitrogen temperatures until, as Allison mentioned, newer plastic configurations became available.

There has been some evolution, but the challenges are still very much present, particularly now that we are adding the complexities of having to maintain container closure in a system that is scalable and automated, and needing to withstand the extreme ranges of temperature during freezing and thawing processes at a specific volume for the specific cell therapy application. There are no standards yet for the kinds of containers that we need. As a result, there is a lot of confusion in the market about what to adopt because your container drives your freezer configuration—the racking systems that you use in your storage container, as well as

"Cryopreservation has given us the opportunity to take into account things like the messiness of scheduling a patient to come in for a visit. We now do not have to throw out a US$500,000 product because it sat out overnight before a patient could get to the point of care."

your controlled-rate freezer and your thawing device. Then ultimately, the container controls how the product is manipulated by the end-user in terms of being able to infuse, transplant, or transfuse it. A lot of design constraints are now being placed on the containers themselves, which is causing some tension as Allison mentioned.

SW: We are talking about bags here in the main, but for small volumes, bags are not necessarily the ideal format. They have some issues with recovery and all kinds of different challenges with smaller volumes. What a lot of people have used instead are screwcap vials. In fact, there are a few commercially approved products out there that use these vials as their final drug container, but those also have real issues. For one thing, there is serious concern with leakage in the screwcap-type vial—both in terms of contaminants getting in, and cross-contamination throughout your storage systems. There is documented evidence of this having happened in the past. Also, if you have a leak and get vapor nitrogen in that vial, when you take it out, the pressure will change and could cause explosions and some dangerous situations.

Another challenge with vials is that if you think about a standard rubber or elastomeric stopper on a vial, those closures are a lot less secure in cryogenic temperatures. You have to do some pretty significant engineering to make sure that you are not getting leakage with those types of stoppers.

So, we need to start looking at what has been developed over the last 10–15 years. There are some options for sterile ready-to-use vials that have specifically been designed for this type of storage.

AH: To circle back on what Sean said, in cell therapy, there can be very, very small volumes of cells that are administered to the eye or the brain, or any other specific organ. There is really a dearth of solutions available to manipulate and to cryopreserve that small cell number.

SW: Yes. As an example of that, we talked to a lot of people in the dendritic cell vaccine space where they are talking about less than 0.5 mL for the final product volume.

Q Can you go deeper on the key challenges with bags and larger volume vials?

SW: There are a couple of things that we have already touched on. One of them is that when we have a soft material, a bag material can fracture. Over the last 10 years, there have

been some significant and important advancements in materials. Processing the bags correctly is not necessarily the issue anymore. However, I will say that biostorage facilities often see more breaks in the bags that come in than people might be aware of.

When you are handling thousands of units through cryopreservation, a 5% or even a 1% fracture rate is still quite significant. At US$500,000 or more for one therapy, it is a big loss for the company. More importantly, that is a therapy that may not be recoverable for the patient. We think that that low fracture rate is still important to consider.

The other thing is that as you go into larger volumes, you must consider the freezing profile and the geometry of the material within that bag. If you want to have a consistent freezing profile, you have to control the geometry. You cannot say, "We are going to scale up in volume and we are going to do a freezing process of a 5 mL vial. Now we are going to put 100 mL in a vial-shaped container and freeze that." You must have a very different freezing profile at the center of that. Bags resolve this issue, but you have to keep the same geometry in order to do that.

One of the big things alluded to earlier concerns scale. When you take a bag and you want to fill that with 1,000 units in a short period of time, or if you are considering some of these large incidence indications with many thousands of patient treatments, that is difficult to do with our current soft bags. You cannot hook those up to a fill system very easily to get that going. There are some challenges in the current configurations, so more rigid, automation-friendly types of containers would be really useful.

Q On that note, what soon-to-arrive innovations can we look forward to that will improve the situation?

AH: You can think of the newly arriving innovations in different categories: in equipment, in reagents, and in techniques.

There has been a steady development of new preservation technology, such as thawing technology, that allows us to record the temperature of the thawing unit as a function of time to go into the batch production record, which is very important. In terms of other technologies, I would love to have the ability to control nucleation in every sample in a controlled-rate freezer. That would help the field considerably.

In terms of other equipment, there are companies working on the bedside process. After the dry shipper comes from the developer and is at the clinical site, how do we create an infrastructure at the clinical site in terms of equipment that really facilitates the proper thawing and dispensation of the product? As for reagents, I have a personal bias here—the reagent that we are most invested in allows the DMSO-free preservation of cells.

We also need techniques to improve processing and to improve proficiency. Those are the things that I see as emerging areas in this field.

JA: Picking up on Allison's wish list, there are certainly exciting new products coming out in the near future. I share Allison's wish to be able to control ice, whether it is nucleation or growth, in ways that allow us to think about the freezing and thawing differently. Those technologies are soon to come to market, which will help to reduce our dependence on the traditional cryoprotectants that are used and introduce other ones that would be more favorable.

We are probably going to see a shift away from the reliance on equilibrium freezing, where you are cooling very slowly, to more kinetic-based freezing methods that are faster. While

they may not necessarily result in ice-free freezing or vitrification, they do result in conditions that are still highly favorable for cell recovery. To make those methods really practical, we are going to need the cooling and thawing technologies to support large-scale production. That will come in the near future, as more advances in cryopreservation sciences are made.

One of the things I am seeing in the industry is an attention to the cryopreservation process from the pre-cryopreservation analytical side right through to the thawing side, and understanding how decisions at each of those stages build on each other. As Allison mentioned, developing the data sets to support that process from a supply chain perspective will be really important. We are starting to see freezer companies, for example, that have built-in automation or tracking either by radio frequency identification or 3D barcode. They are able to track the thermal profile of a sample throughout its lifespan by indirectly monitoring time-out-of-temperature or time-in-environment. That is incredibly valuable data to understand because the thermal profile of these products will predict the outcome.

In the very near future, I expect to see transient warming excursions being taken a lot more seriously by regulatory agencies. Having that kind of data is going to be absolutely essential to knowing how many times your freezer was opened, and as a result, the exposure conditions for every sample that you have in that inventory. Without the technology to support that innovation, it will be unachievable to implement. Again, there are a lot of small innovations happening that are going to collectively help the field.

SW: To pick up on that last comment about smaller innovations, it is interesting if you look at the independent products that are available out there. A lot of the capability now exists to do these things that you were describing, Jason, and the things that we think we need in the industry for success. We do not have to develop and do anything brand new. We just need to put together these innovations in the way that the people need it to function. It is all already there.

One of the things that we on the tool provider side require is an understanding of exactly what is needed by industry so that we do not bring through something that does not make a lot of sense. We are close to being able to provide tools that allow you to do things like at-scale cryopreservation, reproducible volume, and novel containers for larger volumes. The more we hear from the end users what they need, the better we will be at hitting that target correctly.

Q What are the most pressing priorities when it comes to standardization in this field?

AH: Standardization would be fantastic, but I do not know if the field is ready for it. I have been a part of developing standards over many decades, none of which were actually used by people! The field must be elevated before standardization can take place. People need to understand the scientific principles behind preservation, and they need to understand how those scientific principles get translated into a protocol that is used for preservation. Then, that end outcome can be done consistently and reproducibly.

From what I have seen, the level of proficiency of people who are in the field actually doing preservation, is very, very low. Standardization will not be adopted and used until that proficiency is improved.

JA: In terms of the standardization, I would share that sentiment. We have to be cautious about what we are standardizing and why we are standardizing it. There has been a push to standardize some elements of the cryopreservation protocol that would work for a specific cell type or specific cell product, but are not necessarily what is going to be needed long-term. What the industry needs to focus on is standardizing elements like tube size, rack systems, and shipping container configurations. We have been focusing too much on standardizing the variables driven by the cell product themselves—the actual cryobiological requirements of that cell therapy. We need to get out of standardizing these variables because we are walking ourselves down pathways with our bioprocessing that are not going to be the best paths forward for cell types and cell products that we know are coming down the track.

The desire would be for us to standardize those steps where control and reproducibility are important. Again, I am hesitant to jump to standardization quite yet because there is some evolution that has to occur within the system first. There are certain needs holding up advances in a few areas. Tools like containers and freezing equipment could be standardized right now, but that does not mean locking down processes. That is a conversation that has to occur with the tool developers and the cryobiologists.

SW: When people raise a question about standardizing something, you have to ask if standards are even the right solution to the problem. Are people asking for standards because there is too much confusion in the process, or is it because standards would make their day easier since they would not have to think about how to solve a particular problem? Having said that, standardization of containers in terms of specific geometry and performance requirements makes sense because it allows you to automate systems. You could buy an automated system that will work with any vial, for instance. There are elements along those lines that you can develop at any time.

The other area of standardization that would really help has to do with the questions that developers need to ask about cryopreservation. I do not necessarily think that everybody is getting the same questions back from their regulatory authorities. For example, we need to know the standard method of qualifying your dry shipper so that you do not have to go through a year of validation just to show that one dry shipper that maintains temperature is going to work just as well as another dry shipper that maintains temperature. These are the kinds of things that we can come up with in standard protocols and standard requirements that would really help therapy developers address supply chain and logistics challenges.

Q Looking to the future, what will be some other key next steps to continue bridging the cold chain knowledge gap between cryobiologists, cell and gene therapy developers, and clinicians?

SW: The first thing that comes to mind is the future students coming into the field. What is great about this industry is that people who have studied cryobiology are getting roles with important companies that are putting cryobiologists on these programs to develop therapies. The answer is to just keep doing that. These are the people who are going to be able to tell their process development and research development scientists, "Hey, don't forget about these elements of this process, if you are going through it, because we cannot change things after you have come a certain distance."

"In terms of other [preservation] technologies, I would love to have the ability to control nucleation in every sample in a controlled-rate freezer. That would help the field considerably."

AH: The most important thing we can do is talk to each other. I will give you a specific context. We host a monthly meeting called CryoChats where we bring in experts to talk on a panel. We bring in everybody from cryobiologists to industry representatives. The point of it is to talk about the challenges people are facing so they can hopefully get help. That kind of community building is important, and can help us bridge the gap between academia and people who are in the trenches using this technology as part of their day-to-day work.

JA: I would echo both Sean and Allison's comments with respect to needing more highly-qualified individuals who have the skills to work with industry in developing the technology. We need to get the academics to be more engaged with the community. It is also really important to recognize that there is a role for the scientific societies and organizations that are bringing people together to make sure that there are applicable and easily accessible content and materials for each of the communities to engage with.

Oftentimes, we see the industry folks talking in their own spheres about the problems that they are facing, and the cryobiologists talking and publishing and presenting material in theirs, but there is not a lot that is crossing between these groups in terms of materials and knowledge. Even this panel is a good example of where we can bring together communities that would normally not necessarily mesh. It is important to find opportunities for collisions, bringing problems forward in an academically interesting way, while still being practical.

That is sometimes the challenge on the academic side. We look at the problem and say, "That's easy to solve," but we do not understand the complexity of the regulatory side, the quality system, the scale, or any of those other elements that are missing. There is a requirement for there to be joint sharing of that information in forums and vehicles that allow for that information to be translated.

The regulatory authorities and the government have a role here as well to engage more broadly. I am seeing that in various forums where the regulatory agencies realize the field is coming into problems and, in an effort to solve those problems, they are trying to bring together the industry and academics through funding vehicles and grant programs. Money can sometimes unlock innovation. If we have additional funding in various forms in both industry and academia, these communities could come together and allow for innovation to proceed much more quickly, as opposed to having to occur more naturally.

The final step is to recognize that cryopreservation does require some tweaking. It is not a standard process. We are trying to standardize a lot of things in advanced therapy manufacturing. There are a couple of ways of transfecting cells in order to actually express a vector, for instance. Cell expansion technologies are becoming a little bit more standard as different manufacturing companies jockey for position in the market, too. But the cryobiology process itself is still fairly open and there is a lot of opportunity for innovation within that. How do we provide encouragement early in process development to allow that innovation to continue, and to be evaluated before processes begin and it becomes more difficult to do?

Looking to the future, opportunities to get those innovative ideas into companies and into academic programs early would really be helpful.

Q Do you have any parting comments?

SW: We are all very lucky to be involved in this really innovative part of the healthcare industry. The promise of cell therapies is one of the most interesting things that has happened in my lifetime. However, we have got to keep communicating because the promise is so big. If we just let it fall down, we will be missing so many opportunities.

JA: We are trying to solve a really hard problem by putting something biologically alive into a state where it is not biologically alive and store it for an extended period of time. The kinds of cells that we are preserving do not do that naturally. The science to make this happen in itself is pretty amazing. Then, to do it at an industrial scale is quite the accomplishment. Now, we have to look at translating what we are doing with cells into more complex biological therapies, like organoids, tissues, and ultimately, human organs.

The same kind of technology path is taking us down that stream. As an industry, how do we start to think about getting ready for this future where biological material that is stored in low temperatures for periods of time becomes the new medicines? We are seeing it now, but the future is so much brighter, and it is going to be enabled by the ability to cryopreserve and store these products.

AH: We have just scratched the surface here today. There are a lot of opportunities to learn more and become more involved in the field of preservation. The Society for Cryobiology will be meeting in Washington, DC in July 2024. People can come, meet cryobiologists, learn some of the science that is there, and really become more immersed in the field. I strongly encourage people to attend that meeting and learn what they can, so that they can continue down the path to wisdom and greater knowledge in the field.

BIOGRAPHIES

JASON ACKER is the Senior Development Scientist with the Canadian Blood Services' Centre for Innovation and a Professor in the Department of Laboratory Medicine and Pathology at the University of Alberta, Edmonton, Canada. His research focuses on understanding the response of cells and tissues to *ex vivo* storage and the development of methods for their preservation and use as therapeutic products. Acker's blood services laboratory has responsibility for developing scientific and technical evidence to support innovative changes in blood product manufacturing, storage, and utilization at Canadian Blood Services. Through the Canadian Blood Services Centre for Innovation, Acker leads Canadian Blood Services' efforts to assess new technology, products and processes that can improve the efficiency, quality and safety of blood products in Canada. Acker is author of more than 225 publications, eight book chapters, and holds 12 patents in the area of cell preservation and microfabrication. Acker serves as an Associate Editor for *Transfusion* and is an editorial board member for four journals and invited reviewer for more than 35 scientific journals. He serves as the Scientific Secretary with the International Society for Blood Transfusion and is a Scientific Member and Co-Lead with the Biomedical Excellence for Safer Transfusion Collaborative. Acker is the past-president and a Fellow of the International Society for

Cryobiology and is actively involved in consulting with and advising companies and organizations developing biobanking and cell therapy programs.

ALLISON HUBEL is a Professor in Mechanical Engineering at the University of Minnesota and Director of the Technology Leadership Institute where she holds the HW Sweatt Chair. She is President of the Society for Cryobiology. Hubel has studied both basic science and translational issues behind preservation. Her work spans from the study of molecular mechanisms of damage during preservation to the development of technology to improve preservation outcomes. She is the Founder of Evia Bio, a startup company based on the preservation technology developed in her lab. As co-PI for the MN-REACH commercialization hub and faculty lead for the University of Minnesota Great Lakes I-Corps Hub site, Hubel has been involved in working with faculty interested in commercialization of academic research. Hubel has published numerous scientific articles in the field of preservation, and she is the author of, *Preservation of Cells: A Practical Manual* published in 2018. She is a former deputy editor of *Biopreservation and Biobanking* and received the Outstanding Achievement in Biobanking Award from ISBER.

SEAN WERNER is the Chief Technology Officer of Cell Processing at BioLife Solutions, a leading provider of bioproduction tools and services to the cell and gene therapy and broader biopharma markets. BioLife acquired Sexton Biotechnologies in 2021 where Sean was President of the company known for providing processing and handling solutions for the cell and gene therapy industry. Sean received his PhD from Purdue University in Biology followed by post-doctoral positions at the Indiana University School of Medicine and Eli Lilly and Company. Sean has previous experience filling various roles in the scientific, global regulatory, and general management functions supporting medical devices, autologous cell therapy, and single use disposable development programs. In his 23 years working in the life science industry, he has guided regenerative medicine research programs, pre-clinical, and clinical testing and submission strategies leading to global commercialization of medical devices and bioprocessing tools and successful initiation of multi-national cell therapy clinical studies.

AFFILIATIONS

Jason Acker PhD
Professor,
Department of Laboratory Medicine and Pathology,
University of Alberta

Allison Hubel PhD
Director,
Technology Leadership Institute,
University of Minnesota

Sean Werner PhD
Chief Technology Officer,
Cell Processing,
BioLife Solutions

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The 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: Acker J has recieved operating grants from CIHR, NSERC, NIH,CBS and consulting fees from Allovir, HemoCue America LLC, Resilience America LLC, and CatamaranBio within the past 36 months. Acker J is part of the scientific advisory board for BioLife Solution Inc., a board member for IBST and BEST, and a shareholder and CEO of PanTHERA CryoSolutions. Hubel A owns stock in Evia Bio. Werner S owns stock in BioLife Solutions.

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

ARTICLE & COPYRIGHT INFORMATION

Copyright: Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

Attribution: Copyright © Sexton Biotechnologies®. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

Article source: Invited. This article was based on an Expert Roundtable discussion, which can be found here.

Expert roundtable recorded: Oct 31, 2023; **Revised manuscript received:** Dec 6, 2023; **Publication date:** Dec 20, 2023.

We hope you enjoyed this transcript of the roundtable.
You can also watch the recorded roundtable here

Sexton Biotechnologies®
Part of Biolife Solutions®

Cell Processing Solutions

Building tools for tomorrow's therapies

We focus on providing tools to therapy developers that improve performance, reduce risks and secure the probability of positive clinical outcomes. We approach manufacturing challenges from two distinct perspectives:

Improving cellular processing methods moving from lab practices to industry standards.

Improving cellular performance with new reagents and methods.

Signata CT-5™

The Signata CT-5 allows users to incorporate automation early in the manufacturing process without eliminating critical flexibility during process development. The system provides multiple consumable options for various manufacturing techniques to add safety and control to CGT manufacturing.

CellSeal® Cryogenic Vials

CellSeal Cryogenic Vials are the first purpose-built rigid containers used in the cell and gene therapy industry with hermetically sealed access ports that withstand the challenges of -196°C storage without compromising container closure integrity (CCI).

Radio Frequency Tube Sealing

Easily access the CellSeal platform using our radio frequency tube sealer. Sealing unit is specifically tuned for optimal sealing of CellSeal vials. Compact system offers consistent and reliable sealing to protect your intermediates or final product.

To find out more please visit:
biolifesolutions.com/cell-processing/handling

CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING & CMC

SPOTLIGHT

INNOVATOR INSIGHT

Empowering the future of cell therapy: leveraging partnerships to support advancement

Juan Patarroyo, Fiona Mack, Xavier de Mollerat du Jeu, and Josh Judkins

As the number of new cell therapy companies steadily rises, a different approach and perspective is needed to ensure correct support is provided to promote the success of new advancements and foster the therapeutic potential that early developers hold. The journey from discovery to market is challenging and collaborative relationships among stakeholders, such as drug developers, academic institutions, and industry partners, offer an effective framework for addressing these challenges. Tools providers specifically play a vital role by developing innovative technologies and platforms for cell therapy manufacturing. In this article, we will explore the significance of these relationships and discuss the related barriers, opportunities, and responsibilities for emerging drug developers, tool providers, and early discovery teams. We will share insights on how collaborative technical work can facilitate knowledge exchange, funding access, and impact the establishment of shared infrastructure, enabling efficient navigation of the complex cell therapy landscape. We will also share experiences relating to training, investment, and how collaborative frameworks can empower early discovery teams and create a thriving ecosystem that supports the advancement of cell therapy research and development.

Cell & Gene Therapy Insights 2023; 9(10), 1423–1433

DOI: 10.18609/cgti.2023.185

INTRODUCTION: LEVERAGING COLLABORATION TO DRIVE INNOVATION IN THE CELL THERAPY SPACE

Product flexibility and customer engagement can enable commercialization success across the development continuum. Critical aspects include ensuring a robust supply chain to support commercialization, GMP compliance, having closed, automatable, and scalable processes, and maintaining a robust library of regulatory documentation to enable IND filings. Thermo Fisher Scientific offers solutions that can be used from the early phases of development onwards, which meet all of these critical aspects. The key aim is to help simplify and accelerate time-to-market through provision of trusted, high-quality products and services backed by collaborative support and a robust supply chain network.

In the cell therapy development journey from research through to clinical and commercial, developers often skip or shorten the process development stage. This can happen due to the need for rapid progress to commercialization, budget constraints, and a lack of accessibility to platforms. However, there are risks downstream when process development is neglected, including overly expensive and unscalable processes, non-compliance with regulated product standards, and reduced appeal for biopharma acquisition, all of which hinder commercialization.

In the context of the bio-incubator space, partnerships are essential to drive the economical use of biotech funding. Having scalable platforms and instruments—for example, the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System, the Gibco CTS Xenon™ Electroporation System, and the Gibco CTS DynaCellect™ Magnetic Separation System instruments—centrally available for early-stage companies to access and adopt during the early phases can help accelerate process development. The backbone of this endeavor is collaboration within a larger ecosystem, with support provided in protocol development and instrument adoption.

EXPERT ROUNDTABLE




Discussion with Juan Patarroyo, Fiona Mack, Xavier de Mollerat du Jeu, and Josh Judkins (moderator)

DOI: 10.18609/cgti.2023.185

Q **JJ:** Can you each briefly introduce yourselves?

JP: I am the Director of Science Operations and Strategy at LabCentral. I am what we term an 'all-site' team member of the LabCentral staff, and my main role is to understand the needs of each of the 120–130 resident companies within our ecosystem, spanning several different sites. They each work in different areas of therapeutics, including cell and gene therapy (CGT). My communication with them is key to understanding their needs and pain points so that we can bring in innovation and technology for them to move projects forward for themselves. I aim to bridge the gap between these companies and our equipment sponsors and suppliers.

FM: I am Vice President and Head of Co.Lab Cambridge, part of Bayer's initiative to bring healthcare to all. An integral part of my role is to look into how we can invest in early-stage innovation to support our entrepreneurs. Co.Lab is a global network of life science incubators that are part of our broader business development strategy to foster early-stage innovation and provide the support of large pharma to help biotech companies bring their ideas to life. Co.Lab Cambridge, our flagship location, recently launched in May, 2023. It is dedicated to supporting companies within the CGT space. We leverage the enterprise-wide expertise that Bayer has in this field—for example, our affiliate companies, BlueRock Therapeutics and AskBio, are both developing novel therapeutics to treat CNS disorders.

