CELL & GENE THERAPY INSIGHTS

CELL THERAPY BIOPROCESSING & ANALYTICS: TODAY'S KEY TOOLS & INNOVATION REQUIREMENTS TO MEET FUTURE DEMAND

SPOTLIGHT

INTERVIEW

Industrializing allogeneic cell therapy bioprocessing: devising streamlined solutions to complex challenges

LIOR RAVIV joined Pluristem in 2011 and currently serves as Vice President of Operations & Development. Prior to that Mr. Raviv served as Process development engineer and Projects manager & Product development Team leader at Pluristem. Prior to joining Pluristem and during the years 2010-2011, Mr. Raviv held the position of R&D Analytical Researcher at Teva Pharmaceutical Industries. Mr. Raviv holds a M.Med.Sec in pharmacology from the Ben Gurion University and a B.S.c, in Biotechnology engineering from the Ben Gurion University.

Cell & Gene Therapy Insights 2020; 6(10), 1457–1464

DOI: 10.18609/cgti.2020.160

Q Can you tell us what you are working on right now?

LR: At Pluristem we are currently active in two phase III trials that will have readouts within the coming year, interim analysis of the Phase III study in Critical Limb Ischemia (CLI), and top line efficacy results of the Phase III study in muscle

regeneration following hip fracture. We are also conducting phase II trials in Covid-19 complicated by ARDS (Acute Respiratory Distress Syndrome) in the United States, Europe and Israel.

Our main focus is on scaling up the entire operation for market readiness. For years we have been developing our in-house 3D proprietary manufacturing facility as well as our operational processes, so they are ready to scale-up and scale-out in the future.

We are also working on projects around cost reduction and further operational independence, such as our in-house developed serum-free media and in-house preparation of solutions. All this preparation is geared towards market entry.

Q Can you give us more background on some specific bioprocessing considerations, and the approach you take at Pluristem with your cell therapy products?

LR: Our cell source is the placenta, which is a unique and diverse source. We are specialists in expanding the cells and developing our allogeneic products.

Our process and product were designed with a view of delivering an allogeneic product that is able to be administered off-the-shelf – meaning that no tissue matching is needed, only thawing and injection.

We designed our product with the philosophy that cell therapies are very complex, but end users don't need to feel the complexity. They will use the products that are the easiest to use. Our thought process was that we needed to have a very unique and complex product that will also be as simple as possible to use for the end user. It is a bit like the cellphone – the technology behind them is extremely complex, but everyone can use them.

The bioprocessing for our product begins with the collection of the placenta from the hospital, then ranges through manufacturing, and extends all the way to the patient's bedside. We need to control the entire process in order to be able to provide the best quality product with the easiest possible use. Right from the start we had to think backwards, beginning with the end in mind.

The best way to utilize the potential of allogeneic products is by working at a large scale. You can take a sample from a donor and treat thousands, or potentially millions, of patients from one collection. Back when we started in 2007, cell therapy processes were more of an art than a science. Even today, most processes are manual and poorly controlled. We realized that if we wanted to utilize the scale, we needed to have a completely industrialized process that could yield a consistent product over and over again. This is why we decided to invest in technology and in-house manufacturing early on, in order to assure reproducibility and control. We believe that to fully control the lifecycle of a cell therapy product, as

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many processes as possible need to be performed in-house in order to understand, control and improve them during the process and product development.

Based on these decisions we built our processes with an emphasis on closed, automated, controllable technologies, and trained the entire operations in-house. Our main focus was to control and assure the quality along the way, from collecting the placenta until the patient's bed.

Our overarching approach is to sketch the current process and how this will be in the future, to understand where we are, and consider our building blocks: which technologies and which processes we need. Next, we scan for available technology and if we don't find technologies that will help us preserve product quality and characteristics, we at Pluristem develop the technology we need.

Q What is your approach to reducing bioprocessing and process optimization timeframes?

LR: Based on our philosophy of in-house manufacturing and reliance on data and technology, we developed a platform process for adherent cells that is based on automation and control.

The platform we built allows us to change process parameters and play with materials and critical material attributes so we can then adjust the process for a variety of products. Having a platform that can be controlled and adjusted for different products allows us to have a lot of freedom to learn about the product, and to test different conditions in order to understand their effects.

