CELL & GENE THERAPY INSIGHTS

PODCAST INTERVIEW

Key factors to consider for successful cell therapy manufacturing: a case study



Dave Humphries, Content Marketing Manager, Thermo Fisher Scientific, **speaks to Valentina Becherucci,** QC Scientist, Children's Hospital Meyer, **Øystein Åmellem,** Director of Cell Therapy, Thermo Fisher Scientific, **and Xavier de Mollerat du Jeu,** Senior Director R&D Cell Therapy, Thermo Fisher Scientific.

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DH: Today, we'll be discussing the key factors to consider for successful cell therapy manufacture. Valentina, can you tell us a little bit more about what you do at the Meyer Children's Hospital?

VB: I work for a small cell factory located in Florence, Italy, at the Meyer Children's Hospital. Our cell therapy is represented by allogeneic bone marrow derived mesenchymal stromal cells (MSCs), and we have two approved clinical protocols. One is for the treatment of steroid-resistant graft versus host disease (GvHD) in pediatric patients. GvHD is a pathological condition that can occur after an allogeneic transplant system. The second clinical protocol is a multi-center Phase 2 study in collaboration with several Italian cell factories, for the treatment of older patients with COVID-19-related pneumonia.

In short, the production process is completely open, as we work under a class A cabinet, with a class B background. The production process starts with the isolation of MSCs from 10ml of bone marrow, and thanks to their ability to grow on plastic surfaces, we use plastic flasks of different sizes for the culture. As you know, MSCs are only 0.001% of bone marrow white blood cells, so they must be isolated and cultured to reach therapeutic doses.

Our production process takes about four weeks of cell culture, with media changes two times per week. Our cell culture medium is composed of the gene element supplemented with a 5% human platelet-lysate, produced completely internally. After one week of culture, the cells reach about 80% confluence on flasks, and they are decanted, counted, and treated again until we reach the therapeutic dose (1 million cells per kg for pediatric patients, and 3 to 4 million cells per kg for older patients). After about three or four weeks of culture and at the last passage, the cells are counted, frozen, and stored into the nitrogen enclosure until they are injected into the patient. It is important to understand that for both protocols we produce one batch for one patient, and for a media of two batches produced in one month. As I said before, we are a small cell factory.

XMJ: Are those autologous therapies?

VB: It is allogenic for both protocols. It is one donor, one batch, one patient.

XMJ: How do you find your donors?

VB: Our healthy donors give bone marrow for transplantation to the international bone marrow bank.

XMJ: Do you do any selection before?

VB: We count the white blood cells.

ØÅ: In the field of MSCs, it is also normal to use a source from umbilical cord blood or adipose derived materials as well as bone marrow. Is there any specific reason you have selected the cells from bone marrow rather than from other sources?

VB: It depends on the type of therapy. In our hospital, we work in the onco-hematology department, so we have practiced bone marrow transplantation for other malignancies. The cells are the same; with MSCs, the potency is the same whether they are derived from bone marrow or from adipose tissue or cord blood. In our case, we used bone marrow-derived because it was easier for us to source.

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ØÅ: That makes sense. When you have a four-week manufacturing time, that means that the cells are undergoing several passages. Do you have criteria for how many passages you run in your manufacturing process, in order to not lose the cell's characteristics? Do you count the number of passages so that you get to the desired end point of your drug?

VB: The data of all culture comes out after process validation. The goal is to reach the therapeutic dosage. The culture can be shorter – you can stop it at three weeks and not four weeks. It cannot be more than four weeks because, according to the literature, if you culture for more than four or five weeks, you can get some unwanted effects on cells. For example, you can get genetic variation that is not good for the patient. The four weeks comes from our process validation, where we produced five batches of MSCs, and in these batches we saw that the variability was low in terms of the number of cells after four weeks of culture. We also checked other parameters of MSCs, for example the antigen expression of specific markers that must be positive or negative according to International Society of Cell Therapy.

XMJ: Valentina, in this four-week process, how do you ensure you maintain sterility? Do you do weekly/daily QC monitoring on your process?