Our site in Cambridge is partnered with LabCentral for operational excellence, allowing a great deal of expertise to reside in the same location. Furthermore, Co.Lab Cambridge is co-located with the Bayer Research & Innovation Center, providing access to internal expertise from oncology to CGT within the R&D organization, in addition to our business development colleagues. (Co.Lab is part of the business development organization for Bayer). Co.Lab Cambridge is a unique center of excellence that can harness all these resources and relationships.

XMJ: I am Senior Director of R&D at Thermo Fisher Scientific, based in Carlsbad, California. Our approach to cell therapy involves enabling our customers to take their drugs to the commercial stage. I represent the product side, helping to make different products to serve our customers from R&D to commercial. Over the past few years, we have created a cell therapy-focused applications team dedicated to supporting our customers. Their key aim is to assemble all of the technology across Thermo Fisher and integrate these technologies into processes to be shared through collaborations with customers. This enables customers to de-risk their processes by leveraging our knowledge directly. Through our collaboration program, we share protocols, know-how, and even instruments to help customers move through to commercialization.

We also work closely with bio-incubators like LabCentral, providing instruments and knowledge to enable their residents' transition from discovery to commercial. Collaboration is win-win: the drugmakers get their drug on the market through our enabling tools, and in

exchange, we receive incredible feedback on how to design our products, which can then be passed on to the product teams to inspire the next generation of products and instruments.

Q **JJ:** As someone who sits in the commercial part of the organization, developing relationships based on trust is what fulfils me the most. Could you expand on how to measure success when engaging with these collaborative partners? What are you getting from this engagement?

XMJ: Success for me is seeing products fulfilling a need for customers. We spend a lot of time and money developing products, so it is important that customers want to use them. This interaction is absolutely critical. When we get positive feedback, that is a huge success for me. As a product maker and developer, these interactions of knowledge sharing allow us to help customers take their products to commercial. Ultimately, we are speeding up processes to enable patient access to these drugs, which is what really matters.

JP: The mission of LabCentral is to provide the infrastructure and operational excellence that many companies need because they are often in the early stages of development without a lot of funding. Working in a collaborative way with our equipment providers is key for our early-stage biotechs to be able to develop essential processes, protocols, and workflows. This enables them to build packages for investors and to attract talent. Collaboration benefits everyone on the wheel, and the equipment sponsors are particularly key to that wheel because they have the know-how. They have the ability to take these instruments to the next level and use 100% of their capability. They can tailor any protocol to any need that a company has to hopefully benefit many of the patients who are waiting.

FM: Starting in a company at an early stage is risky but hopefully, rewarding. What can give you an edge over your competition is technical expertise that allows you to know what not to do. In many cases, it is important to fail fast in this business. We learn that not only through our strategic partnerships but from collaborations with companies in incubators. The experience of failing and restarting can be leveraged by being in an incubator and having that sense of community. With our Co.Lab incubators, Bayer hopes to move this beyond a regional level, across a global incubator network spanning Berlin, Japan, Shanghai. Success to me is knowing that a company has reached a milestone that they may not have been able to reach had they not used the Co.Lab incubator knowhow.

Q **JJ:** Can you expand on how you specifically support cell therapy in your respective incubators?

FM: The Co.Lab Cambridge was specifically designed to support early-stage CGT companies to utilize the world-leading expertise that we have within Bayer and its affiliates that

DOI: 10.18609/cgti.2023.185

are co-located within the sites. That access to expertise drives much of our decision-making. The space is designed to support lab work at the early stage, meaning there are many BCL2+ goods, for example, and analytical equipment available. Our strategic partnership with LabCentral 238 allows our residents to access more biomanufacturing equipment to enable process scalability at an early stage. Physically, the site is an open shared lab space with some private lab and office space depending upon individual needs. We have the ability for companies to grow and graduate into the LabCentral 238 space.

Besides the physical facilities, we provide mentoring discussions to match the needs of our residents with levels of Bayer's internal expertise. These discussions can be very technical—perhaps about how to differentiate iPSC cells, for example—but could also be related to finding the best regulatory approach or designing a clinical trial that shows value for the drug. Through our various mentoring one-on-one sessions and groups, roundtable discussions, and scientific symposia, we bring in that scientific and industry expertise.

Bayer also has a manufacturing arm. In Berkeley, California, we have a CDMO business focused on contributing to early-stage process development. This group is willing to do things at reduced fees or even deferred payments for pilot studies for process development as companies begin to scale, with no strings attached. It can help reduce those transition timelines from one company to the other and help streamline time to clinic.

JP: From the LabCentral perspective, partnering with Bayer Co.Lab has been an incredible thing to do in terms of moving companies focused on CGT to the next level.

In the LabCentral 238 Main Street building, our labs are much larger, and they can support much larger teams in expanding their own processes and networking. These labs have been built specifically for process development work in many ways. For companies beginning to think about how to accelerate their drug discovery process, the labs and equipment are specifically picked for that purpose. It allows companies to test their system and protocols ahead of eventually take them to a CDMO. They have the ability to own these processes from the beginning, to make mistakes or be successful. At 700 Main Street, there were no process development capabilities, which posed challenges for some of the companies that came out of there. With that in mind, the LabCentral team decided to build this new facility at 238 for the pipeline of companies coming through.

It can be challenging for companies to know how to progress, as CDMOs often want different things. If we give companies the ability to start working on these processes from the beginning, it gives them more power to transfer this information to bigger CDMOs to start expanding their drug products. Partnering with equipment providers such as Thermo Fisher gives us the capability to offer the specific complex instrumentation required for process development.

XMJ: Equipment is paramount. Access to that equipment as early as possible helps developers to establish what they do not yet know. Drug developers can often focus on the disease and not fully appreciate what it takes to reach commercialization. Having access to Bayer, Thermo Fisher, or LabCentral helps to build an understanding of these process decisions. Moving a process to manufacturing is daunting. Leveraging an incredible amount of support from

experts can dramatically speed up the process of making that drug available. It is a lot more than that just space and products; it is people around you who create a valuable knowledge ecosystem.

FM: We have recently started assembling a panel of experts ranging from discovery to regulatory to commercialization. We ask our Co.Lab residents for questions and then pose these to our panel. Developing long-term relationships with solution providers, incubator companies, and residents, is all key to building a strong ecosystem in a good position for success.

JP: The ecosystem of LabCentral involves companies talking and learning from each other. These companies coexist in the same building or area, and they learn what to do and what not to do through mentoring each other. The beauty of an incubator is that you are housing all these brains in one place in a way where they can feed each other to reach an ultimate goal.

Q **JJ:** I was an account manager at LabCentral for a number of years. When I first learned about this new facility, I knew Thermo Fisher was well placed to offer support as we have the infrastructure in place to do training and protocol development, and can connect residents with our CTI team. Our flagship instruments—the Rotea for cell processing, the Xenon for electroporation, and the DynaCellect for cell isolation based on Dynabeads—can offer transparent cell therapy process development for everyone. Xavier, could you shed more light on what a collaboration would look like with the Thermo Fisher CTI team?

XMJ: Many people see us as a toolmaker but with the collaboration team, we have created something new over the past few years. We want to show the value that we bring beyond just providing customers with products.

One of our issues is the sheer number of products we offer. Once these offerings are narrowed down to meet individual needs, then the collaboration begins. This can involve a visit to one of our sites to see things in action, including how to connect tools together. We freely share our protocols. We can also offer weekly or bi-weekly meetings to discuss problems and fix them or develop protocols to help customers. So, we are not just making tools—we also provide expertise in how to use them. We can also offer early access to future products so customers can prepare themselves for what is coming. We are together on this journey to commercialization.

Q **JJ:** How do these partnerships benefit us? Since this is provided for free, what do we get out of this transaction?

XMJ: The benefit to us is incredible. I make products for our customers, and if I do not get the intel on how they use them and what they want to do with them, then I will never develop the right products.

DOI: 10.18609/cgti.2023.185

It also drives adoption. I want people to see the tools that we have. We act as the central point to connect people to other divisions of Thermo Fisher. As we have an overwhelming amount of tools, customers may only see the tip of the iceberg, but there is always more that we can offer to help. And ultimately, I get to see drugs all the way through to commercial, which is an incredible benefit in itself.

FM: A similar question often gets asked about open innovation on the pharma side. In order to continue to grow our business, we need access to what is going to be coming next, and hopefully, to have a hand in guiding that to benefit both our company and the patient. We are giving a lot of our time and internal research to this, but these ideas that may lead to another acquisition of an early-stage company, another investment, or a licensing deal, could have a tremendous impact on the value of our portfolios. There are lots of parallels to what we are doing here. It is great to see the different strategies and perspectives that we each have whilst trying to reach the same end goal.

JP: Hopefully, LabCentral is facilitating that collision between all of these different partners to create an ecosystem of collaboration. As CGT is such a complex modality, you need all the help you can get. The expertise from Bayer, Thermo Fisher, and other equipment suppliers can all synergistically come together.

Q **JJ:** If you could say anything to a toolmaker about what is required to support these kinds of advanced therapies, what would you tell them to do to ensure success?

JP: At LabCentral, we talk to companies and understand their needs and how they want to build their workflows and protocols. Then we work backwards, identifying what is needed for success. There is a lot of complexity to consider, including the chosen modality, and not all instruments may work in the same way.

We try to either bring in instruments that will be widely used, or place our companies in contact with others with the capabilities to help them. Again, that complexity can come in many different forms. We want to bridge that connectivity by talking to these resident companies, understanding their needs, and teaching them how to fulfill those needs.

XMJ: In cell therapy, we are at the beginning of the journey from a tool perspective. The tool makers have been working on first-generation products. We used a lot of bioprocessing knowledge when we made 5,000 L bioreactors. Then, we realized that was not going to work, and we needed to make things smaller.

Now, we are at the stage where these tools and products are on the market, and people are using and tweaking them. This is where we start refining, redeveloping, and building the next generation to fit developers' needs. This is where collaboration is so critical. With intimate collaborations where people share their processes or what they want to do in the future, we can design better products.

Q **JJ:** What is the future of the incubator as new tools and therapeutic targets come to light? How will partnerships and collaborations drive the field another decade down the line?

JP: We keep our fingers on the pulse with regards to the science. CGT has been around for quite some time, but there are still waves of innovation taking place. We adapt as things change over time. To enable us on our mission to help companies deliver, we need to ensure we understand the science and what is coming up through the pipeline, whether it is CGT, small molecules, biologics, or delivery platforms. This enables us to bring in the right tools, the right equipment, and the right experts to help companies move forward, as ultimately, patients are waiting for these therapies.

FM: Since our opening, our focus has been on CGT companies, which is a broad field. Ultimately, we are looking for companies that can benefit from working with Bayer, and that could enable these platforms to become more ubiquitous. For example, we have been thinking about companies focused on small molecules for immunology to mediate some of the immune suppression to allow repeat dosing for some advanced therapies. We are also looking for the next generation of non-viral gene delivery, any diagnostics that could help with that, and novel ways to do clinical trial design or manufacturing to reduce costs to become part of our Co.Lab network. For me, the next generation means thinking broadly to expand our community, including expanding our virtual network. There, companies could tap into all the expertise in Bayer and within our community of partners. Those companies could be beyond the incubation state but still looking to solve the challenges of manufacturing.

As we begin to expand globally, we may not be so modality-focused, allowing us to think about companies that could be aligned overall with Bayer's focus areas of cardiovascular diseases, oncology, and immunology. We want to ensure that we harness the full utility of the Bayer enterprise to enable growth.

Q **JJ:** Xavier, how are we at Thermo Fisher going to address the changing market?

XMJ: We have to try to understand the future because it takes 5–7 years to develop a product. In 7 years' time, the cell therapy field will look different. Will there be centralized or decentralized manufacturing? What will the dosages be? Will people be using lentivirus or non-viral gene editing tools? It is incredibly challenging to predict. At Thermo Fisher, we are trying not to predict the future, we are trying to predict the different features of the future, such as predicting that hospitals may start manufacturing, or that large biopharma will need substantial amounts of centralized manufacturing.

We are trying to develop products that will fit in all those different scenarios. That is why we are keen on platforms and modularity. We know automation and closed systems are must-haves.

DOI: 10.18609/cgti.2023.185

We also want to predict how the processes will develop. The processes have already shortened from 12 days to 24 hours, so how fast will they be in the future?

We have processes in place to review new trends on a quarterly basis, to ensure our programs are consistently relevant. This is critical: the cell therapy industry is unique because it moves so fast.

BIOGRAPHIES

JUAN PATARROYO is Director of Science Operations and Strategic Relationships, providing in-depth scientific, technical and strategic support to resident companies via direct engagement with each resident company. He also supports sponsor relationships identifying new technologies for LC resident companies. Prior to LabCentral, Juan was Principal Scientist at Novartis designing and developing new assays for drug development and identifying new targets for autoimmune conditions. He has over 20 years of drug development experience working in big pharma as well as star-up companies like the ones housed at LabCentral. His in-depth drug development knowledge will complement the science operations team engaging with resident companies to aid in their success. Juan obtained his BS in Microbiology and Chemistry from San Francisco State University.

FIONA MACK is currently Vice President, Head of Bayer Co.Lab Cambridge, USA, which is a part of the Business Development & Licensing/Open Innovation function. In this role, she is building a new cell and gene therapy incubator to accelerate innovation within the field. Prior to join Bayer, as Head of JLABS@TMC, Fiona was responsible for external engagement, innovation sourcing, company onboarding, portfolio management, operational excellence, educational programming, and P&L. She catalyzed and supported the translation of science and technology into valuable solutions for patients and consumers across the pharmaceutical, medical device, consumer, and healthtech sectors. Fiona has held senior leadership positions at Ipsen, Roche and Pfizer supporting external innovation efforts to expand rare eisease, neuroscience and oncology portfolios. Fiona earned her PhD in Cell and Molecular Biology from the University of Pennsylvania and her undergraduate degree in biology from Cornell University. Her innovative work has been published in high impact journals and she also has several granted patents.

XAVIER DE MOLLERAT DU JEU is the Senior Director of Research and Development of the Cell and Gene Therapy business unit at Thermo Fisher Scientific, first joining the company in 2005. His team is currently responsible for integrating new closed modular platforms into cell therapy production workflows. In his prior role, Xavier and his team developed cell therapy processing platforms and identified new DNA delivery approaches for hard to transfect cell lines and primary/stem cells—inventing Lipofectamine® 3000 and authoring several patents around nucleic acid delivery in the process. Xavier studied molecular biology and plant physiology at the University of Montpellier II in France and received his PhD in Human Genetics in 2003 from Clemson University in South Carolina. His thesis work involved identifying the gene(s) responsible for split hand/split foot malformation 3 (SHFM 3). His post-doctoral fellowship research was in the laboratory of Dr Michael G Rosenfeld at UCSD, where he studied the roles of microRNAs in pituitary gland development.

JOSH JUDKINS earned his BS in Biochemistry from Ohio Northern University, and MS and PhD in Chemistry and Chemical Biology at Cornell University. After graduate school,

Josh completed a postdoc in neuroscience chemical biology at Pfizer in Cambridge, MA and has been active in the biotech community in eastern MA since. After Pfizer, Josh joined Thermo Fisher Scientific where he has been for over 7 years holding various life science-focused technical roles, and is currently the Eastern MA Cell and Gene Therapy Business Development Manager.

AFFILIATIONS

Juan Patarroyo
Director of Science Operations and Strategic Relationships,
LabCentral

Fiona Mack
Vice President, Head of Bayer,
Co.Lab Cambridge

Xavier de Mollerat du Jeu
Senior Director of Research and Development,
Thermo Fisher Scientific

Josh Judkins
Cell & Gene Therapy Business Development Manager,
Thermo Fisher Scientific

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors have no conflicts of interest.

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

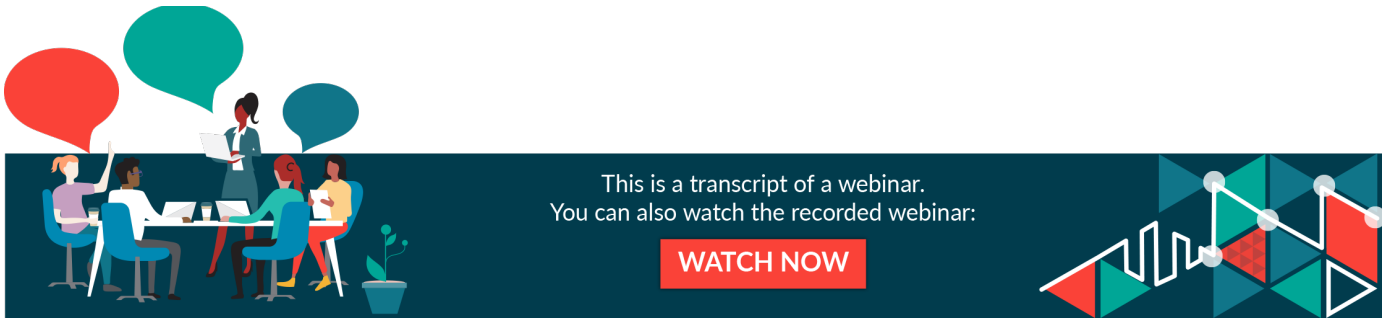
ARTICLE & COPYRIGHT INFORMATION

Copyright: Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

Attribution: Copyright © 2023 Thermo Fisher Scientific. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

Article source: This article is based on a transcript of a webinar, which can be found here.

Webinar recorded: Sep 21, 2023; **Revised manuscript received**: Nov 13, 2023; **Publication date:** Nov 23, 2023.



DOI: 10.18609/cgti.2023.185

Closed cell therapy manufacturing solutions

Modular, standardized, adaptable, and scalable

We offer a cell therapy manufacturing workflow that serves as a foundation for digital integration of your cell therapy process and data management, while enabling the interaction of production and control layers to manage all aspects of clinical manufacturing. When used in concert with our comprehensive portfolio of Gibco™ Cell Therapy Systems (CTS™) consumables, reagents, and instruments, you can transition to the clinic with ease.

- Physical and digital connectivity
- 21 CFR Part 11 compliance
- In-process analytics
- Exceptional reagents and protocols

For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. Caution: Not intended for direct administration into humans or animals. © 2021, 2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. **COL024705 0423**

CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING & CMC

SPOTLIGHT

INTERVIEW

Addressing the risk presented by extractables and leachables in cell therapy manufacturing

The identification and measurement of extractables and leachables (E&L) is a critical element of advanced therapy QC. David McCall (Senior Editor, *BioInsights*) speaks to Jason Creasey (Managing Director, Maven E&L Ltd) about regulators' expectations and the importance of adopting a risk-based approach to E&L for cell therapy developers.

Cell & Gene Therapy Insights 2023; 9(10), 1125–1131

DOI: 10.18609/cgti.2023.149

Q What are you working on right now?

JC: Right now, I am continuing to run my consultancy business, which is devoted to extractables and leachables (E&L). It allows me to continue working full-time in the area of E&L—a journey that began for me in the mid-1990s. This actually coincided with the beginning of interest in E&L in general, which started in the inhalation space with metered dose inhalers. As well as supporting clients, I also like to find time to present on the topic at conferences and seminars, and I work with the Extractables and Leachables Safety Information Exchange (ELSIE) Consortium as a scientific advisor. So, I guess you could say I am mildly obsessed with the topic!

Q Why are E&Ls so important to the cell and gene therapy field in particular? What is really 'need to know' about them for those working in the space?

JC: When it comes to advanced therapies such as cell and gene therapies, E&L should be considered, since it studies and manages a specific risk to the safety and purity of these medicines. Managing the risks to safety or purity of medicine is a key step in every pharmaceutical development program. What changes is the size of the risk for different types of products. For example, products that are delivered by the parenteral route, which is the case for many advanced therapies, are among those that are at higher risk from leachables.

Let me define what is meant by E&L, because I think that is important to understand. Starting with the 'L' part, the term 'leachables' has been used by the pharmaceutical industry to describe an impurity that enters the drug product formulation as a result of an interaction of that formulation with materials of construction of either the manufacturing process and/or the packaging and delivery device. These systems contain a lot of plastics or elastomers, which can be a source of substances (small molecules) that leach from the materials into the drug product and are then delivered to patients receiving the medicine.

In order to study these leachables, experiments are designed to study this possibility and the specific substances in these materials, because of the risk that leachables may either be toxic to the patient themselves, or may affect the quality of the drug product, which in turn has a negative impact on the patient. These designed experiments are the 'E' part. Doing extractable experiments helps in a number of ways. It helps predict what the leachables may be, and it helps you develop your leachable methods of analysis (by defining targets). It is also helpful to know which of your materials of construction are the source of a leachable. This then gives you the choice to replace that material, if the leachable derived from it is a concern.

Earlier in my career, I led a team at GlaxoSmithKline tasked specifically with looking at E&L. One of our major responsibilities was to develop analytical methods to look for extractables in the materials of construction, and methods to look for leachables in the drug product. This was not a trivial task since it is a wide and varied set of substances that may be released from plastics and elastomers, and in many instances, we were uncertain as to what these substances were before we developed and tested the methods of analysis.

The development, validation, and application of such analytical methods is a complex and time-consuming activity, which needs planning and dedication as well as advanced analytical chemistry skills and equipment. That is why the typical model for E&L analysis today is to leverage a mixture of in-house and outsourced resources.

As well as designing and operating the analytical methods, you then have to understand the results and make decisions on whether the risk from leachables is high enough to warrant doing something about it.

Like every drug, advanced therapy products are evaluated and approved by a regulatory authority prior to use, so any information generated must be communicated effectively to that authority. As I have explained, leachables have the potential to affect both the safety and efficacy of a medicine. To determine the associated risk, measurements are made to demonstrate both what is present in the medicine and how much of it might be dosed to patients. In the

DOI: 10.18609/cgti.2023.149

case of cell or gene therapies, their methods of manufacture and delivery can increase the risk that leachables affect patients. In many ways, they are unique, because the patient's cells or genetic material may be placed directly in contact with formulations of the medicine both during manufacture and when the medicine is finally introduced back into the patient. With most other medicines, the leachables are only introduced in that final delivery step.

"Right now, we do not have universal guidance on E&L. The guidance that does exist can be quite general or indeed contradictory in places."

Q How and why might the importance of E&Ls increase further as the regulatory environment for advanced therapies continues to evolve and mature?

JC: The regulatory environment has certainly developed over time. For advanced therapies, this area continues to innovate and evolve in an attempt to deliver better and better therapies to patients. This may be through the introduction of novel materials of construction and novel methods of delivery—in principle, both could offer mechanisms for a greater risk from leachables, if risk assessment is not fully considered in their design. Traditional approaches to the evaluation of impurities were based around knowledge of the active pharmaceutical ingredient (API) and its potential impurities, as these were considered the highest risk items. However, regulators are now much more likely to ask companies to consider any element that may offer a risk to patients.

Q Are there any particular concerns or considerations around divergence in regulatory opinion and guidance relating to E&Ls in the global setting?

JC: I think the concern here is a lack of consistency in expectations. Right now, we do not have universal guidance on E&L. The guidance that does exist can be quite general or indeed contradictory in places. However, there is an ICH guidance document under construction (ICH Q3E) and there is hope that this may lead to a greater consensus of opinion on this area—that would be most welcome. For now, the best advice is to be science-led in justifying the approaches you take to E&L analysis.

Q What are some of the keys to adopting a risk-based approach to E&L requirements, and what is the optimal timing for planning and then implementing such an approach?

JC: In many ways, the risk-based approaches to E&L are the same as those in use for the other aspects of pharmaceutical development. Unfortunately, though, people do sometimes neglect to use them for this particular area.

"One of the ways in which I think I can help [reduce uncertainly in E&L analysis] is to bring groups together in areas of joint benefit."

The most critical item is probably to employ QbD. Selecting good materials for construction is key to this. There is a concept in pharmaceutical development (outlined in ICH Q8) of creating a quality target product profile (QTPP). This can be extended to the selection and requirements for the materials of construction of both manufacturing equipment and packaging and delivery devices. If you select materials with a knowledge and understanding of their potential to produce leachables then of course, this lowers the risk. That is not always easy to achieve, but I think the attempt is worthwhile.

This QTPP then influences the timing of events. It means that you have to be prepared to consider activities around E&L at three specific points (and to potentially repeat activities, if required). Firstly, as I have implied, there is the design stage. Here, you may be selecting and specifying materials of construction and thus, a period of risk assessment and evaluation might be undertaken. Secondly, you have the period of clinical development leading up to and including regulatory approval of the marketed product. As I mentioned earlier, regulators will expect a package of information that will confirm the risk from leachables is low. Thirdly, there is the lifecycle period, post-approval. During this period, there may be changes made to the approved product and these changes need to be evaluated for their potential to introduce new leachables, or to increase the amount of existing ones.

The studies around E&L are linked to these three stages, and each stage is linked together by a risk management process designed to ensure planned activity is connected to any changes made. This is quite difficult to achieve; you want to conduct studies only when the materials of construction are defined and not subject to change, but of course, those studies need to be completed prior to point where you need to present them for regulatory approval.

Q What are the key E&L-related analytical tools and assays available today, and what might be coming down the innovation pipeline next?

JC: I guess this is one of the reasons I have kept within this area throughout my career. It has always been the case that we have needed to adopt cutting-edge analytical tools to study extractables or leachables. This is because we are frequently asked to perform screening exercises to detect 'everything' that may be present in a given material of construction or a drug product formulation. Clearly, 'everything' must have some caveats, but it frequently means pushing the limits of analytical science in terms of detection limits and the ability to identify and quantify at the trace level. In order to achieve this, it is now generally agreed that it is appropriate to subdivide the screening into three subgroups of organic substances (volatile, semi-volatile and non-volatile). Whilst there is some overlap, this can be translated into three complementary

DOI: 10.18609/cgti.2023.149

analytical methods—two gas chromatography-based assays (covering volatiles and semi-volatiles) and a liquid chromatography-based assay. Each of these methods is typically coupled with mass spectrometry (MS) to allow detection and identification, although other detectors are sometimes used to support and supplement MS.

Regarding the future pipeline of tools, each of these analytical approaches is subject to almost continual innovation and development, driving either better detection limits or mechanisms of identification. Certainly, over the period of my involvement in the E&L field, MS has developed significantly—for example, the use of higher-resolution MS has allowed for significant improvements in the identification of substances. I think one area linked to this, which still needs further development, is our ability to transform the large quantities of raw output into meaningful results and knowledge. It is now quite possible to collect vast amounts of data, but the attempt to successfully interpret and transform that into useful knowledge has only just begun.

Q You have been actively involved with a variety of initiatives, groups, and publications aimed at developing best practices relating to E&Ls—can you pick out any particular conclusions or directions from these activities that you would like to see regulatory bodies acting upon or adopting in future?

JC: I have been very lucky to be involved in a large number of initiatives relating to E&L over the last two decades. I was involved in the review of the original 2006 PQRI OINDP recommendations for E&L, and it is interesting to see how this document has influenced the direction of E&L ever since. Arguably, its most important achievement was to set a safety-based risk threshold for leachable study, and then link it to the analytical method requirements. Prior to this document, researchers were forced to use the instrumental detection limits as a guide for how low to detect leachables. I think this principle of linking analytical requirement to a knowledge-led risk assessment of leachables is still very important, the most important part being the risk-based aspect. We are still sometimes held back by not conducting our assessments of leachables based around the risk they pose. Determining the risk is not always easy, but it is something I think we can do. Very often, the absolute true risk from a leachable is low—however, we struggle to demonstrate this because we lack certainty in the information available to us. There is uncertainty in the analytical measurements made, and there is uncertainty in the safety assessments conducted due to lack of information on toxicity. Therefore, I would ask that regulatory authorities do all they can to facilitate the collection and circulation of tools and information that work to reduce this uncertainty.

Q Finally, can you highlight one or two key goals and priorities that you have for your work over the foreseeable future?

JC: My goal is to continue to aid in reducing the uncertainty I have mentioned. Right now, there are lots of different groups spending resources conducting analysis on their drug

products for leachables. This is a time-consuming and potentially expensive activity. One of the ways in which I think I can help is to bring groups together in areas of joint benefit. I don't believe this is an area where there is anything to be gained from not collaborating for best effect. Many of the systems used are common throughout the industry and I think that at the moment, there seems to be a lot of repetition in the testing being done by different groups. If we can move to a position of better agreement both on what is required to be done, and on what is an effective means to then share and disseminate risk outcomes, perhaps this area can move forward more rapidly. As a field, we can then focus on areas of true risk while wasting less time on low-risk situations.

BIOGRAPHY

JASON CREASEY is a graduate analytical chemist. In 2019, he established Maven E&L Ltd, as its Managing Director and Principal Consultant. Maven E&L was setup to provide advice to clients working in the pharmaceutical industry on all aspects relating to the topic of extractables and leachables (E&L) and the risks that leachables pose to the quality and safety of drug products. Prior to this, he worked for GlaxoSmithKline, where he was the director of their R&D E&L Team. He has worked in the topic area of E&L since the mid 1990s on a wide range of modalities and dose forms seeing this area expand and grow in significance for the pharmaceutical and medical device industries. In addition to running his consultancy, he is a scientific advisor to the ELSIE consortium. Since setting up Maven E&L; he continues to present, discuss, and write about E&L. He now publishes a regular E&L blog through LinkedIn and his website (www.MavenEandL.com), for the exchange of ideas and discussion. As well as supporting client projects, among recent E&L activity, he is presenting and commenting on risk-based approaches to E&L requirements within the pharmaceutical industry, that he hopes will form part of an ICH guidance in the not-too-distant future, and has helped ELSIE publish and discuss their white papers linked to concepts in leachable risk management and develop their database further.

AFFILIATION

Jason Creasey
Managing Director,
Maven E&L Ltd

DOI: 10.18609/cgti.2023.149

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has 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

Copyright: Published by *Cell and Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

Attribution: Copyright © Creasey J. Published by *Cell and Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

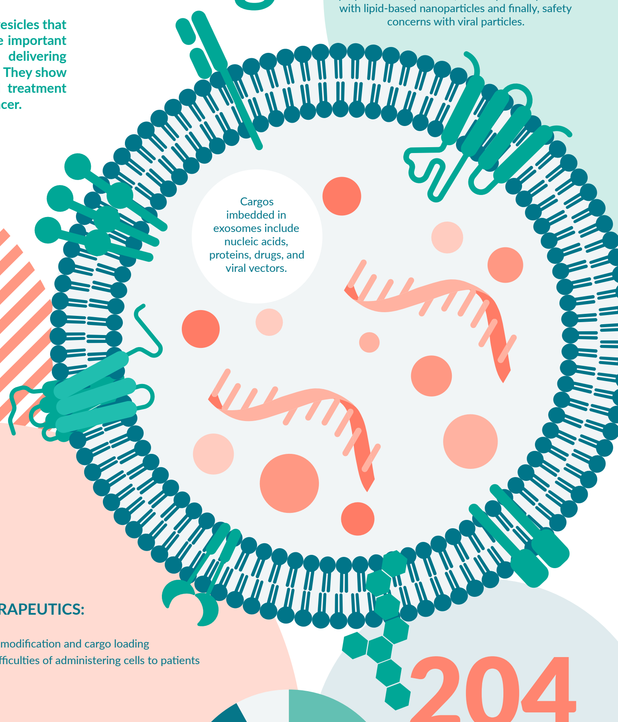
Article source: Invited.

Interview conducted: Aug 4, 2023; **Publication date:** Sep 27, 2023.

Exosome manufacturing

Exosomes are small (30-150 nm) extracellular vesicles that are released by exocytosis from cells. They are important mediators of cell-to-cell communication, delivering genetic and bioactive molecules to target cells. They show significant promise in novel diagnostic and treatment strategies for a range of diseases, including cancer.

Exosomes can offer an alternative to nanoparticles and viral particles because they circumvent some of the issues associated with them. Metallic toxicity is associated with metallic nanoparticles, low yield and biodegradability associated with polymeric nanoparticles, biocompatability issues with lipid-based nanoparticles and finally, safety concerns with viral particles.



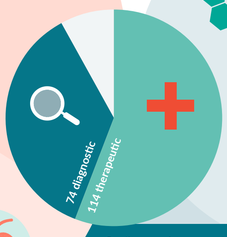
Cargos imbedded in exosomes include nucleic acids, proteins, drugs, and viral vectors.

THERAPEUTIC USES OF EXOSOMES

- Biomarkers for diagnostics
- Cell-free therapies
- Drug-delivery systems:
  - Direct method: exosomes are loaded with therapeutic agents
  - Indirect method: cells are genetically engineered or cocultured with therapeutic agents to produce artificial exosomes
- Cancer vaccines
- Personalized medicine

ADVANTAGES OF EXOSOMES AS THERAPEUTICS:

- Ability to cross the blood brain barrier
- Ability to engineer them including labelling, targeted modification and cargo loading
- As a cell-free therapeutic, they avoid the risks and difficulties of administering cells to patients
- High blood circulation clearance
- High cellular uptake
- Stability in the body
- Biocompatability
- Ubiquity



204

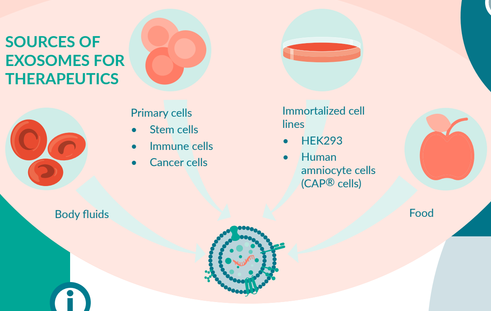
clinical trials using exosomes

For clinical trials, exosomes are required to comply with CGMP. CGMP manufacturing comprises

- Cell type and origin
- Culture method and medium
- Purification
- Characterization and identification method

Quality, consistency and functionality of exosomes is greatly impacted by the type, quality and heterogeneity of the source material. Therefore, in order to minimize exosome heterogeneity, tight control over the heterogeneity of the source material is essential.
Assessment of therapeutic indexes of source materials such as age should be considered.

SOURCES OF EXOSOMES FOR THERAPEUTICS



MANUFACTURING CHALLENGES

- Lack of standardization in isolation methods
- Primary cells usually grown in serum-containing media. Serum needs to be exosome free and removed prior administration.
- Achieving high yields coupled with high purity
- Exosomes derived from different sources have different features and therefore require different manufacturing, characterization, and purification protocols.

EXOSOME MANUFACTURING

CELL LINE CULTURE AND EXPANSION

- Rounds of division and expansion to generate a sufficient number of exosome-producing primary cells
- Expansion of suspension cells in controlled bioreactors when manufacturing at scale
- Challenge: scale up of adherent primary cell culture method

EXOSOME ISOLATION

- Normal flow filtration (NFF) to separate exosomes from extracellular vesicles (EVs)

CONCENTRATION AND PURIFICATION

- Traditionally ultra centrifugation but it can disrupt exosome integrity and does not remove macromolecule contaminants. Therefore it has a lower yield and purity.
- NFF or tangential flow filtration (TFF) - higher yield and better batch-to-batch consistency
- Size exclusion chromatography
- Affinity column chromatography
- Anion-exchange chromatography
- Magnetic beads

Purification challenges:
The size overlap between exosomes and other extracellular vesicles such as microvesicles make them difficult to separate.

ANALYTICS: CHARACTERIZATION AND IDENTITY TESTING

Exosome characteristics must be analyzed in order to determine the quality of the produced exosomes.

CHARACTERIZATION

Exosome particle number per volume
- Nanoparticle tracking analysis (NTA)

Presence of positive exosome markers
- Immunoblotting

Presence of negative exosome markers
- Immunoblotting, qPCR, MS, ELISA

Size and structure of the lipid bilayer
- Cryo-electron microscopy

Total protein
- BCA protein assay

IDENTITY

Assays detecting originator cell-specific markers or common exosome markers, such as proteins, lipids, or nucleic acids, can be used to analyze exosome identity

Exosome-specific proteins
- Western blotting, fluorescence activated cell sorting (FACS) with a specific antibody

Cargo loading/active pharmaceutical agent
- Immunoblotting, nano-flow cytometry, multiple reaction monitoring, ELISA, PCR

Protein identification
- Liquid chromatography, LC-MS, MS, Western blotting, flow cytometry

Surface marker profiling
- Flow cytometry, Western Blot, ELISA

Lipid identification
- LC-MS, MS

Transcriptomics
- Sequencing

There is still a lack of quality control guidelines over exosome stability, safety, potency, and quality requirements.

PURITY

Purity
- SE-HPLC

Host cell protein
- ELISA

Host cell RNA
- HPLC or gel electrophoresis

Host cell DNA
- Residual DNA quantitative assay

SAFETY

Mycoplasma
- Microbiological culture method, qPCR

Bioburden
- Membrance filtration

Adventitious virus
- *In vitro* assay, qPCR

Sterility
- Direct innoculation

Endotoxin
- Gel clot, photometry

***In vitro* potency assays – cell based biological assays**

References

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9095511
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9473149
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8766409
- https://www.frontiersin.org/articles/10.3389/fimmu.2022.865245/full
- https://www.frontiersin.org/articles/10.3389/fbioe.2021.811971/full

ThermoFisher SCIENTIFIC

CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING & CMC

SPOTLIGHT

INTERVIEW

Enabling patient access to CAR-T cell therapy in India

David McCall, Senior Editor, *Cell & Gene Therapy Insights*, speaks to **Shashwati Basak**, Vice President, Cell and Gene Therapy, Intas Pharmaceuticals, about the availability of autologous CAR-T cell therapies in India, the current state of regulatory guidance in the country, and the phase-appropriate analytical control of cell therapy manufacture. As engineered cell therapy products rapidly increase in complexity, it is critical that costs are reduced to ensure affordability to patients worldwide, including those in India.

Cell & Gene Therapy Insights 2023; 9(10), 1379–1384

DOI: 10.18609/cgti.2023.178

Q What are you working on right now?

SB: Currently, my team and I are focused on developing novel gene-modified cell therapy and gene therapy (CGT) products. The idea is to develop otherwise inaccessible and expensive advanced therapies at a fraction of the cost of current commercial products in order to provide accessibility and affordability to the Indian patient population.

Q Tell us more about the R&D pipeline for cell and gene therapies at Intas Pharmaceuticals

SB: Intas Pharma was the first Indian company to establish cell and gene therapy development programs in India. The company began with the vision to develop the next generation

of cell- and gene-based therapeutics to treat a wide range of genetic disorders and cancers. The CGT unit is involved in the drug discovery and process development (with full-fledged analytical support) of various gene and cell therapy products, including CAR-T cell therapy.

Q What is the current situation in India in terms of the availability of autologous CAR T cell therapies to patients? How is Intas seeking to help in this area in particular?

SB: At the moment, eligible patients for CAR-T cell therapy must travel to the USA, or other countries that offer these therapies at an exorbitant price. There is no accessibility to these therapies in India for patients, and the majority of such patients would not afford these therapies even if they were able to travel. There is a huge unmet need in India.

Although the evolving regulatory aspect needs attention given the novelty of CGT here, support from the government and other funding agencies, combined with the efforts of a few industry visionaries, has enabled several start-up companies and hospitals to get involved. They are dedicated to working on bringing autologous CAR-T cell therapies to Indian patients. In addition to Intas, there are two other companies leading this effort—ImmunoACT, a spin-off of an academic lab in the Indian Institute of Technology, Bombay, and Immuneel Therapeutics, which is located in Bengaluru. Both these companies have conducted phase 2 trials in India. ImmunoACT recently received the approval of its indigenously developed CD19 CAR-T cell therapy product in India. I believe this milestone is just the beginning for Indian companies.

At Intas Pharma, our team is developing several CAR-T cell products, including one targeting CD19 alone and two more targeting CD19/20 and CD19/22. The former is in the preclinical stage while the latter two are in the discovery stage. We also have a collaboration with the Tata Medical Center (TMC) in Kolkata and Miltenyi Biotec in Germany to allow us to initiate our phase 1 trial with Lentigen/Miltenyi's CD19 LV soon.

The collaboration with TMC serves as a demonstration of proof of concept to establish a point-of-care unit, following the decentralized manufacturing model. This will be a fully functional and self-sufficient manufacturing unit with both QC and QA capabilities in a hospital setting. We believe this can speed up the vein-to-vein or turnaround time, which is crucial for the patients' survivability since most of these patients are terminally ill with no other treatment options available. Intas Pharma is actually unique in that the parent company is located in the Westernmost part of India, while the medical center is in the East part of India, which contributes to the appeal of a point-of-care manufacturing unit.

"There is no accessibility to these therapies in India for patients, and the majority of such patients would not afford these therapies even if they were able to travel. There is a huge unmet need..."

DOI: 10.18609/cgti.2023.178

In parallel, we are developing a novel CD19 CAR-T product and also building the capability to manufacture plasmids and LVs in-house-an important consideration in terms of bringing down costs and increasing accessibility for Indian patients. Both of our autologous CAR-T cell therapy initiatives are being funded in part by a grant from the Biotechnology Industry Research Assistance Council under the National Biopharma Mission of the Government of India's Department of Biotechnology. This is a great example of government support to both industry and hospitals expediting the launch of these therapies.

Q What is your perspective on recent regulatory guidance relevant to the cellular immunotherapy space, particularly that which relates to QC and release testing of T cell therapy products? What are the key considerations for cell and gene therapy developers such as Intas?

SB: In terms of Indian regulatory guidance, the first draft of the national guidelines for gene therapy product development and clinical trials was published by the Indian Council of Medical Research in 2019. Due to a few unique features of these therapies, the aim of this guidance was to provide a framework to Indian cell and gene therapy developers over and above the guidelines already outlined in the existing Drugs and Cosmetics Act, which is more suitable for small molecules and biologics.

Since CAR-T cells and other CGT products are categorized as drug products, the quality expectations for product release remain the same as those for any other drug product. All the critical quality attributes (CQAs) must be identified during the development phase so that all relevant analytical tests can be identified, developed, and qualified or validated as fit-for-purpose prior to conducting the trials.

However, there are few exceptions to the rule given the living nature of the cell-based products. For example, the compendial sterility testing as per USP <71> takes a long time, which can pose a challenge. Fortunately, the regulators have recommended alternative strategies for the testing and release of living drug products. The US Pharmacopeia now has chapter 1071, which includes a risk-based approach to rapid sterility testing for product release. Examples of other alternative methods that may be needed for live cells include rapid mycoplasma and rapid endotoxin tests.

For non-compendial tests, the US FDA recommends qualification or validation to ensure they are fit for their intended use. In addition, the guidance states that for *ex vivo* genetically modified cells administered immediately following manufacturing, in-process sterility testing of a sample can be performed 48 to 72 h prior to final harvest. This may include a Gram stain and a sterility test compliant with 21 CFR 16.12. Under this approach, the release criteria for sterility would be based on a negative result and no growth resulting from the 48 to 72-h sampling.

At Intas Pharma, we are incorporating both the Indian guidance and the global guidances in the early product development phase so that we are aligned with both global and local regulatory expectations to ensure safe and high-quality products for our patients.

"Manufacturers are constantly looking to simplify the process without compromising on quality and capacity with the overall aim of reducing the cost of goods."

Q Are novel cell therapy analytical tools and technologies delivering the required degree of repeatability and precision, for you (e.g., cell counting)? If not, where are the key innovation shortfalls at the moment?

SB: Cell counting measurements are used in cell and gene therapy applications to evaluate cell viability and concentration in order to assess the quality and quantity of cells for use in a variety of processes. This also determines the dose that needs to be infused into the patient. Automated cell counters are used in combination with flow cytometry-based absolute cell counts to cross-validate the data in order to avoid manual counting, which is prone to errors.

Real-time cell counting while the cells are in different phases of the process would be a key innovation to track critical in-process parameters, rather than just the endpoint.

Q What does phase-appropriate analytical control of cell therapy manufacture look like, particularly in the early stages of development?

SB: Analytical methods are critically important throughout process and product development. They are used to support manufacturing investigations and characterize process changes. As the CQAs for most cell therapies are poorly defined and vary from product to product, selecting assays for process development can be challenging. Given the complexity in using a living cell as a product, it is important to develop many orthogonal assays early on in the product development pathway. This approach ensures we characterize and measure the unique physical and biological characteristics of the product that may affect the CQAs.

The idea is to identify the matrix that is both biologically meaningful and sensitive to variations in the process. As the product advances through the different clinical development phases, our understanding of the process and the product characteristics will increase. In parallel, an understanding of the manufacturing process and test methods is expected to evolve. Manufacturers are constantly looking to simplify the process without compromising on quality and capacity with the overall aim of reducing the cost of goods. As the sequence becomes clearer, the assays will be refined and improved, and new assays will be identified to reduce both the turnaround time of these living drugs and the cost of goods to increase patient affordability.

DOI: 10.18609/cgti.2023.178

Q Where and how are you applying automation in your cell therapy processing? And how can we move further towards the automation of data analysis in cell therapy manufacture?

SB: At Intas Pharma, we have not yet delved very deeply into automation, as we are in the discovery and early clinical stages. However, the field as a whole is clearly moving towards the adoption of automation, mainly to reduce variability in the manufacturing process. This is important because of the nature of cells, especially in the autologous setting where the starting material itself is inherently variable.