VB: In our process, we perform initial sterility before starting the culture directly on the bone marrow. Then, we perform an in-process control of sterility after two weeks of culture, and at the end of the culture, before freezing. In our process, cells will be frozen after four weeks of culture and then stored in liquid nitrogen until you get the patient. In this case, the sterility is performed both on cells and on the cell culture media, on the supernatant.

Q **DH:** What are the QC or analytical tests you implement in your process to ensure the safety and quality of the product?

VB: According to the regulatory specification, the testing methods must be validated, and mandatory regular testing includes testing of the sterility, endotoxin, mycoplasma, and karyotype, and in our case, we also perform cell identification with flow cytometry. All these tests are performed as in-process control at different steps of the process, and also for the lot-release at the end of the process.

ØÅ: Valentina – as you are using flasks, you operate in Class A cell culture conditions. Have you tested bags, or a more closed system that you could operate in a hood?

VB: We have tested different kinds of flasks with more surface for culture. However, we do not use bags. Bags are only used in the final step for freezing and storage in liquid nitrogen. We only use open system and flasks.

XMJ: You mentioned it is a Phase 2 process. As you move to Phase 3 and commercial, you will need to scale this process. How are you thinking about doing this?

VB: This is a good question. The goal is to reach therapeutic doses. One way to get good results and to scale up the process for the final production in Phase 3 and commercialization could be, for example, the use of a bioreactor. We are not planning to change the process, because the process is validated and works well like this. We could increase the number of rooms for the production, or we could introduce a closed system.

Q **DH:** Xavier and Øystein, you are working very hard to solve some of the challenges Valentina mentions. What things have you identified? What things are we working on at Thermo Fisher Scientific to address the challenges in Valentina's process?

XMJ: What we hear from Valentina is very common in cell therapy. Cell therapy has incredible potential to cure cancer and other disease, such as long COVID-19. A lot of the process is taught at the bench in R&D. The main question is how to take this to the commercial or industrial phase and scale-up.

Typically, they are either donor- or patient-specific, so you cannot just scale-up the volume and have 1000 doses. It is a scale-out model, where you have many different donors. You not only need to find a way to scale-out, but you also need to find a way to constantly monitor the culture because you are dealing with a moving target. Each donor behaves a little differently. We heard from Valentina, it can go from two weeks to four weeks, so you have to find ways to adapt to this.

At Thermo Fisher, we are trying to develop the tools to allow you to do this. For example, having a closed- system, meaning a platform that can be in bags outside the hoods, so you can do it in different classes of rooms to reduce cost. This will increase your ability to put multiple instruments in the same room and treat many patients in the same room. The other way is the idea of in-line analytics: being able to constantly monitor your process so you can adapt and change the culture based on how the donor behaves.

The goal is to enable commercialization and industrialization at scale, and we think through technology, you can achieve that. We are very connected to a network of collaborators and researchers, like Valentina, to really understand each different process. We want to learn more about those process, and apply what we know about technology, working together in partnership and collaboration so the drugs become available to all.

ØÅ: One of the clear trends in the cell therapy industry is that everything is changing all of the time. We see scale-up, we see scale-down, we see scale-out. It is a very dynamic environment depending on the drug platform of choice. That gives the industry quite a lot of challenges to solve, because there is no (or very limited) standardization. Everyone wants to see more standardization, but as long as everything changes all the time, that is a difficult game.

The important thing for us is to work closely with the customer. Manufacturing and developing technologies that will work in a GMP environment takes quite a long time. Working closely with customers allows us to find a better way to improve the system and make it more

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efficient, more tailored, and more potent. To solve some of the biggest challenges in this industry and target difficult application areas, for example solid tumors, we require more tailor-made technologies and systems. Scalability is a key word for us, in terms of providing systems but also the technologies that can be scaled, either up, down, or out.

XMJ: Not only do we want to make and provide good products that people need, but we have also created a network of labs, some based in US, some based in Singapore, to work in collaboration with our customers. We are going beyond just the product and are now thinking about solutions. I agree with Øystein about the importance of collaborations. Once we better understand what a researcher wants to do in a process, then we can apply different tools and work together in a partnership to help them scale.