There are already a few fully automated, closed, and integrated systems available on the market that can be used for manufacturing cell therapies such as CAR-T cells. These include Miltenyi's Prodigy, Lonza's Cocoon, and OMPUL by Orgenesis. The latter is a fully integrated, closed-loop, all-in-one mobile bioprocessing unit encompassing the entire GMP suite. Another example is Cytiva's Chronicle automation software, which is GMP-compliant software providing a unified digital platform to monitor cell therapy manufacturing operations and supply chain logistics. The field is moving in the right direction in terms of automation.

Q What are the keys for you in formulating and executing a successful CMC compliance strategy, particularly in light of the ever-increasing complexity of engineered cell therapy products?

SB: The key is to align with the regulatory expectations for successful CMC compliance. An ongoing conversation with regulators at each stage of the product development process is helpful to understand their perspectives, especially as the area is relatively new in India. In addition, as drug developers, we need to provide regulators with the scientific rationale to increase the awareness of these novel products and their uniqueness.

We need a back-and-forth dialog between drug developers and regulators so that everyone can be on the same page in terms of successful CMC compliance. In India, we can always take guidance from the already well-established FDA and EMA guidance.

Q Finally, can you sum up one or two key goals or priorities, both for you in your own role and for Intas Pharma as a whole, over the next 12–24 months?

SB: The major goal would be to progress a few of these cell and gene therapy programs into the clinical phases in India.

BIOGRAPHY

SHASHWATI BASAK is the Vice President and Head of the Cell and Gene Therapy Unit at Intas Pharmaceuticals (Biopharma Division), Ahmedabad, India. She is leading the development of several programs in gene and cell therapies, spanning from early-stage research to clinical stage. Prior to this, she served as the Head of Quality and Regulatory Operations at another leading Indian CGT company called Immuneel Therapeutics Ltd, focused on

developing CAR-T cell therapies. Basak has a PhD in Molecular and Cell Biology from the Indian Institute of Science, Bangalore. She did two postdoctoral fellowships from the Salk Institute for Biological Sciences and Stanford University, studying gene expression and regulation, and cancer signaling pathways. She has over 20 years of scientific leadership experience in translational research, clinical biomarkers, analytical assays, technology platforms, quality and compliance. She has worked in several Biotech and Biopharma companies, including Biocon Bristol Myers-Squibb R&D Center, Aurigene Discovery Technologies and Immuneel Therapeutics Ltd, and held positions of increasing responsibilities in varied roles.

AFFILIATION

Dr Shashwati Basak
Vice President and Head,
Cell and Gene Therapy Unit,
Intas Pharmaceuticals (Biopharma Division)

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given her approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has 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

Copyright: Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

Attribution: Copyright © 2023 Basak S. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

Article source: Invited.

Interview held: Oct 12, 2023; **Revised manuscript received**: Nov 5, 2023; **Publication date:** Nov 22, 2023.

DOI: 10.18609/cgti.2023.178

CELL & GENE THERAPY INSIGHTS

CELL THERAPY DOWNSTREAM PROCESSING & CMC

SPOTLIGHT

INTERVIEW

Key considerations in expanding global manufacture of a commercialized CAR-T cell therapy

Following the successful commercial launch of CARVYKTI®, partners Legend Biotech and Johnson & Johnson are busy expanding global production capabilities. **Sarah Snykers,** Head of Operations, Legend Biotech, took time out to talk to **David McCall,** *Cell & Gene Therapy Insights* about the ins and outs of setting up commercial CAR-T manufacturing facilities in Europe.

Cell & Gene Therapy Insights 2023; 9(10), 1435–1439

DOI: 10.18609/cgti.2023.186

Q What are you working on right now?

SS: Legend Biotech is creating a global manufacturing footprint to produce cell therapy products. Currently, in collaboration with Johnson & Johnson, the company manufactures its CAR-T cell therapy, CARVYKTI® (ciltacabtagene autoleucel; cilta-cel), in the US. Two additional facilities based in Ghent, Belgium are anticipated to come online over the next two years to add to global supply.

CARVYKTI® is a B-cell maturation antigen (BCMA)-directed, genetically modified autologous T cell immunotherapy indicated for the treatment of adult patients with relapsed or refractory multiple myeloma, after four or more prior lines of therapy including a proteasome inhibitor, an immuno-modulatory agent, and an anti-CD38 monoclonal antibody. The therapy is being evaluated in a comprehensive clinical development program across multiple settings.

In a nutshell, the patient's T cells are encoded with a CAR that can find and destroy BCMA-expressing cells. BCMA is highly expressed on the surface of malignant multiple myeloma B-lineage cells; it is also expressed on the surface of late-stage B-cells and plasma cells.

I lead the CAR-T production operations of CARVYKTI® at the two production hubs at Ghent: the brownfield facility, Obelisc, and the greenfield facility, Techlane. I support the commission and certification of the buildings and the tech transfer from the US to Belgium, including the execution of the preclinical package, such as engineering runs, comparability and stability runs, and the Process Performance Qualification. In particular, I focus on building up an agile and flexible organization that consists of people with the right mindset and a passion for healthcare.

Q What are some of the key high-level challenges and considerations in establishing commercial CAR-T cell therapy manufacturing operations in Europe?

SS: In the European Union, and even more specifically in Belgium, there is a noticeable scarcity of CAR-T companies, especially when compared to the United States. This situation impacts the availability of resources and the acquisition of talent in the region.

The production process for CAR-T is distinct when compared to small molecule drugs. It is a personalized and innovative technology that involves many open manipulations. These manipulations necessitate individuals to possess specific skills and the right mindset, which includes being aseptic, precise, agile, and having a problem-solving capability.

Specifically, for autologous cell and gene therapies/CAR-T treatments, deviation management requires quick resolution. While the process is robust, there is intrinsic variability due to the biological nature of the product. Achieving a fast or timely closure of investigations is crucial for the prompt release of the product, ensuring it's made available to patients without delay. This process demands a unique set of skills and needs experienced investigators.

For commercial production, the focus is primarily on maximizing production. To achieve this, there is a significant need for many new hires, along with timely training and requalification. Furthermore, according to Annex 1 regulation in the EU, operators must undergo aseptic requalification every six months, which takes up a considerable amount of production slots.

When it comes to commercial production and operations, maximizing the production process is key. However, a manual process has limitations when it comes to scaling up, particularly in terms of space, capacity, and resources.

Therefore, it's highly recommended that companies choose production or process technologies that are easily scalable. Additionally, when designing facilities, it's essential to anticipate potential future changes.

Q What for you are some key recent breakthroughs and opportunities in terms of reducing manufacturing timeframes?

SS: For optimal operations, it is essential to organize and train teams so that we can function like a well-oiled machine with reduced idle time. Adjustments to the production process, such as closing off certain steps or implementing closed automation, allowing us to facilitate grade C environments based on regulations. This would result in less intensive gowning, cleaning, and environmental monitoring sampling. Furthermore, efficient flows of materials and personnel are crucial for smooth operations.

...and regarding reduced Cost of Goods (COGs)?

SS: Again, resources, materials, and maintaining a facility up to the required grade are the main cost drivers. One way to improve efficiency is by adjusting the production process, such as making changes that can lead to reduced environmental monitoring and facility costs. Reducing the number of required resources can be achieved by building an efficient, well-trained organization and eliminating open manipulations in the process. As a longer-term strategy, expanding the recycling of plastic consumables can be an environmentally friendly and cost-effective measure.

...and lastly, in terms of enhancing process control?

SS: Enhancing process control can be achieved through predictive data modeling and data analytics.

Q How to optimize cell therapy fill-finish—what are the key innovation gaps there?

SS: Whilst several technologies exist to fill and finish in closed vials, the opposite is true for bags. For autologous CAR-T therapy, the number of bags per production is very limited.

Q How can we move further towards the automation of data analysis in cell therapy manufacture?

SS: It is crucial to embrace the full potential of digitalization technologies. The reliance on manual data handling methods not only introduces a risk of human error but also presents scalability challenges as the volume and complexity of data grow.

The path to automation includes the adoption of integrated digital systems, such as electronic batch records or manufacturing execution systems, which automatically capture data

in real-time across various stages of the manufacturing process. These systems facilitate end-to-end traceability and provide a robust framework for ensuring the consistency and reliability of data, which is vital for meeting the regulatory requirements in the pharma industry.

Advanced analytical techniques can be leveraged to explore and go through the extensive datasets involved, identifying anomalies and optimizing processes. Predictive modelling can become a powerful tool here, enabling anticipation and adjustment of production parameters for better outcomes.

Cloud-based solutions can significantly help in this automation journey by offering real-time data access and analysis capabilities. This ensures that decision-making is timely, informed, and can even be conducted from remote locations, enhancing monitoring and control flexibility.

However, as we integrate these digital systems, we must ensure they meet regulatory standards, such as the US FDA's 21 CFR Part 11 and the EMA's Annex 11. It is also crucial to consider the related costs and challenges, such as integrating new technologies with existing infrastructures and ensuring there is personnel skilled enough to maintain and manage these advanced systems.

In conclusion, it is important to bear in mind that while automation is key, the expertise and insights of scientists, engineers, and analysts remain indispensable. The aim of automation is not to replace the human element but to support it by freeing up time to focus on more strategic, creative, and complex tasks, driving innovation and efficiency in cell therapy production.

Q Finally, can you sum up one or two key goals or priorities, both for you in your own role and for Legend Biotech as a whole, over the next 12–24 months?

SS: In my role, I aim to achieve successful clinical and commercial production of CARVYKTI® at both production hubs. As for Legend Biotech, one of the company's goals is to establish a global clinical and commercial CAR-T production footprint to meet the increasing demand.

BIOGRAPHY

SARAH SNYKERS is Senior Director of Operations at Legend Biotech, Ghent Europe. She has 20 years of experience in cell and gene therapy. She has headed several departments in biotech companies, including manufacturing, QC, R&D and manufacturing, science, and technology; all focused on clinical or commercial production of autologous and allogeneic cell and gene therapeutic products. Over the last 15 years, she was involved in three greenfield production hubs, and in several global tech transfer projects for clinical and commercial production of cell and gene therapy.

AFFILIATION

Sarah Snykers PhD
Head of Operations,
Legend Biotech

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has 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

Copyright: Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

Attribution: Copyright © Snyker S. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

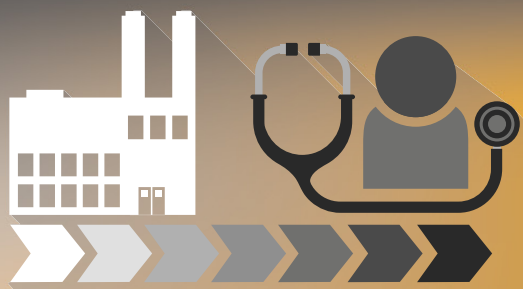
Article source: Invited.

Revised manuscript received: Nov 19, 2023; **Publication date:** Nov 30, 2023.

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN CHANNEL EDITION

Navigating the Final Mile



NOVEMBER 2023
Volume 9, Issue 10

VIEWPOINT
Navigating the final mile: how are hospitals providing capabilities for commercial cell
Alexey Bersenev

VIEWPOINT
Navigating the final mile: how are Australian hospitals delivering autologous cell and gene therapies?
Sharon Sagnella

CELL & GENE THERAPY INSIGHTS

NAVIGATING THE FINAL MILE: ENSURING THE HEALTHCARE SECTOR CAN DELIVER THE CELL AND GENE THERAPIES OF TOMORROW

CHANNEL CONTENT

VIEWPOINT

Navigating the final mile: how are hospitals providing capabilities for commercial cell therapy product delivery?

Alexey Bersenev
Director, Cell Therapy Labs, Yale New Haven Hospital, and Assistant Professor of Clinical Laboratory Medicine, Department of Laboratory Medicine, Yale University

"During the onboarding process, besides the clinical and laboratory side, financial, administrative, and contracting departments may be involved until a hospital is ready to deliver the product routinely."

VIEWPOINT

Cell & Gene Therapy Insights 2023; 9(10), 1397–1402

DOI: 10.18609/cgti.2023.181

On September 22 2023, **Abigail Pinchbeck**, Assistant Editor, *Cell & Gene Therapy Insights*, spoke to **Alexey Bersenev**, Facility Director for Cell Therapy Laboratories, Yale New Haven Hospital about how hospitals, including Yale New Haven, are accommodating current commercial cell therapy products whilst preparing for the future of delivery to patients. This Viewpoint article is based on that interview.

INTRODUCTION

As Facility Director of Yale New Haven Hospital, which is affiliated with Yale University, Alexey Bersenev's role is to take care of daily facility operations. Two labs exist within one facility, for cell therapy processing and advanced cell therapy. The cell processing lab deals with standard of care and commercial cell therapies whilst the advanced cell therapy lab takes care of all investigational cell therapy products.

OUTLINING THE FINAL MILE OF DELIVERY FOR CGT PRODUCTS

The final mile describes the distribution of industry-manufactured products from the central manufacturing plant into hospitals where the products are administered to patients. There are multiple departments involved with the final mile within any hospital. During the onboarding process, besides the clinical and laboratory side, financial, administrative, and contracting departments may be involved until a hospital is ready to deliver the product routinely.

Every stem cell transplant program consists of three major components: the patient bedside, the collection facility (usually apheresis), and the cell processing facility. Besides these three major components, the intensive care unit may be involved in managing potential adverse reactions. Coordinators are not involved directly in patient care, but in coordinating, scheduling, and placing orders. The hospital pharmacy is involved in product traceability through pharmacy label generation and product verification. Commercial cell therapy products are labeled as 'Rx', or prescribed medicine, meaning they must go through the pharmacy inventory.

On the cell processing side, handling involves receiving apheresis collection or procuring starting material, packing this material, and shipment out to the manufacturer. When the product has been manufactured in the central manufacturing plant, the product is returned to the cell processing facilities. When this is received, chain of identity and chain of custody is completed and documented. Typically, these products would be transferred into cryogenic liquid nitrogen freezers for storage before infusion.

When the patient is ready, a notification is received from the coordinator, and a doctor releases the order for product administration. The release from storage is authorized, and then the product is taken from the lab to the infusion bedside in an infusion cart. Yale New Haven Hospital procedure is to thaw at the bedside, where chain of identity/chain of custody verification is once again performed. Other centers prefer to thaw in the lab. The nurse infusionist will infuse the product. If adverse reactions occur and the product cannot be infused, the product can theoretically be returned to storage for later use or discarded.

EVOLVING SUPPLY CHAIN CAPABILITIES FOR CGT PRODUCTS

One challenge for hospitals is that although manufacturers of commercial products view hospitals as the initiators of the GMP supply chain, stem cell processing labs historically were not compliant with GMP regulations. Only good tissue practice is required in the US, which differs from GMP in a few small ways. This can be challenging for stem cell processing labs because as part of the onboarding process, an audit may unveil that

some processes are not being compliant with GMP. Hospitals may require some investment and time to become fully compliant. Another requirement in the US is for facilities and hospitals that collect, receive, administer, and store commercial cell therapy products to be Foundation for the Accreditation of Cellular Therapy (FACT)-accredited.

For some CAR-T cell commercial products, including Kymriah, there is the additional challenge of cryopreserving starting material, which is a lengthy procedure, but routine for cell processing labs supporting stem cell transplant programs. Commercial CAR-T cells are a new cell type for these labs.

Due to the nature of autologous commercial cell therapy products, the hospital acts as the supplier of starting material for commercial manufacturers, which is highly unusual. Historically, hospitals have never been the supplier to big pharma or biotech. The paradigm is changing, which is challenging and necessitates investment and time. Some hospitals may not have the resources to facilitate these changes and adopt wide use of commercial cell therapy products.

ACCOMMODATING COMMERCIAL CAR-T PRODUCTS IN HOSPITALS

The onboarding process can pose many challenges for hospitals. When Yale New Haven Hospital first started delivering CAR-T cell therapies in 2019, there were roughly a few hundred people from many different departments involved in the processes from start to finish, so completing training took time and certification took almost a year. If a hospital is not certified in the US, the hospital cannot administer this product to the patients. Coordinating certification cross-departmentally can be difficult. The US FDA also requires risk evaluation mitigation strategy training for anyone related to product handling and patient care.

There are further activities on the contracting side, with agreements negotiated between the hospital and the manufacturer. The manufacturer can audit several departments and any findings must be addressed. Audit findings or ongoing changes in commercial product manufacturer's procedures require changes to the processing lab's standard operating procedures, which must be approved before staff undergo retraining. With multiple commercial CAR-T products, this can be hard to keep up with.

Back in 2018, the onboarding process took almost one year as standard. Since then, hospitals have generally accelerated after introducing the first two products, Kymriah and Yescarta. The onboarding process now takes around 3–6 months depending on the product.

In the future, fewer people will require training due to similarities between the products and training will be faster due to increased familiarity. Once the first or second certification has been achieved, other certifications will become quicker and easier to obtain. Despite this, some hospitals simply may not have the resources to certify for multiple commercial cell therapies. The major resources required are twofold: the qualified personnel and the physical constraints, such as the number of available beds or devices (apheresis machines, cryogenic storage freezers, etc.).

As a greater variety of commercial cell products are introduced to the market, hospitals may become more selective about certification. For example, there are currently three CAR-T products for adult diffuse large B-cell lymphoma: Kymriah, Yescarta, and Breyanzi. Kymriah and Yescarta came on the market first, but Bristol Myres Squibb still decided to go forward with Breyanzi as they were confident that it would become the best in class. Now, in some hospitals, prescriptions for Kymriah and Yescarta for adult lymphoma are decreasing, whilst Breyanzi prescriptions are going up. If a hospital wants to become certified now, they may simply choose to only certify for Breyanzi, rather than all three.

STANDARDIZATION OF THE FINAL MILE

Standardization is the million-dollar issue in the field currently. Hospitals are beginning to show difficulties in keeping up with new products, manufacturer requirements, and audits especially due to a lack of qualified personnel, the physical constraints of facility size, and overall capacity. Standardization would make wide clinical adoption of commercial cell therapies easier, but the conversation between involved parties is still in its infancy. Questions have been raised in the recent annual meeting of the International Society for Cell & Gene Therapy and the American Society for Transplantation and Cellular Therapy about how to standardize workflows across multiple cell therapy products. The field is at the stage of having these conversations and publishing proposals. The next stage is to talk to manufacturers and discuss hospital needs. The next actor to ask would be the regulators and accrediting organizations, who should agree on specific proposals and make standardization possible.

It is challenging to identify what exactly could be standardized. One area of possible standardization could be labeling and product handling. In cell therapy, labeling is generally unified to fit ISBT 128 standards, a well-established standard for blood and cell therapy products. However, commercial manufacturers of cell products may not be fully compliant with ISBT 128 labeling. Another area to standardize could be product delivery and storage. For example, it could be proposed that all products must be stored in liquid nitrogen vapor, with a standardized storage temperature. Most commercial products require a storage temperature below –120 °C or –130 °C, although some require below –150 °C. A standard storage requirement of below –120 °C could be proposed across the industry. Other potential areas of standardization could include packaging and shipping, storage devices, and whether to thaw the product at the bedside or in the lab. These may seem like small individual changes, but with many various factors standardized, the time and cost savings would be exponential.

THE FUTURE OF THE FINAL MILE

Right now, Yale New Haven Hospital is certified for six commercial cell therapy products. By the beginning of next year, this number may include two more, possibly involving treatment for solid tumors. Possible approvals coming shortly in the US include Iovance Biotherapeutics' lifileucel for melanoma and Adaptimmune's Afami-cel for advanced synovial sarcoma. Products may also be approved in benign hematology for sickle cell disease by Vertex Pharma and CRISPR Therapeutics. A decade from now, there may be 50 or 100 products on the US market.

It is unlikely that stem cell processing labs will be able to keep up with a large continuing increase in new commercial cell therapy products coming to the market. One solution is that instead of cell therapy or stem cell processing labs handling these products, hospital pharmacies could do so instead. Another solution could be that some of the high-complexity products could still be handled by cell therapy labs, with less complex products handled by the hospital pharmacy. To handle commercial cell therapy products, pharmacies will need to train staff and build the infrastructure to maintain the products, such as liquid nitrogen freezers and supply tanks.

Currently, about 85% of US hospitals utilize stem cell processing labs [1] to handle commercial cell therapy products. Some hospitals have already started to create a separate unit called 'cell pharmacy', specifically designed to handle commercial cell therapy products. Successful cases include Stanford University Hospital in California. At Yale New Haven Hospital, there is not yet a separate cell pharmacy unit, but cell pharmacy terminology is used throughout the standard operating procedures.

It is important to note that the delivery of commercial cell therapy products in hospitals could differ in different regulatory jurisdictions. In some European countries, pharmacists may be required to handle these products. For example, at one of the past International Society for Cell & Gene Therapy annual meetings, a pharmacist from the Netherlands spoke about how pharmacists were trained to process cells for Kymriah manufacturing. As it is a prescribed medicine labeled SRx, regulation requires it to go through a pharmacy.

In the future, when these therapies become safer and more numerous, besides large academic medical centers, community and mid-size hospitals will become involved. Community hospitals do not have FACT-accredited facilities or established stem cell transplant programs. At some point, the field will likely need to move beyond FACT-accredited programs as cell therapies become safer, less toxic, and easier to deliver and administer in an outpatient setting. It likely will happen when commercial cell therapies expand outside the use of immune-engineered cells in oncology into autoimmune diseases or regenerative medicine applications. For example in musculoskeletal diseases, administration will become a minimally invasive procedure that a trauma surgeon can perform locally. For this, there would be no reason to require FACT accreditation.

REFERENCE

1. Bersenev A, Gustafson MP, Hanley PJ. ISCT survey on hospital practices to support externally manufactured investigational cell-gene therapy products. *Cytotherapy* 2022; 24(1), 27–31.

BIOGRAPHY

ALEXEY BERSENEV received his medical education and certification as a general surgeon in Russia. He holds a PhD in Transplantation/Pathology. He gained expertise in immunology, hematology, stem cell biology and published scientific papers during post-doctoral training in the US in Philadelphia at the Thomas Jefferson University and the Children's Hospital of Philadelphia. He worked as a cell manufacturing specialist at the University of Pennsylvania and trained in clinical cell processing in a GMP cell manufacturing facility and was involved in the manufacture of CAR T-cell products for clinical trials and technology transfer to industry. He has expertise in clinical manufacturing of cellular products for clinical trials, including product and process development, cell processing and culture, operations of academic GMP facility and compliance with regulations. In addition to his position as Director of the Advanced Cell Therapy Lab at Yale-New Haven Hospital, he is an Assistant Professor of Clinical Laboratory Medicine at the Department of Laboratory Medicine at Yale University.

AFFILIATION

Alexey Bersenev PhD
Director,
Cell Therapy Labs,
Yale-New Haven Hospital; and
Assistant Professor of Clinical Laboratory Medicine,
Department of Laboratory Medicine,
Yale University

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has 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

Copyright: Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

Attribution: Copyright © 2023 Bersenev A. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

Article source: Invited.

Revised manuscript received: Nov 2, 2023; **Publication date:** Nov 29, 2023.

CELL & GENE THERAPY INSIGHTS

NAVIGATING THE FINAL MILE: HOW TO ENSURE THE HEALTHCARE SECTOR ARE PREPARED TO DELIVER THE COMMERCIAL CELL AND GENE THERAPIES OF TOMORROW TO PATIENTS?

CHANNEL CONTENT

VIEWPOINT

Navigating the final mile: how are Australian hospitals delivering autologous cell & gene therapies?

Sharon Sagnella
Research and Development Manager, Department of Cell and Molecular Therapies, Royal Prince Alfred Hospital

"Differences in software and quality control tests can be tedious. Standardization can help at every step of the process."

VIEWPOINT

Cell & Gene Therapy Insights 2023; 9(10), 1335–1340

DOI: 10.18609/cgti.2023.171

On October 9 2023, **David McCall**, Senior Editor, *Cell & Gene Therapy Insights*, spoke to **Sharon Sagnella**, Research and Development Manager, Department of Cell and Molecular Therapies, Royal Prince Alfred Hospital, about overcoming the final mile to deliver autologous therapies to patients in Australia. They discuss the challenges hospitals in Australia face, and how they are developing the personalized supply chain capabilities to overcome them. This article is based on that interview.

BACKGROUND

Royal Prince Alfred Hospital (RPAH) is one of Australia's oldest tertiary referral public health care institutions. The Department of Cell and Molecular Therapies (CMT) at RPAH is responsible for handling any cell and gene therapies (CGT) delivered to clinical patients. This may involve storage and infusion of products, complex formulations, and cell manipulation. At present, CMT is servicing 12 active CGT clinical trials with around 11 new CGT trials in the pipeline that are earmarked to begin within the next 12 months. Indications span a number of different clinical departments including hematology, neurology, oncology, and cardiology. CMT comprises four GMP cleanroom suites, a production team, a quality team, a clinical trials team, an operations and administrative team, and a research team for process development and translational projects (approximately 20 staff members). In addition to servicing clinical trials, the department is responsible for the delivery of Yescarta and Kymriah, the two approved and funded CAR-T therapies in Australia. Furthermore, CMT provides clinical CGT manufacturing capabilities to clinicians, academics, and industry partners.

CAR-T therapies are available at six different locations in Australia including RPAH, Royal Brisbane Hospital, Sydney Children's Hospital, Westmead and the Children's Hospital at Westmead, Peter MacCallum Cancer Centre, and the Royal Children's Hospital. In addition to Kymriah and Yescarta, Tecartus and Carvykti are Therapeutic Goods Administration (TGA)-approved.

THE FINAL MILE IN HOSPITAL SETTINGS

A fastidious approach and planning are necessary for the implementation of CGT in a hospital setting due to the complexities of delivering this type of specialist service. This approach must be multi-disciplinary with dedicated funding to meet clinical, scientific, logistical, and regulatory requirements. Hospitals require specialist cell handling capabilities including proper infrastructure and trained staff to meet the necessary regulatory requirements. An accredited apheresis unit is an essential component for the delivery of a range of cellular therapies. Furthermore, clinical expertise spanning a range of specialties with direct experience in managing patients receiving CGT is also required.

CMT has been a pioneer in the clinical implementation of CGT having been involved in the first gene therapy clinical trial in 2001 for the treatment of hemophilia which utilized AAV delivery directly to the liver. Building of the CMT cleanroom facility at RPAH, a vital piece of infrastructure for the future service delivery of CGT, was completed in 2012. RPAH was approved for the provision of CAR-T cell therapy service in 2019 and the last three years have seen a large uptick in service delivery due to the steady increase in CGT clinical trials.

CMT in consultation with RPAH Pharmacy, RPAH institutional biosafety committee, and RPAH research and governance office has a set of guidance documents provided to RPAH clinical departments on how to implement CGT in the clinic. The first stage of that process is consultation with CMT to ensure that the necessary

infrastructure and trained staff are available to feasibly service the clinical trial. This involves a number of considerations including the number of patients expected to be on a trial, the cold storage requirements, formulation requirements, additional onsite manufacturing requirements, etc., thereby ensuring the resources, including staff, are available to handle the trial. This process has become more essential in recent years due to the large uptick in the number of CGT clinical trials in the pipeline worldwide.

Once a trial is considered feasible, it must go through regulatory approval. In Australia, if the trial involves a genetically modified organism (GMO), the sponsor or commercial entity must establish if they require a GMO license from the Office of the Gene Technology Regulator. Institutional Biosafety Committee applications may be required through the specific site in addition to human research ethics approvals before a site-specific application can be submitted to obtain final research governance approval to conduct the clinical trial at RPAH.

Staff involved in the trials must complete the required training in investigational product handling and storage conditions. Chain of custody must be maintained from the product's receipt on site until it goes into the patient. This process involves a great deal of logistics, communication, and adequate staffing. Additionally, extensive documentation is required to demonstrate all processes and procedures have adhered to the product handling specifications and that chain of custody has been properly maintained throughout the delivery of the CGT.

CHALLENGES FOR AUSTRALIAN HOSPITALS IN DELIVERING CGT

Currently, one of the major issues the CGT field is facing is the trained workforce gap. Public hospitals are limited in staffing numbers, and the CGT pipeline is ever-increasing. In Australia, and around the world, there is a small pool and pipeline of experienced staff who are able to do the work, including experienced clinical staff who understand the clinical management side of CAR-T therapies and the challenges that come with delivering those. This is in addition to the lack of trained technical staff responsible for handling, manipulation, and formulation of CGT under GMP-compliant processes.

Three years since service delivery of Kymriah began in 2020, the clinical hematologists at RPAH have now treated more than 80 patients and are well experienced. In other parts of Australia, that experience is limited. A lack of trained, experienced workforce poses challenges to every aspect of the logistics pipeline. There are no training programs in universities for training the CGT workforce at present; most training happens through well-developed in-house training programs. Furthermore, the management of CAR-T and other CGT by clinical staff is not necessarily something that would be covered through their basic training.

Chain of custody of the product also poses challenges, as each sponsor has their own software or system. Some of the smaller ones still remain paper-based. Every time a new trial is taken on, staff require training for each new product and the procedures are never the same from one trial to the next. By next year, RPAH is expecting to have two dozen trials ongoing, and each of the seven production staff will have to be trained in all processes involving receipt, storage, processing, and formulation for infusion.

Yet another challenge in Australia is the distances existing across a big country. There are a limited number of places that can realistically deliver these types of therapies to patients. Reaching the patients living in more rural and remote places to deliver therapies is a specific challenge within Australia.

Other challenges in Australia in CAR-T and other CGTs are based on a reliance on offshore manufacturing. All commercial therapies are currently manufactured offshore, and there is limited availability of manufacturing infrastructure for CAR-Ts

or cellular therapies in Australia. There are a handful of smaller facilities, including RPAH, and a couple of larger ones, but capacity remains a challenge. Another challenge is the high costs of CAR-T therapies and other CGTs and how to best be able to provide these through a public health system. The federal government must find pathways to fund these into the future. For example, while there are four TGA-approved CAR-T cell therapies in Australia, currently, only two are funded. As a way to manage the high cost of these therapies and ensure the right patients receive them, a national steering committee meets on a regular basis involving clinicians from CGT-qualified centers. If a clinician wants to place a patient on one of these therapies, it must be discussed and decided by a multidisciplinary team across the nation.

As the CAR-T field moves towards solid tumors, the issue of funding in Australia will become more pertinent. A hemophilia B gene therapy has recently been approved by the TGA, with a price tag of approximately 5 million AUD. The Australian government needs to have discussions and make some tough decisions about funding these types of therapies going forward. Hopefully, they will become cheaper, but they will always be more expensive than a small molecule or biologic.

CGT REGULATION IN AUSTRALIA

Australia regulates gene-based therapies via the Gene Technology Act 2000 and the Therapeutics Goods Act 1989. The Australian regulatory system is unique in that it has a centralized regulator–the Office of the Gene Technology Regulator which strictly regulates GMOs in Australia. Cellular therapies in Australia are regulated as biologics, while gene therapies are regulated as prescription medicines by the TGA. Biologics can be classified as class 1–4, with CAR-Ts classified as 'high risk' class 4 biologics. TGA approval for conducting clinical trials in Australia can occur via two different pathways: clinical trial notification and clinical trial approval. Clinical trial notification is used for therapies that have already been approved by a different regulatory body, such as the US FDA or EMA. This is simply a notification to document that a product is going to be used in a trial. The clinical trial approval pathway is a more complex full application process that is needed if using a therapy that has not previously been used. This is similar to the IND in the US. Approval of a product for commercial use requires an extensive review process by the TGA to assess safety, quality, and efficacy.

DEVELOPING SUPPLY CHAIN CAPABILITIES TO HANDLE AUTOLOGOUS CGT PRODUCTS

Access to GMP vectors in Australia can pose difficulties. There are no companies within the Australia and New Zealand region at present that can produce GMP vector, with the closest being in Singapore. GMP vector is in high demand, and not having it within the region has been a barrier to much development. Hopefully, next year GMP manufacturing will be available in New Zealand. In addition, a vector manufacturing facility is being built at Westmead but is still awaiting its GMP license.

As the move towards more onshore manufacturing takes place, the question of booking spots in manufacturing will be raised. A few centers have GMP licenses for CAR-T manufacturing already. Having centers work well in conjunction with each other would be ideal, but unfortunately, a lot of state-to-state competition is seen in the country, creating unnecessary barriers.

As only six hospitals in Australia have been approved and qualified for the delivery of commercial CAR-T cell therapies, there is a large portion of Australia that must travel long distances for access. The logistical challenges posed by moving these therapies around are huge. Moving towards a hub and spoke model with other hospitals could help

address some of these challenges, however, more trained technical and clinical staff will then be required.

Another change is a push towards point-of-care manufacturing, which many hospitals are looking into. This could be beneficial, but it may take a while before the field is ready. In addition, there are continual technological advancements in the manufacturing processes and software that will bring down the cost of these therapies.

The COVID-19 pandemic highlighted many difficulties in Australia's reliance on overseas. A lack of production onshore means an increased frequency of supply chain issues, such as from the standpoint of getting technicians into service facility equipment or replacing equipment parts as well as access to necessary consumables. Due to Australia's location, it has the unique challenge of supply chain vulnerability.

LOOKING TOWARDS THE FUTURE OF THE FINAL MILE

It is hoped that processes and procedures can be standardized along the logistics chain in the future to ease implementation. Differences in software and quality control tests can be tedious. Standardization can help at every step of the process.

In CGT, there will likely be a move towards more allogeneic therapies, with less reliance on autologous. Allogeneic is still in its early stages, but this would remove some of the logistical steps, including apheresis, and allow us to move more quickly. It is unlikely that autologous therapies will entirely disappear, but a reduction in reliance on them is likely.

In the CAR-T therapies, other immune effector cell types continue to be explored, including natural killer cells and γδ T cells. There is a huge range of different types of immune effector cells, each with various benefits. These could be more allogeneic, in that they can be created in batches without the requirement for manufacturing slots for each patient.

A recent push within New South Wales to standardize the hospital pharmacy requirements has occurred. They are beginning to put out guidance for hospital pharmacies for handling and delivering CGT products to the clinic. This is not yet Australia-wide, but it is a step in the right direction.

More guidance from the people on the ground is needed. Dr Sharon Sagnella and Professor John Rasko participate in a number of steering committees relating to CGT implementation at RPAH. In a decade, the field will hopefully have identified ways to improve access in Australia to rural and remote communities, which is one of the biggest challenges. Ensuring equitable access to these therapies across Australia, including many remote Indigenous communities, remains a priority.

BIOGRAPHY

SHARON SAGNELLA is the Research and Development Manager for the Department of Cell and Molecular Therapies at Royal Prince Alfred Hospital, Sydney, Australia. After completing a PhD in Biomedical Engineering at Case Western Reserve University, Cleveland, Ohio, she gained extensive experience in process development and commercialisation during time spent in CSIRO and industry. Throughout her career, she has contributed extensively to preclinical development, clinical trials, and the clinical implementation of new therapies.

AFFILIATION

Sharon Sagnella PhD
Research and Development Manager,
Department of Cell and Molecular Therapies,
Royal Prince Alfred Hospital

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given her approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has 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

Copyright: Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

Attribution: Copyright © Sagnella S. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

Article source: Invited; externally peer reviewed.

Revised manuscript received: Oct 27, 2023; **Publication date:** Nov 10, 2023.

NOVEMBER 2023
Volume 9, Issue 10

CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES

COMMENTARY
Building rings with spheres: a cell therapy approach to incontinence
Martha Gilbert, Simona Čaputová, Delielena Poli, Manou Kooy, Richard M Day, Georgia Sturt, and Josephine Parker

FAST FACTS
Cell culture in a chemically defined environment
Chengkang Zhang

FAST FACTS
Case study: media optimization control strategies
Alex Sargent

FAST FACTS
Reliable detection reagents in CAR-T/NK cell flow cytometry
Annika Graband

FAST FACTS
HEK293 suspension culture in stirred 3D-systems for gene therapy development
Jorge Escobar

WEBINAR DIGEST
Three distinct analytical ultracentrifugation methods for virus and viral vector characterizations
Susumu Uchiyama

CELL & GENE THERAPY INSIGHTS

COMMENTARY

Building rings with spheres: a cell therapy approach to incontinence

Martha Gilbert, Simona Čaputová, Delielena Poli, Manou Kooy, Georgia Sturt, Josephine Parker and Richard M Day

Fecal incontinence is a prevalent condition, that remains vastly underreported. The condition impacts the patients' quality of life and has negative socio-economic and environmental impact on the society. Current patient management guidelines recommend a stepwise approach to treating fecal incontinence, from conservative treatment options, through minimally invasive surgical options, all the way to first- and second-line surgical options. Unfortunately, the conservative treatments remain ineffective, and, in many cases, the surgical options are either not desirable or not suitable. Regenerative medicine, and specifically, cell therapy, has the potential to offer a curative treatment that is less invasive, more effective and efficient. Cell therapy technologies, while still under development, can improve the current state-of-play in the realm of fecal incontinence at the clinical, patient, and socio-economic level. The aim of this article is twofold. Firstly, it is to raise awareness about the silent affliction that fecal incontinence is and about the impact that it has on patients and society. Secondly, it is to position cell therapy, relative to the current treatment approaches, including, for example, sacral nerve stimulation and sphincteroplasty, as to emphasize its potential to provide a suitable treatment alternative.

Cell & Gene Therapy Insights 2023; 9(10), 1293–1306

DOI: 10.18609/cgti.2023.167

THE PROBLEM: FECAL INCONTINENCE IS A PREVALENT CONDITION THAT IS CURRENTLY LACKING APPROPRIATE TREATMENT OPTIONS

Fecal incontinence: a brief introduction to the condition

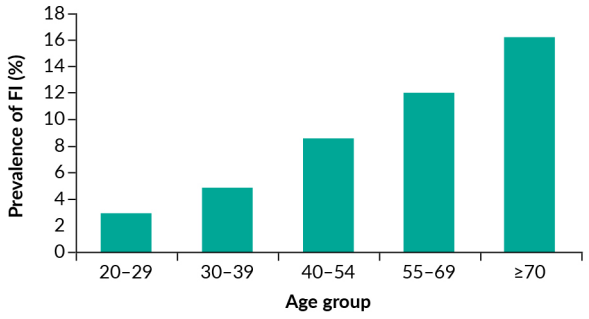
Fecal incontinence (FI) is a condition in which control of bowel movement is impaired, leading to leakage of feces. According to the symptomatic profile, FI is classified in three categories [1]:

1. Passive incontinence;
2. Urge incontinence;
3. Fecal seepage.

In case of passive incontinence, patients are unaware of the discharge that involuntarily arises due to overflow of the full rectum [2]. Passive incontinence indicates the malfunctioning of the anal sphincter, anorectal reflexes, or a neurological disease. As opposed to passive incontinence, urge incontinence occurs when patients consciously defecate while being unable to control sudden bowel movements [3]. Urge incontinence suggests impairment of the anal sphincter or the rectum to prevent discharge. Lastly, fecal seepage is the complication of involuntary discharge after the occurrence of normal continence and bowel movement [4]. The disruption of the anal organs, notably to the sphincter muscle that creates a ring structure around the anal canal, can be structural or functional, respectively meaning that FI has occurred after injury, trauma, or childbirth, or naturally due to neurological disorders [1].

FIGURE 1

Overall prevalence of FI by age group analysed by the US National Health and Nutrition Examination survey (US data) [7].



While underreported, FI is a prevalent condition, most common in parous women, frail older patients and patients with neurological disorders

Although an estimation of European adults affected by FI is recognized, its precise and worldwide prevalence is unknown, as the lack of patients reporting their FI-related symptoms and diagnosis discrepant symptoms thus leads to a potentially underestimated prevalence of patients suffering from FI [5]. Despite lacking precise data, it is estimated that in Europe approximately 57 million adults are affected with FI [6]. Specifically, FI occurs in up to 15% of the Western population. Both men and women of all ages worldwide are affected by FI (Figure 1), and although FI in men has received little attention in the past, it is still as much of a problem in men as it is in women [7].

Groups with the highest incidence rates of FI are parous women with sphincter muscle damage or dysfunction, frail older patients, and patients with neurological or spinal disease/injury. These groups are described below, respectively.

Parous women may suffer from sphincter injury as a result of pregnancy and childbirth

It has been reported that 11% of postpartum women globally have sustained sphincter muscle injury [8]. While the prevalence is primarily studied in high-income countries [9], it is apparent that women from low- and middle-income countries (LMICs) are at a high risk of developing FI, too. Specifically,

DOI: 10.18609/cgti.2023.167

women that develop uncommon communication between the gastrointestinal tract, the urinary tract and/or the genital tract, so-called obstetric fistulas (OF), during labor are at a higher risk of developing FI [9,10]. The majority of these OF cases are in sub-Saharan Africa and South Asia, followed by Latin America and the Caribbean [11]. Those affected by OF in low- and middle-income countries (LMICs) are young, primiparous, impoverished women that have little, or no access to health care [11]. Pregnancy and childbirth are risk factors for transient postpartum FI. Studies have shown that during pregnancy and childbirth, women encounter issues such as pelvic floor injury and stretching or tearing of the nerves, muscles, and supporting tissues [12]. Vacuum or forceps-assisted vaginal delivery is seen as a risk factor for developing FI as these methods increase the risk of anal sphincter ruptures [13]. There are studies that suggest that caesarean section protects against developing sphincter injury, specifically fecal incontinence beyond the postpartum period [14]. Of the 11% of women with sphincter muscle injury, between one-third and two-thirds will suffer from FI [8]. To illustrate the impact of this, of the 4.09 million women who gave birth in Europe in 2021 [15], 449,900 are likely to have a sphincter muscle injury, of which 150,000 to 300,000 will be affected by FI. The type of birth and delivery method also plays a role in the prevalence of FI in parous women [13]. In some cases, postpartum FI is only temporary and neuromuscular injury sometimes improves during the first year after giving birth [13], while in others the condition gets progressively worse due to the confounding effect of aging and the menopause.

Frail older patients have a high risk of bowel disturbances

FI in older patients can be a challenging and stigmatizing condition to deal with alone. Patients often do not seek help for their condition. The main risk factors for FI in the elderly include bowel disturbances such as diarrhea and irritable bowel syndrome (IBS) [4]. Bowel disturbances are more amenable to therapeutic intervention as they are often easier to correct than neuromuscular injuries to the pelvic floor [4]. Several causes have been reported for the onset of FI in frail older patients, of which examples include living a sedentary lifestyle or having a decreased fiber intake. No differences between sexes have been analyzed in older patients, as opposed to lower age groups that suffer from FI [16]. There are certain comorbidities associated with FI, such as diabetes mellitus, stroke, and neurological disorders [4]. Prevalence rates of FI increases in patients over the age of 50, among hospitalized patients and in patients that are institutionalized. According to a systematic review of older patients in care homes in Europe, approximately 50% of older people living in care homes are affected by FI, compared to an estimated 18% of the general population [17].

Patients with neurological injury or disorder suffer from disruption of nerves that control storage and excretion of waste

Other examples of patients suffering from FI include those with neurological disorders, metabolic disorders or other types of disorders that affect the functioning of the sphincter muscle. Individuals with a neurological disease have a higher risk of FI than the general population [18]. The nervous system controls storage and excretion of fecal waste, hence the disruption to the nerves enhances the likelihood to develop FI [19]. For example, the incidence of FI is higher in patients with multiple sclerosis, spinal cord injuries, cerebrovascular diseases, and Alzheimer's disease [19,20]. Patients with dementia are prone to FI due to use of medication, dietary intolerance, or the decreased cortical control over stool release [21].