Q **DH:** Valentina, can you detail your experience with Ilaria Scarfone implementing the MycoSEQ analytics that are critical to the process?

VB: I agree with Xavier in that the collaboration between the final user – in our case our cell factory – and Thermo Fisher specialists was very important in our experience. At the beginning of our process, we had problems with the validation of the mycoplasma testing. We were using the MycoSEQ, and we had some problems because we did not have any amplification of our cells or our medium. We were unsure of the problem with this inhibition, as we did not get any amplification during the PCR reaction. We started a collaboration with Thermo Fisher specialists in Italy, and thanks to this collaboration we solved the problem. It was due to an inhibition caused by the presence of the heparin in our culture medium. After many trials, it was with these Thermo Fisher specialists that we finally solved the problem, and we can now use and validate the MycoSEQ in our process.

Collaboration is very, very important. It is also useful for the development of new QC testing, for example sterility testing. The future of the sterility testing is dependent on the molecular way, instead of classic culture testing.

XMJ: That is a great point and a great example of why this is so critical. When we developed those products, we had standard assays. We used typical media and we tested. Once we put the product on the market, if it does not work, we cannot just say sorry – we need to follow up. Then we found the heparin in the media, and that allows us to better understand our products, and design better.

The second point Valentina made, which is very important to us, is getting feedback so we can develop the right products. It takes a long time, up to four or five years to make a product, so you have to choose it right. If you do not have constant feedback, then you may spend five years in development to launch the same product as someone else, and it will not help anybody. A constant back and forth helps to better design. In cell therapy, where things move so fast, we need to always be able to adapt if needed. Ultimately, it is about providing the right products that allows standardization, scale-up, commercialization, and make those drugs available to everybody. That is the goal.

Q DH: The MycoSEQ product is an interesting example because it has been around for a while and is used in many processes. As Xavier said, cell therapy is moving so fast, and suddenly there are requirements to test for mycoplasma in sample matrixes that contain heparin, which is critical to cell processes. Solving that problem, alongside our customers, is critical to meet regulatory requirements and bring these therapies safely to the clinic. Are there any other features we are working on now in Thermo Fisher, or from your side Valentina, that you think will be critical in the future?

VB: The most important innovation for the future in cell therapy field is the sterile processing. Manufacturers of synthetic products must address the requirements for sterile processing, especially for autologous settings. We use allogenic cells, but there are many cell therapy products used in autologous settings. They have short shelf lives, so sterility testing is critical for these kinds of products. Good sterility testing must be as fast as possible, so it is important to think about molecular assays for sterility testing. The European Pharmacopeia is going to approve rapid sterility testing with PCR assay. I think this will be the future of sterility testing.

ØÅ: Valentina, you mentioned that you free the cells after manufacturing. Have you met any challenges regarding thawing and stability of the product after freezing?

VB: After testing the stability of the product, we found that our product is stable for ten years after freezing. We tested during the process validation, and after freezing the cells they were stored for liquid nitrogen, six months, one year, and two years. To test the cells, they were thawed, then cultured again, then we evaluated the ability and adherence to plastic surfaces. We found the ability of overall 90% after thawing after six months, one year, and two years, meaning cells were stable.

XMJ: Valentia, we hear what you said about rapid sterility a lot. As you mentioned, it can take too long, and people want to release the drugs. We see processes getting shorter, but it is important to still wait for the sterility before you inject your patients. We are working on rapid sterility tests like you mentioned, using molecular tests. Just as importantly, you want to ensure you work with the regulatory body to make sure the tests are being validated and approved for this kind of use.

Even to do a QC analysis, people can use up to 50% of the total cells. They make the cell product and 50% of it is used just for QC. We are trying to make smaller miniaturizations, e.g., multiplex assays, to reduce the need for those cells. This way, you save the products instead of using everything for QC. QC takes a lot of labor and people to run those assays, so we believe that automation would help the field, especially as you scale.