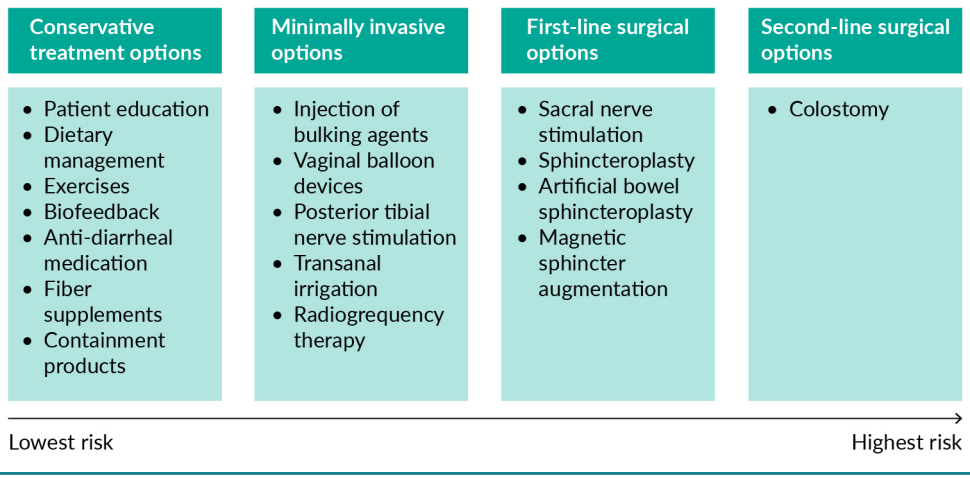
Current treatment options for treating FI are often either not effective, or not desirable and/or suitable

Current patient management guidelines recommend a stepwise approach to treating FI—from conservative treatment options, through minimally invasive surgical options, all the way to first- and second-line surgical options (Figure 2). Conservative treatments designed to minimize symptoms are typically used in first-line therapy, especially in those with mild symptoms. Such treatment options include dietary modifications, patient education, bowel management exercises, biofeedback and anti-diarrheal or antimotility medication [22]. If conservative treatments do not have the desired effect (estimated to fail in 40–75% of the cases), patients will be treated with minimally invasive options including injections of bulking agents, balloon devices, posterior tibial nerve stimulation, transanal irrigation and radiofrequency therapy [6,23]. In case of ineffective treatment by means of non-surgical options, first-line and second-line surgical options are proposed. In first-line surgical treatment, the sacral nerve is stimulated or sphincteroplasty is performed to strengthen weakened muscle areas [24]. Of the patients recommended for first-line surgical treatment, approximately 80–90% receive SNS and 10–20% undergo sphincteroplasty. The success rate of these treatment options is approximately 60% and 80%, respectively, with potential declining effect over time [25,26]. The efficiency of sphincteroplasty can be enhanced by magnetic sphincter augmentation [27]. The last resort is second-line surgical treatment, to create a colostomy, where stool into a collection bag is diverted through an opening in the abdomen.

The effectiveness of different treatment options for FI depends on the severity of the condition and the patient group. The treatment considerations differ for the three patient groups: parous women, frail older patients, and patients with neurological disorders. The most suitable stepwise approach for parous women is to first attempt conservative treatment (electrical stimulation of the pelvic floor muscles, physiotherapy, dietary management, etc.), and then minimally invasive and first-line surgical treatments (SNS). In frailty, patients are prescribed dietary modification or referred for the minimally invasive and first-line surgical treatments (SNS). For patients with neurological disorders, treatment depends on the symptoms but most often starts with conservative treatment options, such as bowel

FIGURE 2

Stepwise treatment approach for patients with FI.



DOI: 10.18609/cgti.2023.167

management programs. Unfortunately, conservative treatment options are generally ineffective and the patient undergoes surgical treatment. However, many patients do not wish to, or are unsuitable for undergoing surgery.

FI impacts patients' quality of life and has socio-economic and environmental implications on the society

FI poses emotional & mental stress on patients' lives

FI heavily affects the quality of life (QoL) of affected individuals. Lifestyle, social interaction, coping behavior, depression or self-perception, and level of embarrassment are aspects of the QoL of FI patients that are influenced by several FI severity factors (i.e., frequency of soiling, quantity and type of fecal loss, and urgency) to different degrees [28]. Due to the associated social stigma of FI, it is often deemed as the 'silent affliction' [29] or the 'unvoiced symptom' [30]. Topics that breach social norms about bodily functions are often regarded as something that should be discussed in private. The fact that patients are aware of the public stigma makes them develop self-stigma, i.e., they internalize the public's negative reactions and interpret the stereotypes as true and accurate. This can lead to the avoidance of help-seeking [31]. Those with mild symptoms may be unwilling to realize that they are experiencing FI symptoms; and those that eventually come to terms with a diagnosis are reluctant to share it with others and seek further help from health care professionals [32]. Approximately 70% of patients with FI do not reach out for medical help [28]. Moreover, FI can lead to social isolation and has an impact on intimate relationships and self-esteem [33]. For example, despite the partners and spouses being generally supportive of their partners' diagnosis, they have also reported avoiding intimate and sexual activities with the affected individual [33].

FI imposes significant costs both for patients and society

Patients suffering from FI have substantial medical costs. Firstly, this includes expenditure for incontinence products, medications, and other healthcare products [34]; secondly, costs are incurred due to greater frequency of health care practitioner visits, which includes costs of transportation, costs of the consultations [33]. Patients with FI have on average 4.21 more healthcare visits per year than patients without FI [35]. Moreover, FI patients need support in their day-to-day activities, in particular, frail older patients that need nursing support. While more recent statistics are not available, in 2012, the average annual cost per person was €4,110, including direct medical and non-medical costs and indirect costs for productivity loss [36]. This causes an overall economic burden, as the money could otherwise be invested elsewhere [35]. Regarding the financial impact to society, affected individuals become less active through increased days off, loss of productivity, and higher rates of unemployment and absenteeism [37]. Considering the EU as the relevant population for this article, patients with large-volume FI report missing an average of 50 days from work or school annually, relative to those individuals without FI symptoms [37].

FI imposes environmental costs due to the increasing use of medication and products

Patients with FI use a wide range of medications and hygienic products. In Europe, the contamination of groundwater is enhanced due to the increasing use of medication [38], with anti-diarrheal medication reportedly being found in groundwater [39]. With the rising need for incontinence products (e.g., pads or diapers), the energy consumption and carbon emissions increase [40]. Similarly, as the products often contain non-biodegradable material, the environmental pollution increases too [41].

Overall, without effective treatments, parous women, frail older patients and patients with neurological disorders suffering from FI will continue experiencing a decreased QoL and the condition has significant economic and environmental impacts on society.

THE SOLUTION: A CURATIVE TREATMENT DECREASING THE BURDEN ON PATIENTS' LIVES & ON SOCIETY

Conservative treatment options are generally ineffective and patients are often referred to undergo different kinds of surgical treatments. While surgical sphincter repair is the most successful improvement of continence, it does not always persist in the long-term [42]. Hence, regenerative medicine approaches have been under investigation as a novel alternative approach due to their success in the treatment of other indications (e.g., hematological, cardiovascular, neurological, digestive, traumatic, endocrine, renal, and metabolic conditions) [42].

Regenerative medicine products

Regenerative medicine aims to restore tissue that is impaired due to injury, aging or disease [43]. Treating fecal incontinence with regenerative medicine is at its infancy, there is a lot more within the field to be explored and developed. While the development of regenerative medicine in the relatively new realm of FI is rather fast, it is still lagging relative to other indications [43]. Among else, this is due to the stigma associated with FI, resulting in patients' reluctance to openly discuss the condition [31]. Consequently, the potential of patient recruitment for clinical trials is limited. Moreover, in the context of sphincter defects, it remains difficult to understand the choice of suitable biomaterial, the cell behavior following implantation and other technological aspects [43]. The most common approaches in the field of regenerative medicine include injection of biomaterials, tissue engineering, cell therapy, and a combination of the therapies [43]. The focus of this article has been narrowed down to, specifically, cell therapy, due to the vast potential that the approach shows in treating FI. While cell therapies for treating FI are still under development, the plethora of ongoing studies shows a clear positive signal regarding their potential as an alternative FI treatment. While the rest of the article focuses specifically on cell therapy, for the sake of completeness, the section below presents an overview of the four different regenerative medicine approaches.

Biomaterials can be used for injection into the anal sphincter to promote the restoration. Biomaterials include materials such as polymer, ceramics, metal, and composite materials [44]. Bulking agents are one type of biomaterial and can be inserted into the individual under local, regional, or general anesthesia. The injection depends very much on the type of clinical indication as well as the substance used. Bulking agents are intended to expand the tissue in the anal canal and prevent fecal leakage [45]. They can be performed in an outpatient setting with a low risk of morbidity, therefore increasing in popularity [45]. The use of bulking agents results in less frequent episodes of fecal incontinence over time as they can guide the healing process [46]. Some examples of bulking agents include the silicon biomaterial (PTQ), carbon-coated microsphere (Durasphere®), and the dextranomer in stabilized hyaluronic acid, also known as NASHA Dx [47]. Among those that are currently utilized, the NASHA Dx is the bulking agent that has shown to be most successful. This agent is approved for use in the USA and was trialed in Europe and in the USA in 2011. The result of the bulking agent was a >50% reduction in incontinence episodes, a 50% or greater reduction in incontinence episodes in 52% of the therapeutic treatment group compared to 31% in the placebo group at a 6-month interval [47]. Follow-up at 12 months presented a 50% or more reduction in FI episodes in 69% of patients in the therapeutic group,

DOI: 10.18609/cgti.2023.167

whilst the placebo group were not measured at 12 months [47]. In 2013, the efficacy of all injectable bulking agents was measured, and it was concluded that the NASHA Dx injectable demonstrated a significant improvement in continence [47].

Tissue engineering is an approach that evolved from the field of biomaterials which involves the growth of functional organs *in vitro* that are then implanted into the body [43]. The goal of tissue engineering is to restore, maintain or improve damaged tissue and organs. Further research should enlighten upon the clinical application of tissue engineering in patients with fecal incontinence. So far, the vascularization and integration of the engineered tissue are challenges that yet need to be overcome before patients can be treated by means of tissue engineering [48]. Although it is stated that the cells' environment and thus cell differentiation can be carefully regulated [49], some of the disadvantages of tissue engineering include the risk of tumorigenicity, immunogenicity, graft rejection and cell migration [50]. Additionally, vascularization of the site of implantation is potentially limited and the formation of the implantation requires time [43]. Autologous tissue repair showed to be an effective surgery. The advantages of autologous tissue repair include a minimal to moderate inflammatory response as well as a good integration with host tissues, however a disadvantage includes a high recurrence rate [51].

Cell therapy is a relatively novel, long-lasting, and effective regenerative therapy that uses stem cells for the purpose of tissue regeneration. This makes it a very interesting option to identify potentially curative treatments for FI when linked to damage sphincter muscles. In the context of FI, there are no approved cell therapies yet, but the ones under development use autologous cells (i.e. patient's own cells) and relocate them to the site of damage to repair the sphincter muscle. Cell therapy uses a variety of stem cell types, most commonly mesenchymal stem cells that can be obtained from a variety of tissues, often bone marrow, adipose or muscle tissue [52,53]. Autologous skeletal muscle derived cells (ASMDCs) are the most common cell types, these are obtained from the isolation of satellite cells from skeletal muscle biopsies that after processing can become myogenic progenitor cells [54]. These ASMDC can regenerate skeletal muscle cells to repair the external anal sphincter muscle. The current cell therapies under development for FI could be of benefit to patients with urinary incontinence [55,56], but also patients with joint or other muscle injuries [52,57]. A study showed that patients with limited FI duration and high incontinence episode frequency (IEF) are most responsive to cells [58]. Unfortunately, the survival rate of cultured cells is influenced due to the altered immunogenicity occurring during the *ex vivo* culturing period [43]. In addition, it is evident that routine use of cell therapy involves high costs [59]. A more cost-effective method for cell transplantation for anal sphincter regeneration has been proposed. Rather than expanding cells into injured anal sphincters, fragmented muscle fibers could be injected [60].

Cell therapy can be combined with biomaterials that provide a scaffold structure that can protect cells and then increase the chances of engraftment to form functional tissue [43]. When they are isolated from the patient, the cells are cultured *in vitro* and, in the case of FI, can be injected in combination with stimulating biomaterials to facilitate the functionality and attachment of the ASMDCs to the damaged site of the anal sphincter. Hence, the microenvironment is of upmost importance to sustain the quality of the ASMDCs. Without a sustaining environment, the ASMDCs are more likely to undergo apoptosis or reduced viability leading to decreased effect of the therapy. Presence of a scaffold has proven beneficial for the proliferation and myogenic ability of satellite cells [61]. Since ASMDC are at a more advanced differentiation stage than satellite cells, the importance of scaffold could be greater for this cell type [61]. Studies

researching cell therapy in combination with biomaterials have proved promising results, due to the ability of the created microenvironment to sustain the implanted cells. Although it is difficult to maintain a promotive microenvironment to sustain the quality of the ASMDCs, many technologies are emerging with the aim to improve the conditions of patients with FI [62]. In the following section we report on the competitive landscape of cell therapies in the field of FI as these therapies have been gaining more popularity over the past few years.

Competitive landscape within cell therapy

By December 2022, the US Food and Drug Administration (FDA) had approved a total of 27 cell and gene therapies [63]. The FDA anticipate approving another 10–20 therapies each year by 2025 [63]. Worldwide, the UK has the third largest cluster for cell and gene therapy production. In 2021, there were a total of 168 ongoing trials which made up around 9% of all global trials [64]. As of 2021, there have been a total of 16 new approvals of cell and gene therapies by the European Medicines Agency (EMA), of which 12 have been granted marketing authorization by the Medicines and Healthcare products Regulatory Agency (MHRA) [64]. Among the emerging technologies intended for FI cell therapy, a few have explored the use of microcarriers alongside different cell types. A comparison of the existing technologies based on stage of development, presence of the scaffold and intellectual property (IP) protection of the technologies is presented in Table 1 [65–67]. Notably, the most advanced therapies currently in phase 3 clinical trials are cell therapy approaches that do not use scaffold technologies. Conversely, the published studies on FI cell therapy with the use of scaffolds are relatively outdated and seem to have paused at the ical stage. The emerging technology with the most competitive advantage is the one developed by Innovacell, an Austrian start-up that has a broad IP coverage and a product at an advanced clinical development phase (phase 3 trials). Emerging evidence suggests that several cell therapies are seen as safe, however their therapeutic application and effectiveness remains a challenge [68].

IMPACT: INTRODUCTION OF NEW CELL THERAPY TECHNOLOGIES WILL IMPROVE THE CURRENT STATE-OF-PLAY IN FI AT THE CLINICAL, PATIENT, & SOCIO-ECONOMIC LEVEL

Clinical level: expansion of available treatment options

Cell therapy technologies have the potential to significantly alter the paradigm of treatment for patients with sphincter damage and for elderly patients. The conservative treatment options are generally not effective enough and the patient is referred further to undergo the different kinds of surgical treatments [46,69], which are often unsuitable or undesirable. Treatment using regenerative medicine products need to be entirely safe to differentiate them from other surgical interventions. For instance, surgical sphincter repair carries a high risk of wound breakdown and infection [70] and can result in permanent stoma in some patients [71]. SNS has the downside of initial cost and necessity for ongoing (lifelong) therapy maintenance (with further cost e.g., for battery replacements) [72]. Cell therapy could be an effective alternative for these patient groups. Due to its lower invasiveness and associated risk of adverse events, it is seen as a breakthrough therapeutical option for these patient groups.

Patient level: improvement of QoL for patients

Cell therapy products have the potential to significantly improve the patients' QoL. Quality of patients' lives is shown to increase after a clinically successful treatment [73].

DOI: 10.18609/cgti.2023.167

Cell therapy products will provide an alternative treatment option for those patients with severe FI that are not responding to conservative therapies, as described in more detail in the section above. These products have the potential advantage over other surgical interventions and conservative treatment methods. As such, they will be a better alternative for several patient groups. An effective clinical treatment would spare patients from risks and inconveniences, ultimately leading to a higher QoL [73].

TABLE 1

Comparisons of key players in the field of cell therapy for FI.

Entity	Cell type	Scaffold	Development phase	Patents	Reference
AMELIE	Autologous skeletal muscle derived cells	Poly DL-lactide-co-glycolide	Preclinical	1 European, 1 international	Amelie-project.eu
Seoul National University College of Medicine	Autologous myoblasts	bFGF-loaded polycaprolactone beads	Preclinical (dog)	Not found	[65]
University of Texas Southwestern Medical Center	Myogenic stem cells	Polyethylene glycol--based hydrogel matrix scaffold	Preclinical (rat)	Not found	[66]
University of Tampere	Human adipose stem cell	Bulkamid, a non-degradable viscoelastic water-based polymer	Preclinical (rat)	Not found	[67]
Yonsei University	Allogenic-adipose-derived mesenchymal stem cells	None, but in one patent they use chitin and ligament stem cells to promote collagen formulation	Phase 1 completed	2 Korean, 1 international	(NCT02384499)
Innovacell AG	Autologous skeletal muscle-derived cell	None	Phase 3 ongoing	6 European, 6 international	(NCT04976153)
Cook MyoSite	Itamocel autologous muscle-derived stem cell therapy	None	Phase 3 ongoing	Not found	(NCT05776277)
Andalusian Initiative for Advanced Therapies	Autologous mesenchymal stem cells from adipose tissue	None	Phase 2 completed	Not found	(NCT02292628)
Cellf Bio LLC	Smooth muscle cells and neural stem cells	None	Phase 1 ongoing	Not found	(NCT05616208)
Wake Forest University Health Sciences	Muscle fiber fragments that contain muscle precursor cells (MPCs)	None	N/A (procedural)	6 European, 11 international	(NCT05396456)
University Hospital, Rouen	Autologous muscle-derived progenitor cell injection	None	Phase 3 completed	3 European, 2 international	(NCT01523522)

Sources: desk research, Wheesbee, and clinicaltrials.gov. Query for clinicaltrials.gov: (regenerative medicine) OR (tissue regeneration) OR (regenerative therapy) OR (cell therapy) | faecal incontinence; Selected status: Not yet recruiting, Recruiting, Enrolling by invitation, and Active not recruiting.

Socio-economic level: reduction of the negative impact of FI on the economy

Treatment of FI with cell therapies would have the potential to create considerable savings for the EU and would increase the productivity of patients. An effective clinical treatment would allow patients to substantially reduce the medical costs associated with the condition, as well as enable FI patients to be economically contributing members in society. Considering the above-mentioned assumption of 2–5% market penetration, these are the predicted socio-economic impacts of cell therapy products targeting FI:

- The new treatments could save EU citizens between approximately €11.74 and €29.34 million per year for women affected by FI arising from obstetric sphincter injury and between approximately €65.90 and €164.75 million per year saved for all patients with FI in the EU; and
- The new treatments could save between 222,650–556,000 working days per year for women affected by FI arising from obstetric sphincter injury and 1.25–3.125 million working days per year saved for all patients with FI in the EU.

RECOMMENDATIONS & CONCLUSION

Where current therapies against FI often are not as effective as required, regenerative medicine often offer less invasive treatments and can be applied to a broad range of patients. Amongst regenerative medicine, new cell therapies are under development, reflecting positive signals for the field of FI. Exploring some of the recommendations below might further support the endeavors within the field of regenerative medicine, especially in the context of FI.

Technological improvements

Among the key obstacles in regenerative medicine therapies based on skeletal muscle-derived cells are insufficient cell count at delivery/survival and recapitulating the features of adult cells [42]. Both these hurdles could be tackled by integrating a biocompatible scaffold, potentially also releasing stimulatory molecules (e.g., growth factors and cytokines) to facilitate both delivery and functionality of the cells [42]. Additionally, most studies for the use of stem cell therapy for FI so far lacked potential for clinical translation [53]. This is thought to potentially be a consequence of the general focus on the external sphincter muscle regeneration and the lack of understanding of role of the internal sphincter muscle. This could potentially go as far as stimulating vascularization to ensure successful survival and regeneration [42,61]. Safety of the cell therapy approaches should be confirmed, as the replicative property of stem cells is associated with the risk of carcinogenicity. Results from ongoing long-term studies using the cell type of choice, ASM-DCs for FI in most cases, should be carefully monitored [42]. Finally, cellular therapy is costly, so once the technology is improved, their commercialization will depend on efficiently scaling up production of the therapy, potentially through the use of allogeneic cells (i.e. from healthy donors) [74].

Educating patients about different treatment options

Alongside the development of cell therapies that potentially offer a curative treatment for patients with FI, patients' families and the society should be made aware of the arising treatment options. Few patients are informed of the different therapies that are available to treat incontinence. Clinicians should be involved not only in the development of new potentially curative cell therapy treatments, but also in the education of the patients regarding the available options. A more effective

DOI: 10.18609/cgti.2023.167

treatment will eventually contribute to the reduction of the stigma associated with FI.

Providing safe platform for discussion and raising awareness

While the charities and patient groups active in the field of FI exist to provide the much-needed support to patients with FI, it still remains true that the disease is highly stigmatized and its prevalence underreported. A community-based approach providing a safe platform for discussion and sharing, combined with an educative element as described above, needs to be in place. Public information campaigns should emphasize that the condition is relatively common, especially among the groups that are at risk, for the lack of conversation around FI contributes to the public under-estimation of is prevalence, making the experiences feel more alone and perpetuating the cycle of stigma further. Similarly, public-facing campaigns could include information on prophylactic actions that doctors might provide to patients in a clinical setting. It is crucial that there is a unified effort across the EU, or at least that such charitable efforts take place in all countries, so that the inequality among countries does not further exacerbate the negative effect on FI on patients. With an increased awareness and decreased stigma, patients may feel motivated not only to share with fellow patients and thus raise awareness further, but they might also feel more comfortable in participating in the clinical development of alternative treatments.

REFERENCES

1. Shah R, Herrero JAV. *Fecal Incontinence*. 2022; StatPearls.
2. Knol ME, Snijders HS, Heyden JT, Baeten CI. Fecal incontinence: the importance of a structured pathophysiological model. *J. Anus Rectum Colon* 2022; 6, 58.
3. Rao SSC. Pathophysiology of adult fecal incontinence. *Gastroenterology* 2004; 126, S14–22.
4. Pasricha T, Staller K. Fecal incontinence in the elderly. *Clin. Geriatr. Med*. 2021; 37, 71–83.
5. Whitehead WE, C Rao SS, Lowry A, *et al.* Treatment of fecal incontinence: state of the science summary for the National Institute of Diabetes and Digestive and Kidney Diseases Workshop 2014. *Am. J. Gastroenterol.* 2015; 110(1), 138–146.
6. Assmann SL, Keszthelyi D, Kleijnen J, *et al.* Guideline for the diagnosis and treatment of faecal incontinence-A UEG/ESCP/ESNM/ESPCG collaboration 2022. *United European Gastroenterol. J.* 2022; 10(3), 251–286.
7. Ditah I, Devaki P, Luma HN, *et al.* Prevalence, trends, and risk factors for fecal incontinence in United States adults, 2005–2010. *Clin. Gastroenterol. Hepatol.* 2014; 12, 636-643.e2.
8. Dudding TC, Lee EM, Faiz O, *et al.* Economic evaluation of sacral nerve stimulation for faecal incontinence. *Br. J. Surgery* 2008; 95, 1155–1163.
9. Swain D, Parida SP, Jena SK, Das M, Das H. Obstetric fistula: a challenge to public health. *Indian J. Public Health* 2019; 63, 3–8.
10. Ndoye M, Greenwell T. Obstetric fistula. *Urinary Fistula* 2016, 197–228.
11. Semere L, Nour NM. Obstetric fistula: living with incontinence and shame. *Rev. Obstet. Gynecol.* 2008; 1, 193–197.
12. Subki H, Fakeeh M, Hindi M, *et al.* Fecal and urinary incontinence associated with pregnancy and childbirth. *Mater. Sociomed*. 2019; 31, 202–206.
13. van Meegdenburg MM, Trzpis M, Broens PMA. Fecal incontinence and parity in the Dutch population: a cross-sectional analysis. *United European Gastroenterol. J.* 2018; 6, 781–790.
14. Rørtveit G, Hannestad YS. Association between mode of delivery and pelvic floor dysfunction 1848–52. *Tidsskr. Nor. Legeforen. Nr*. 2014; 19, 1848–1852.
15. Eurostat. How many children were born in the EU in 2021? Products Eurostat News; Eurostat 2023 (accessed Aug 2, 2023).
16. Abrams P, Andersson K-E, Apostolidis A, *et al.* Evaluation and treatment of urinary incontinence, pelvic organ prolapse and faecal incontinence.