Right now, for a single product you need to run many assays, which means as you scale-out, you need to multiply those assays, and therefore there is an incredible demand for labor. We think automation could be the way to scale-out.

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VB: I agree. You have to eliminate the operator dependence, even for mycoplasma testing. As you know, the European Pharmacopeia allows three methods for mycoplasma sterility. One is the culture media, but this is operator dependent. If you use PCR or MycoSEQ, you eliminate this variability, and this standardizes the whole process.

ØÅ: Valentina, in your network of academic partners, have you discussed transferring the manual, open process to an automated, closed, bioreactor-type process?

VB: The reality here in Italy is that we are academics in hospital cell factories. We are a small group, not an industrial or commercial group. Considering scalability, we have discussed the use of closed systems. On the market, there are some examples of closed systems, such as bioreactors, which could be advantageous for our production, for example to reduce the personnel that operate inside clean rooms. As you know, you have to understand how to work inside a clean room and pay attention to how you move and the products that you bring inside. There are some issues in dealing with the presence of people inside the production room.

ØÅ: The MSC space is a perfect example of the need for people coming together with different abilities: the industry, academia, and all to progress the field. It seems obvious that it is hard for an academic environment to take on a huge industrialization and transfer to an automated, closed system. It speaks to the importance for more collaboration across the industry to advance this field.

VB: One of the issues in cell therapy production is control of the variables in the process. In our process, even if validated, but controlling variability, you are still using human-derived products, starting from the cells. In our case, even the culture media is derived from humans. One of the goals in future cell therapies is to develop tests to control this variability and to standardize both QC testing and the production itself.

XMJ: Regarding your last point Valentina, we should have better chemically-defined media to avoid variability. You already have variability with your donors, so we should not add variability with the media of the products. This highlights **Øystein's** point that it is a concerted effort between academia, industry, and tool providers like us to work together and provide the best technology to be able to manufacture those drugs.

This is a case where we know the potential of this therapy. Now, we need to figure out how to make them at scale. Usually, it is the opposite: you make them, and you hope they are going to work. The difference here is that we already know the results of those drugs, we just have a huge challenge to be able to manufacture them and it will take a concerted effort between the different industry areas.

BIOGRAPHIES

Valentina Becherucci

QC Scientist, Children's Hospital Meyer

Valentina Becherucci is a QC Scientist at Children's Hospital Meyer. Valentina has a master's degree in medical and pharmaceutical biotechnologies, and a PhD in biochemistry and clinical pathology from the University of Florence, Italy. She has worked since 2010 as a senior scientist in the field of advanced therapy medicinal products (ATMPs), with a special focus on drafting and execution of analytical validation protocols, in compliance with international requirements. During her 10 years as medical and pharmaceutical biotechnologist, Valentina has gained experience in process development, technology transfer, process validation, manufacturing, and biological characterization of cell-based products.

Øystein Åmellem

Director of Cell Therapy, Thermo Fisher Scientific

Øystein Åmellem is the Director of Cell Therapy at Thermo Fisher Scientific For more than 20 years, Øystein has held a variety of leadership positions in R&D, Product Management and Business at Thermo Fisher Scientific. He was responsible for development and commercialization of several products and services, including many for the cell therapy market. He received by PhD from the University of Oslo in the field of molecular cell biology. During his academic career, he focused on the study of physiological & molecular mechanisms of tumor cell growth and was involved in investigating the method of actions for a novel group of anti-cancer compounds developed by Norsk Hydro.

Xavier de Mollerat du Jeu

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Xavier de Mollerat du Jeu is a Senior Director in the Cell and Gene therapy group at Thermo Fisher Scientific, developing new products and solutions for cell therapy manufacturing. Xavier's team is dedicated to new viral and non-viral delivery solutions for T cells engineering and manufacturing, including automation and closed systems. He 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, focusing on identifying the gene(s) responsible for Split Hand/Split Foot Malformation 3 (SHFM 3). During his post-doctoral fellowship at UCSD he studied the roles of microRNAs in pituitary gland development.

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