Neurourol. Urodynamics 2018; 37(7), 2271–2272.

17. Musa MK, Saga S, Blekken LE, Harris R, Goodman C, Norton C. The prevalence, incidence, and correlates of fecal incontinence among older people residing in care homes: a systematic review. *J .Am. Med. Dir. Assoc.* 2019; 20, 956–962.e8.

18. Coggrave M, Norton C, Cody JD. Management of faecal incontinence and constipation in adults with central neurological diseases. *Cochrane Database Syst. Rev.* 2014; 13(1), CD002115.

19. Mawer S, Alhawaj AF. *Physiology, Defecation.* 2022; StatPearls.

20. Russell B, Buswell M, Norton C, *et al.* Supporting people living with dementia and faecal incontinence. *Br. J. Community Nurs.* 2017; 22, 110–114.

21. Goodman C, Malone JR, Norton C, *et al.* Reducing and managing faecal incontinence in people with advanced dementia who are resident in care homes: protocol for a realist synthesis. *BMJ Open* 2015; 5(7), e007728.

22. Rao SSC. Current and emerging treatment options for fecal incontinence. *J. Clin. Gastroenterol.* 2014; 48(9), 752–764.

23. Adil E, Bharucha A, Satish S, Rao M, Shin A. Surgical interventions and the use of device-aided therapy for the treatment of fecal incontinence and defecatory disorders. *Physiol. Behav.* 2018; 176, 139–148.

24. Fecal incontinence - Diagnosis and treatment. Mayo Clinic 2022 (accessed Aug 2023).

25. Leo CA, Thomas GP, Bradshaw E, *et al.* Long-term outcome of sacral nerve stimulation for faecal incontinence. *Colorectal Disease* 2020; 22, 2191–2198.

26. Berg MR, Gregussen H, Sahlin Y. Long-term outcome of sphincteroplasty with separate suturing of the internal and the external anal sphincter. *Tech. Coloproctol.* 2019; 23, 1163–1172.

27. Pakravan F, Helmes C, Alldinger I. Magnetic sphincter augmentation in patients with fecal incontinence after failure of an implanted artificial bowel sphincter. *Coloproctology* 2018; 40, 127–129.

28. Bartlett L, Nowak M, Ho L, Bartlett Y-H, Chiarioni G. Impact of fecal incontinence on quality of life. *World J. Gastroenterol.* 2009; 15, 3276–3282.

29. Gallagher DL, Thompson DL. Identifying and managing fecal incontinence. *J. Wound. Ostomy Continence Nurs.* 2012; 39, 95–97.

30. Leigh RJ, Turnberg LA. Faecal incontinence: the unvoiced symptom. *Lancet* 1982; 319, 1349–1351.

31. Devendorf AR, Bradley SE, Barks L, Klanchar A, Orozco T, Cowan L. Stigma among veterans with urinary and fecal incontinence. *Stigma Health* 2021; 6, 335–343.

32. Norton C, Whitehead W, Bliss D, Harari D, Lang J. Conservative and pharmacological management of faecal incontinence in adults. *Neurourol. Urodyn.* 2010; 29(1), 199-206.

33. Meyer I, Richter HE. Impact of fecal incontinence and its treatment on quality of life in women. *Womens Health* 2015; 11, 225–238.

34. de Miguel Valencia MJ, Margallo Lana A, Pérez Sola MÁ, *et al.* Economic burden of long-term treatment of severe fecal incontinence. *Cir. Esp*. 2022; 100, 422–430.

35. Dunivan GC, Heymen S, Palsson OS, *et al.* Fecal incontinence in primary care: prevalence, diagnosis, and healthcare utilization. *Am. J. Obstet. Gynecol.* 2010; 202(5), 493.E1–493.E6.

36. Meyer I, Richter HE. Impact of fecal incontinence and its treatment on quality of life in women. *Womens Health (Lond.)* 2015; 11(2), 225–238.

37. Xu X, Menees SB, Zochowski MK, Fenner DE. Economic cost of fecal incontinence. *Dis. Colon Rectum* 2012; 55, 586–598.

38. Bunting SY, Lapworth DJ, Crane EJ, *et al.* Emerging organic compounds in European groundwater. *Environmental Pollution* 2021; 269, 115945.

39. Baltic Marine Environment Protection Commission. Vieno N, Hallgren P, Wallberg P. International Initiative on Water Quality. Pharmaceuticals in the aquatic environment of the Baltic Sea region. A status report 2017.

40. Gu X, Sun M, Long X, Deng H, Wang Y. Environmental impact of adult incontinence products in China in the context of population aging. *Sci. Total Environ.* 2023; 875, 162596.

41. Muthu SS, Ng FSF, Li Y, Hui PCL, Guo Y. Carbon and eco-footprints of adult incontinence products. *Fibers and Polymers* 2013; 14, 1776–1781.

42. Trébol J, García-Arranz M. Stem cell therapy for faecal incontinence: current state and future perspectives *World J. Stem Cells* 2018; 10(7), 82–105.

DOI: 10.18609/cgti.2023.167

43. Parmar N, Kumar L, Emmanuel A, Day RM. Prospective regenerative medicine therapies for obstetric trauma-induced fecal incontinence. *Regenerative Med.* 2014; 9, 831–840.

44. Han F, Wang J, Ding L, *et al.* Tissue engineering and regenerative medicine: achievements, future, and sustainability in Asia. *Front. Bioeng. Biotechnol.* 2020; 8, 495216.

45. Mellgren A, Matzel KE, Pollack J, *et al.* Long-term efficacy of NASHA Dx injection therapy for treatment of fecal incontinence. *Neurogastroenterol. Motil.* 2014; 26, 1087–1094.

46. Camilleri-Brennan J. Anal injectable and implantable bulking agents for faecal incontinence. In: *Current Topics in Faecal Incontinence* (Editor: Camilleri-Brennan J). 2020. IntechOpen.

47. Price AD, Paquette IM. Best conservative options for fecal incontinence. *Ann. Laparosc. Endosc. Surg.* 2022; 7.

48. Williams DF. Challenges with the development of biomaterials for sustainable tissue engineering. *Front. Bioeng. Biotechnol.* 2019; 7, 456529.

49. Brown PT, Handorf AM, Jeon WB, Li W-J. Stem cell-based tissue engineering approaches for musculoskeletal regeneration. *Curr. Pharm. Des.* 2013; 19, 3429.

50. Seppänen-Kaijansinkko R. Tissue engineering—pros and cons. *Int. J. Oral Maxillofac. Surg.* 2017; 46, 50.

51. Lin M, Lu Y, Chen J. Tissue-engineered repair material for pelvic floor dysfunction. *Front. Bioeng. Biotechnol.* 2022; 10, 968482.

52. Li P-C, Ding D-C. Stem-cell therapy in stress urinary incontinence: a review. *Tzu Chi Med. J.* 2023; 35, 111.

53. Kim M, Oh BY, Lee JS, Yoon D, Chun W, Son IT. A systematic review of translation and experimental studies on internal anal sphincter for fecal incontinence. *Ann. Coloproctol.* 2022; 38, 183–196.

54. Frudinger A, Marksteiner R, Pfeifer J, Margreiter E, Paede J, Thurner M. Skeletal muscle-derived cell implantation for the treatment of sphincter-related faecal incontinence. *Stem. Cell Res. Ther.* 2018; 9, 1–20.

55. Despite high prevalence, urinary incontinence is still very much a taboo. Uroweb 2021 (accessed Aug 3, 2023).

56. Sam P, Jiang J, LaGrange CA. *Anatomy, Abdomen and Pelvis, Sphincter Urethrae.* In: *StatPearls* (Editors: Sam P, Jiang J, Leslie SW, *et al.*) 2022, StatPearls Publishing.

57. Seval MM, Koyuncu K. Current status of stem cell treatments and innovative approaches for stress urinary –incontinence. *Front. Med. (Lausanne)* 2022; 9, 1073758.

58. Frudinger A, Gauruder-Burmester A, Graf W, *et al.* Skeletal muscle–derived cell implantation for the treatment of fecal incontinence: a randomized, placebo-controlled study. *Clin. Gastroenterol. Hepatol.* 2023; 21, 476–486.e8.

59. Luca M De, Cossu G. Cost and availability of novel cell and gene therapies: can we avoid a catastrophic second valley of death? *EMBO Rep.* 2023; 24.

60. Balaphas A, Meyer J, Meier RPH, *et al.* Cell therapy for anal sphincter incontinence: where do we stand? *Cells* 2021; 10, 2086.

61. Jiang Y, Torun T, Maffioletti SM, Serio A, Tedesco FS. Bioengineering human skeletal muscle models: recent advances, current challenges and future perspectives. *Exp. Cell Res.* 2022; 416, 113133.

62. Zhao X, Li Q, Guo Z, Li Z. Constructing a cell microenvironment with biomaterial scaffolds for stem cell therapy. *Stem Cell Res. Ther.* 2021; 12, 1–13.

63. Viscomi S. What's Holding Cell Therapies Back? A Look Into The Evolution And Future Of Stem Cell Tech 2023 (accessed Aug 3, 2023).

64. Osborne A. Catapult's Cell and Gene Therapies Vision for the UK - Phacilitate 2022 (accessed Aug 3, 2023).

65. Oh HK, Lee HS, Lee JH, *et al.* Coadministration of basic fibroblast growth factor-loaded polycaprolactone beads and autologous myoblasts in a dog model of fecal incontinence. *Int. J. Colorectal Dis.* 2015; 30, 549–557.

66. Montoya TI, Acevedo JF, Smith B, *et al.* Myogenic stem cell-laden hydrogel scaffold in wound healing of the disrupted external anal sphincter. *Int. Urogynecol. J.* 2015; 26, 893–904.

67. Kuismanen K, Juntunen M, Girish N, *et al.* Functional outcome of human adipose stem cell injections in rat anal sphincter acute injury model. *Stem Cells Transl. Med.* 2018; 7, 295–304.

68. Ntege EH, Sunami H, Shimizu Y. Advances in regenerative therapy: a review of the literature and future directions. *Regen. Ther.* 2020; 14, 136–153.

69. Demirci S, Gallas S, Bertot-Sassigneux P, Michot F, Denis P, Leroi AM. Anal incontinence: the role of medical management. *Gastroenterol. Clin. Biol.* 2006; 30, 954–60.

70. Okeahialam NA, Wong KW, Thakar R, Sultan AH. The incidence of wound complications following primary repair of obstetric anal sphincter injury: a systematic review and meta-analysis. *Am. J. Obstet. Gynecol.* 2022; 227, 182–191.

71. Van Koughnett JAM, Wexner SD. Current management of fecal incontinence: choosing amongst treatment options to optimize outcomes. *World J. Gastroenterol.* 2013; 19, 9216–9230.

72. Thaha MA, Abukar AA, Thin NN, Ramsanahie A, Knowles CH. Sacral nerve stimulation for faecal incontinence and constipation in adults. *Cochrane Database Syst. Rev.* 2015; 8, CD004464.

73. Deutekom M, Terra MP, Dobben AC, *et al.* Impact of faecal incontinence severity on health domains. *Colorectal* Disease 2005; 7, 263–269.

74. Chan AML, Ng AMH, Yunus MH, *et al.* Safety study of allogeneic mesenchymal stem cell therapy in animal model. *Regen. Ther.* 2022; 19, 158–165.

AFFILIATIONS

Martha Gilbert
ttopstart, Rijswijk, The Netherlands

Simona Čaputová
ttopstart, Rijswijk, The Netherlands

Delielena Poli
ttopstart, Rijswijk, The Netherlands

Manou Kooy
ttopstart, Rijswijk, The Netherlands

Georgia Sturt
Bowel Research UK, London, UK

Josephine Parker
Comprehensive Clinical Trials Unit, University College London, London, UK

Richard M Day
Centre for Precision Healthcare, University College London, London, UK

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Richard Day is a Director and shareholder of Luna Therapeutics Ltd.

Funding declaration: ttopstart operates as a beneficiary within the Anchored Muscle cELls for Incontinence (AMELIE) project, receiving funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 874807. Bowel Research UK operates as a beneficiary within the Anchored Muscle cELls for IncontinencE (AMELIE) project, receiving funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 874807. UCL operates as a coordinator within the Anchored Muscle cELls for Incontinence (AMELIE) project, receiving funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 874807.

ARTICLE & COPYRIGHT INFORMATION

Copyright: Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0 which allows anyone to copy, distribute, and transmit the article provided it is properly attributed in the manner specified below. No commercial use without permission.

Attribution: Copyright © 2023 Gilbert M, Čaputová S, Poli D, Kooy M, Sturt G, Parker J & Day RM. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

Article source: Invited; externally peer reviewed.

Submitted for peer review: Aug 9, 2023; **Revised manuscript received**: Oct 5, 2023; **Publication date:** Dec 7, 2023.

DOI: 10.18609/cgti.2023.167

Cell culture in a chemically defined environment

Chengkang Zhang, Associate Director R&D, Lonza

Serum has historically been used for *in vitro* cell culture, though due to associated safety and quality risks, moving to a chemically defined environment is recommended. In this poster, the risks associated with using serum or plasma in cell culture will be explored, and several best practices for obtaining optimal results when removing serum from your process will be shared.

SAFETY AND QUALITY RISKS OF SERUM/PLASMA

Serum has historically been used for *in vitro* cell culture due to its growth-promoting effects, however, it does have some undesired properties. Serum, plasma, and even blood-derived human albumin invariably carry the risk of transmitting infectious substances, such as viruses. Common filtration methods for serum and plasma use a 0.2 µm filter, but many viruses, such as HIV (100–130 nm) or influenza (80–120 nm), are smaller than that and therefore cannot be removed by standard filtration methods. To reduce these safety risks, serum may be heat inactivated by heating to 56 °C for 30 minutes, though this process is known to degrade certain biomolecules, such as growth factors, vitamins, and amino acids.

There are also some quality concerns associated with the use of serum or plasma, as the quality often varies from lot to lot and vendor to vendor. Significant effort is needed to screen and stockpile high-quality serum or plasma.

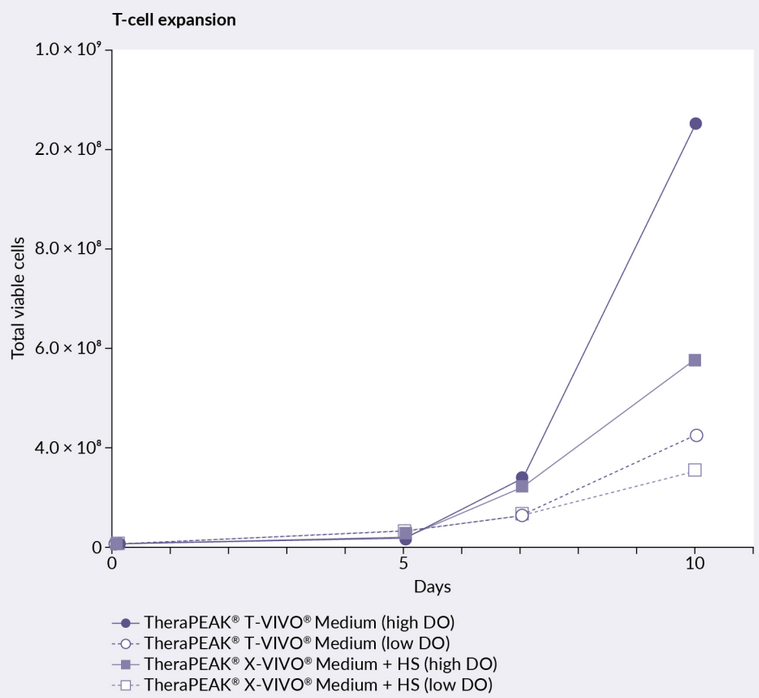
Due to these safety and quality risks, serum can adversely affect a user's cell culture. To reduce patient risk and eliminate the variability linked to human sourced components, it is recommended to move to a serum-free environment.

CONSIDERATIONS IN MOVING TO A CHEMICALLY DEFINED ENVIRONMENT

Removing serum from a cell culture process requires some adjustment to procedures to be successful. A key consideration is maintaining a good dissolved oxygen level. *In vivo*, the distance of a cell from its nearest capillary rarely exceeds 200 µm. In commonly used multi-well plates and T-flasks, O_2 exchanges at the surface of the medium and diffuses through the medium, the rate of which decreases proportionally to the thickness of the medium. It is recommended that medium thickness remains below 3 mm. Higher cell density and higher cell proliferation rate increase the demand for oxygen.

Figure 1. Reduced CD3 T cell expansion when oxygen exchange is limited.

	High DO condition	Low DO condition
Starting cell number	0.5 × 10⁶ CD3 T cells	2.0 × 10⁶ CD3 T cells
Medium thickness	2 mm (5 mL in T-25)	6 mm (15 mL in T-25)



TheraPEAK® T-VIVO® Cell Culture Medium is a chemically defined media containing no animal-origin components that achieves high performance without serum. This medium was used in a study to investigate how CD3 cell expansion is affected when oxygen exchange is limited **(Figure 1)**. CD3 T cells were activated and expanded for 10 days in a T-flask in TheraPEAK® T-VIVO® Medium (no serum) or TheraPEAK® X-VIVO® 15 Medium + 5% HS. Cells were re-seeded into new flasks when viable cell density exceeded 2 × 10⁶ cells/mL. Oxygen exchange was limited by increasing the medium thickness from 2 mm to 6 mm, which significantly reduced the CD3 T cell expansion in both media, despite starting with more cells.

TheraPEAK® is not for human or animal *in vivo* or diagnostic use, including use as a diluent or as an excipient. TheraPEAK® Media is suitable for GMP manufacturing. It is the end user's responsibility to ensure full compliance with all regulations based on their use of Lonza's products. The information contained herein is believed to be correct, however, no warranty is made, either expressed or implied. All trademarks belong to Lonza, and are registered in the USA, EU and/or CH or belong to third-party owners and are used only for informational purposes. All third-party copyrights have been reproduced with permission from their owners. For more details: www.lonza.com/legal.

© 2023 Lonza. CT-MI105 11/23.
All rights reserved.

CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2023; 9(10), 1471; DOI: 10.18609/cgti.2023.192

Copyright © 2023 Lonza. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

In partnership with: Lonza Cell & Gene

Case study: media optimization control strategies

Alex Sargent PhD, Director, Process Development, Cell Therapy CDMO Services, Charles River

T cell-based therapies such as CAR-T and TCR-T hold great promise for the treatment of cancer and other diseases. This poster presents findings on optimized media replacement and perfusion strategies to promote T cell expansion and function across different platforms in cell therapy. These findings demonstrate a fine balance to media replacement in culture systems, whereby over or under perfusion can diminish T cell expansion and differentiation.

THE IMPORTANCE OF PERFUSION IN T CELL CULTURE

To maintain high-density T cell cultures, perfusion is paramount. T cell activation, expansion, and function are regulated by glucose and lactate. Replacing old media with new ensures the total viable cells in the T cell bioreactor culture is maximized, due to replenishing glucose and cytokines in addition to the removal of growth inhibitors such as lactic acid.

NEXT-GENERATION PROCESSES FOR T CELL SCALE UP

In this case study in stirred-tank bioreactors, the following process targets were set:

- Higher cell density (40–50 × 10^6 cells/mL)
- T cells to be fit (low exhaustion) and functional (central/stem cell memory phenotype)
- Serum-free process with all components chemically defined.

The process developed was able to meet these three targets **(Figure 1)**.

Unfortunately, to meet these targets in the high perfusion process, media consumption was significantly greater than for a fed-batch or intermediate perfusion process. This presents a potential roadblock in the scale-up of T cell therapies due to high media costs, especially at the 50 L scale.

Figure 1. High perfusion process enables higher T-cell density (1 L scale).

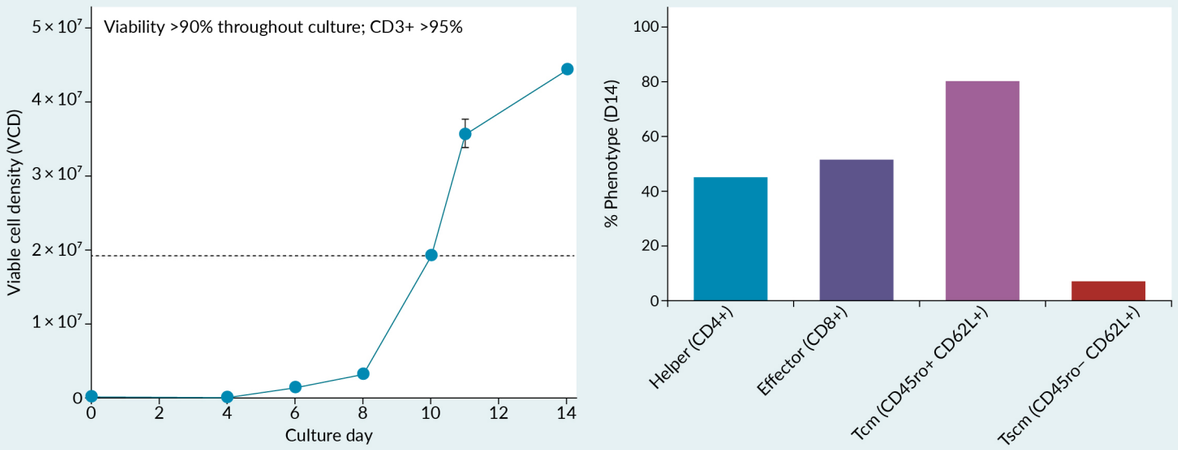
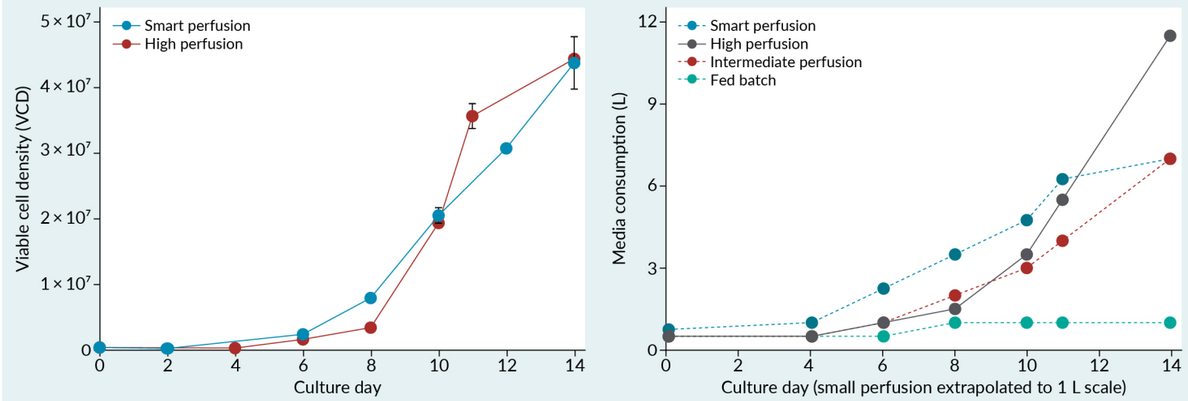


Figure 2. Viable cell density and media consumption of varying perfusion strategies.



Viability >90%; CD3+ >95% (phenotype data pending)

PROCESS OPTIMIZATION: LEVERAGING AUTOMATION & DESIGN OF EXPERIMENT

To overcome this obstacle, the aim of process optimization was to find a method of achieving higher T cell densities using less media in order to realize the cost-effective scale-up of T cell therapies.

Design of experiment (DOE) and novel artificial intelligence tools were used to develop and optimize more advanced media control strategies for T cells and other cell types. For this study, the Ambr15, a robotic liquid handling system was used. Metabolic profiling of T cells using a Python-based machine learning strategy was employed to design an optimized media control paradigm. This strategy leveraged data from multiple runs/donors using a Random Forest model. The key output of this algorithm was high T cell expansion (40–50 × 10^6 cells/mL), and the key input was efficient control of nutrients and metabolites (glucose/lactate).

The 'Smart Perfusion' paradigm developed by machine learning achieves high viable T cell density at half the media cost **(Figure 2)**. This translates to delivering a reduction in the material cost of goods by 30–50%. Serum-free 'Smart Perfusion' reduces material costs 50–70% versus the standard serum process, giving cost savings of US$0.5–1 million per batch at a 50 L scale.

Cell & Gene Therapy Insights 2023; 9(10), 1385; DOI: 10.18609/cgti.2023.179

Copyright © 2023 Charles River. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

In partnership with: charles river

FASTFACTS

Reliable detection reagents in CAR-T/NK cell flow cytometry

Annika Graband, Global Product Manager, Flow Cytometry Reagents, Miltenyi Biotec B.V. & CO. KG

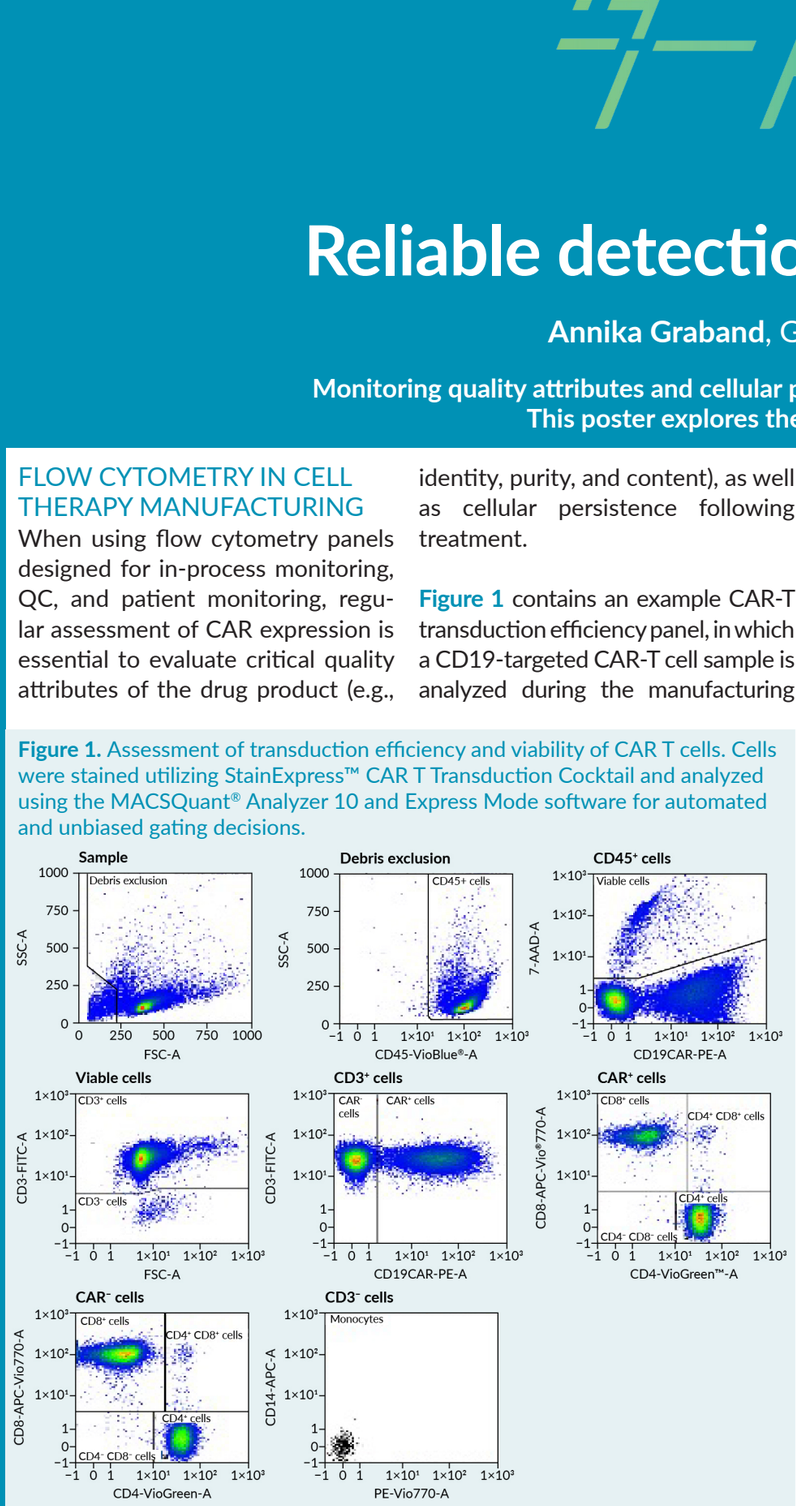
Monitoring quality attributes and cellular persistence of CAR-expressing cells is essential to ensure the safety and effectiveness of engineered cell therapies. This poster explores the benefits of dependable CAR⁺ cell evaluation via flow cytometry using CAR detection reagents.

FLOW CYTOMETRY IN CELL THERAPY MANUFACTURING

When using flow cytometry panels designed for in-process monitoring, QC, and patient monitoring, regular assessment of CAR expression is essential to evaluate critical quality attributes of the drug product (e.g., identity, purity, and content), as well as cellular persistence following treatment.

Figure 1 contains an example CAR-T transduction efficiency panel, in which a CD19-targeted CAR-T cell sample is analyzed during the manufacturing process. Following the establishment of standard scatter settings, CD45⁺ cells are isolated and 7-AAD is employed to exclude non-viable cells. The transduction efficiency within the CD3⁺ cell population is ascertained using a CAR detection reagent (DR), such as the Miltenyi Biotec CD19 CAR Detection Reagent. The panel also provides insight into the CD4 and CD8 cells among CAR⁺ and CAR⁻ cells. The most critical data typically relates to CD19 CAR⁺ cells, either within the viable cell fraction or among the viable CD3⁺ cells. These measurements are relevant in determining the drug product's optimal formulation and dosage.

Figure 1. Assessment of transduction efficiency and viability of CAR T cells. Cells were stained utilizing StainExpress™ CAR T Transduction Cocktail and analyzed using the MACSQuant® Analyzer 10 and Express Mode software for automated and unbiased gating decisions.



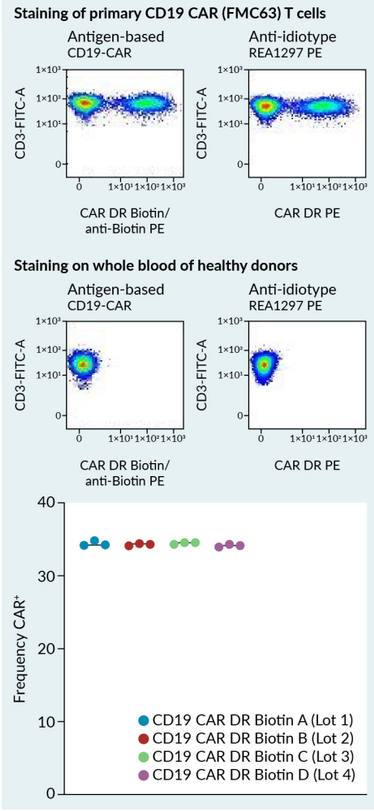
STRATEGIES FOR CAR DETECTION

There are two strategies for CAR detection suitable for clinical studies.

Antigen-based strategies bind the antigen-binding domains of CARs and utilize the binding affinity of the CAR for its target antigen. These methods confirm functional CAR recognition of the target antigen and are compatible with flow cytometry.

Anti-idiotype strategies specifically bind variable regions (scFv) of a particular CAR construct. This enables discrimination of different CAR constructs targeting the same antigen. These methods are compatible with flow, imaging, or cytometry by time of flight.

Figure 2. Specificity and lot-to-lot consistency testing of CAR DRs.



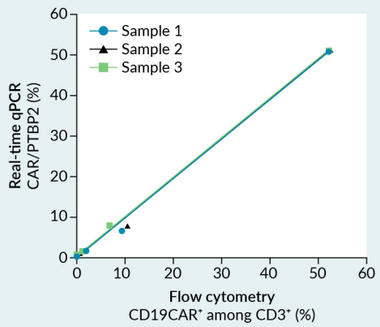
CAR DR QUALITY TESTING

In **Figure 2**, the staining performance of Miltenyi Biotec's CAR DRs is assessed using primary CAR-T cells. Both antigen-based and anti-idiotype CAR DRs consistently yield a high staining index when applied to samples from different donors. CAR⁺ and CAR⁻ cells are easily distinguished, background noise is minimal, and lot-to-lot consistency is preserved.

When tested on blood samples from healthy donors, Miltenyi Biotec's CAR DRs showed high specificity and sensitivity. There was no non-specific binding on whole blood samples, allowing reliable detection of small quantities of CAR-T cells.

To establish a correlation between flow cytometry and qPCR findings, CD19 CAR⁺ transduced T cells were spiked to ethylenediaminetetraacetic acid-anticoagulated whole blood of healthy donors at serial dilutions **(Figure 3)**. Decreasing CAR frequencies were observed when serial dilution samples were analyzed by flow cytometry and real-time qPCR. Spike-in samples of all donors showed a strong correlation. By employing orthogonal methods to validate the accuracy of the flow cytometry assay, the reliability of analytical data was demonstrated.

Figure 3. Sensitive monitoring of CAR⁺ T cells in peripheral blood by flow cytometry and real-time qPCR.



	Sample 1	Sample 2	Sample 3
Slope	0.9765	0.9738	0.9708
R square	0.9976	0.9981	0.9993

CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2023; 9(10), 1321; DOI: 10.18609/cgti.2023.169

Copyright © 2023 Miltenyi Biotec B.V. & CO. KG. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

MACSQuant, the Miltenyi Biotec logo, StainExpress, Vio, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec B.V. & CO. KG and/or its affiliates in various countries worldwide.

In partnership with: Miltenyi Biotec

FASTFACTS

HEK293 suspension culture in stirred 3D-systems for gene therapy development

Jorge Escobar, Senior Applications Specialist Cell & Gene Therapy Applications, Eppendorf Inc.

Well-established cell culture platforms, such as HEK293, can be used to produce viral or non-viral delivery vehicles to introduce a gene of interest. Bioreactors can be a suitable option to establish optimal culture conditions during process development. The Expi293F cell line shows significant improvements over traditional HEK293 cell lines due to its robust growth under suspension culture conditions and its stable and transient expression.

AN INTRODUCTION TO HEK293 & AAV2

HEK293 is one of the most versatile mammalian cell lines with a wide range of applications including the expression of recombinant proteins, antibodies, and viruses. HEK293 cells are low-maintenance, robust, show rapid proliferation, and have convenient application to transient and stable expression.

AAV is a leading platform in gene delivery and has recently emerged as an important tool for the vaccine industry. The upstream AAV2 capsid production workflow begins with plasmid selection of the Rep/Cap plasmid, containing the AAV structural and packaging genes, and the helper plasmid, containing adenovirus-associated genes critical for recombinant AAV assembly.

AAV2 CAPSID PRODUCTION INOCULUM PREPARATION

Cells were cultured in a New Brunswick S41i CO_2 incubator shaker (at 37 °C, 8% CO_2, and an agitation speed of 125 rpm), and over 900×10^6 cells were obtained in the third passage. An inoculum was prepared containing 400×10^6 cells in 200 mL of Expi293F expression medium on each inoculation bottle. The BioBLU 1c single-use bioreactor was connected to a SciVario twin bioreactor control system. 800 mL of medium was transferred into each bioreactor. Culture conditions are given in **Table 1**.

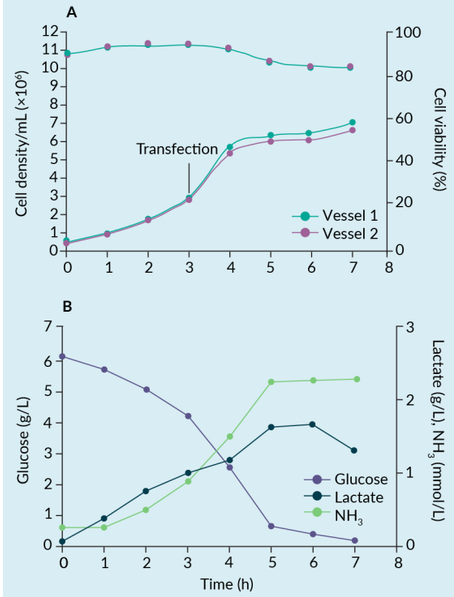
Table 1. Bioreactor culture conditions.

Parameters	Setpoints
Starting volume	800 mL
Ending volume	1 L
Initial agitation	155 rpm (0.4 tip speed)
Temperature	37 °C
Inoculation density	0.4×10^6 cell/mL
Cell culture medium	Expi293™ Expression Medium
DO setpoint	40% (P=0.1; I=0.001)
pH setpoint	7.0 (deadband=0.2), cascade to CO_2 (acid) cascade to 0.45M sodium bicarbonate (base)
Gassing range	Set O_2 at 30% controller output to 21% and at 100% controller output to 100%. Set flow at 0% controller output to 0.04 SLPH, and at 100% controller output to 30 SLPH.

EXPI293F CELL TRANSFECTION & CELL GROWTH

Samples were collected daily from the BioBLU 1c single-use bioreactors to determine the cell viability, cellular density, and concentration of metabolites. The results are shown in **Figure 1**. Transfection cell density was approximately 3×10^6 cells/mL 3 days after bioreactor inoculation. Plasmids at a 2:1 molar ratio were diluted in Expi293F expression medium and FectoVIR was briefly added into the plasmids/medium solution and incubated for 30 minutes at room temperature. A rapid increase of cell growth in both bioreactors was observed between days 1–4 of culture, reaching a peak in viable cell density at 7×10^6 cells/mL.

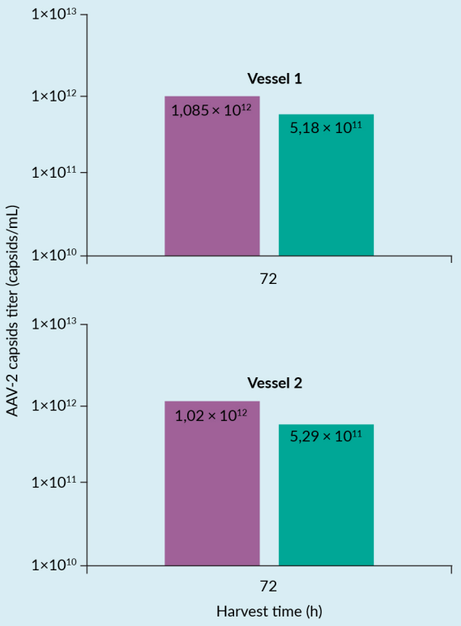
Figure 1. Expi293F cell transfection and cell growth.



AAV2 CAPSID QUANTIFICATION

Excellent reproducibility is shown in **Figure 2**, due to control by the SciVario twin bioreactor control system demonstrated by highly similar AAV2 capsid titers in both BioBLU single-use bioreactors. Higher AAV2 capsid titers were seen in the cell pellet in comparison to the supernatant. We conclude that single-use stirred-tank bioreactors are a valuable tool to establish optimal culture conditions in the process development of current and future therapeutic applications.

Figure 2. AAV2 capsid quantification.



Cell & Gene Therapy Insights 2023; 9(10), 1105; DOI: 10.18609/cgti.2023.145

Copyright © 2023 Eppendorf SE. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY NC ND 4.0.

In partnership with: eppendorf

WEBINAR DIGEST

Three distinct analytical ultracentrifugation methods for virus and viral vector characterizations

Susumu Uchiyama, Department of Biotechnology, Department of Engineering, Osaka University

The demand for characterization and quality control (QC) of viral vectors and viruses for therapeutic purposes is rapidly increasing. One vital critical quality attribute (CQA) is a quantitative description of the particle size distribution, including analysis of empty, partial, and full particles as well as aggregates. This poster will explore three analytical ultracentrifugation methods for the characterization of AAV vectors.

Analytical ultracentrifugation (AUC) was developed for the size distribution analysis of particles in solution. Modern AUC instruments, which utilize direct boundary fitting of sedimentation boundary data, and the development of advanced analytical software have greatly extended the applications accessible by AUC. Sedimentation velocity AUC (SV-AUC) is considered a gold standard method for the size distribution analysis of viral vectors for gene therapy, but several orthogonal AUC methods are appropriate, including band sedimentation AUC (BS-AUC) and density gradient equilibrium AUC (DGE-AUC).

SV-AUC

This method analyzes particles or molecules in solution through homogenous bulk solution centrifugation. S-value distribution depends on molecular weight and shape. SV-AUC data analysis is based on the superposition of non-interacting Lamm equation solutions.

BS-AUC

This method is similar to SV-AUC in that it gives an S-value based size distribution profile, but the sample amount required for analysis is much smaller than what is required for SV-AUC. This smaller sample is layered on top of a bulk solvent and then centrifuged.

DGE-AUC

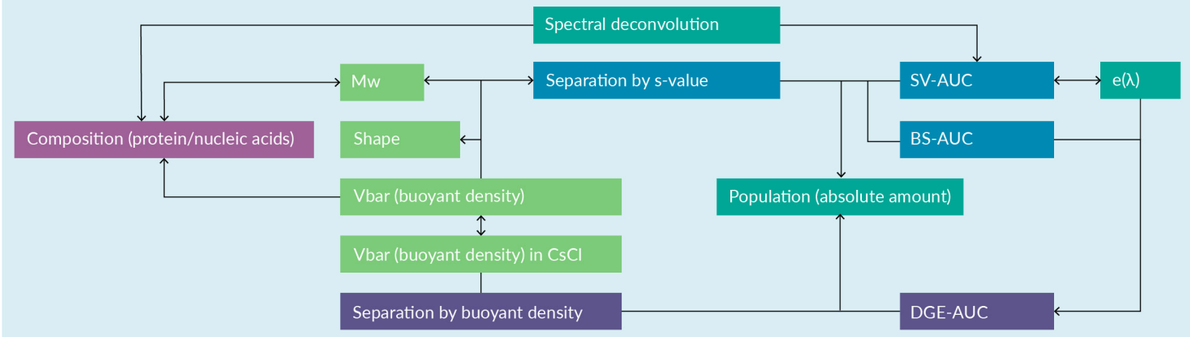
In this method, separation of empty, full, and partial viral capsids is based on a particle's buoyant density differences at the isopycnic point, rather than being based on molecular shape.

Table 1. Summary of the three AUC methods for AAV characterization.

Method	Measured values	Estimated values from specialized softwares	Estimated values from general software	Sample amount
SV-AUC	Sedimentating boundary ≥C(r, t)	S distribution f/f_0 Peak area	Abs (λ); e (λ) Composition (capsid:DNA) molecular weight (Mw) population (incl. F/E/PP/ExP)	5 10^{11} vg
BS-AUC	Sedimentating band ≥C(r, t)	S distribution f/f_0 Peak area	Abs (λ) Composition (capsid:DNA) molecular weight (Mw) population (incl. F/E/PP/ExP)	10^{10} vg
DGE-AUC	C(r) (at equilibrium)	Peak area Abs (λ)	Buoyant density Composition population (incl. F/E/PP/ExP)	5 10^{01} vg

C: Concentration; r: Radius from the axis of rotor.

Figure 1. Flowchart of the three AUC methods.



COMPARISON OF THE THREE AUC METHODS

Table 1 summarizes the key capabilities and characteristics of each method and provides a means for directly comparing the three methods based on measured values, estimated values from general and specialized software, and required sample amount.

WHEN TO USE EACH METHOD?

Figure 1 demonstrates the process flow for the three AUC methods. The size distribution of particles is based on either the s-value or the buoyant density of the solution. When determining the population of full and empty particles, molar extinction coefficient conversion is necessary. Particle molar extinction coefficients can be determined with SV-AUC, and this value can be used for data analysis in chromatographic and other analytical methods. Finally, the composition of the particles and their absolute concentration can be identified by the deconvolution of spectral data obtained from SV-AUC.

SUMMARY

This poster has demonstrated the advantages and use cases for SV-, DGE-, and BS-AUC, explaining how each method may be utilized to obtain a more holistic description of AAV particles.

Watch the webinar here

Cell & Gene Therapy Insights 2023; 9(10), 1405 • DOI: 10.18609/cgti.2023.183
Copyright © 2023 Beckman Coulter. Published by *Cell & Gene Therapy Insights* under Creative Commons License Deed CC BY C ND 4.0.

In partnership with: BECKMAN COULTER Life Sciences