Based on these capabilities we developed an approach we call 'killing a project'. Once we decide to implement a new idea, or to test an improvement for the process, we try to 'kill' the idea and see where it fails. This puts you in the right frame of mind for searching for failure modes in the idea that you are trying to implement. Then, we can tackle these failure options from the beginning of the design. This method of development allows you to build a very robust process or technology.

In order to optimize and shorten bioprocessing times the most important thing is to have increased understanding of both the processes and the product itself – understand the product characteristics, the critical quality attributes, and what the product intends to do.

We have also built a close collaboration between the research and the clinical teams. We always work together in order to understand what critical product attributes we need to preserve when we are implementing changes for process automation.

Characteristics of critical quality attributes and quality assays give us, as developers, a map of where we need to search for changes. We are dealing with a live product that interacts with the environment, so different changes can affect it. Once we build the knowledge space on what the important quality attributes are for the product, we can look for these changes with any new technologies we are implementing through the process. Having unique technologies and working through the years from very low scales to very large scales, has given us a platform to check and test the environment that we are introducing, and how it affects the cells.

Any new ideas that we test start at the lowest scale. We make the change, and then we start introducing new features in the technology for the specific part of the process we are looking

at. We do this step-by-step and the automated processes allow us to do this in parallel. We can utilize many different machines in parallel and check for different conditions in each experiment, learn how these changes are affecting the product, and then advance to the next scale. In this way we learn which parameters we need to guard during changes in order to preserve product quality.

Last but not least, we learned that one of the most important features we need in order to reduce the timeline when optimizing a process is to build a team composed of the different disciplines needed for the product lifecycle. Of course, we have our development teams and engineers, but we add representatives from Quality Assurance, engineering, manufacturing, and regulation. With all of these partners in our development project team, we can accommodate different viewpoints and their needs right from the beginning.

To summarize, based on increasing product knowledge and by using our platform technology which allows us freedom to experiment with critical process parameters, we can create a very fast bioprocess. We measure different conditions in parallel, learn how they are affecting the product, and have a team that supports by providing different perspectives on how to implement the process in the manufacturing environment. This means we can quickly develop and implement robust testing to measure the degrees of potential failures.

Q Can you comment on any particular parts of the cell therapy bioprocess you have/haven't been able to successfully automate to date?

LR: Firstly, it is important to remember that automation in itself is not the goal. The goal of automation is to improve the quality and control over the product.

Our philosophy of quality means we continuously look at the process, from placenta collection to the patient's bedside, to find points that need to be improved. Generally, our approach is always to look for points where we want to improve quality, understand the process parameters, and then find the best solution possible in order to control and improve the quality of that step. Once we have done this, we look for existing technologies in the cell therapy field that could give us the solution.

What we have learned through the years, being one of the first companies that worked on these large scales in mesenchymal-like cells (MSCs), is that many of the solutions we were looking for, did not exist.

Whenever we can't find a ready-made solution, we develop it ourselves, often in collaboration with partners. By way of an example, when we started working on producing cell therapy products, the existing technology for large-scale manufacturing of mesenchymal-like cells involved cell factories, either 10- or 40-stack. We understood that it would not be possible to have an industrialized process at large-scale using this technology, so we started working on a unique bioreactor system, which created the required environment in a closed and controlled system. This technology did not exist for cell therapy at the time - we developed a vast proprietary data and large number of patents around how to adjust this platform for cell therapy, and how to harvest cells in closed systems from this environment.

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We took this approach because we understood there was a gap, and we built a technology that could grow with us through the years. We were one of the first, if not the first, to enter into phase I with a bioreactor technology based on this line of thinking.

Now that we had a technology for growing cells at large-scale, we needed to implement the same steps in the downstream processes: cell concentration, washing, and fill and formulation. Again, we searched for reliable technologies. We found a continuous flow centrifuge we could add, and we were one of the first to implement the kSep technology back in 2011. Next in line was fill and formulation, and there was no solution for large-scale formulation of mesenchymal-like cells that would be filled into vials. So again, we designed our own automated formulation systems.

Another area we tackled was thawing using water baths – when you think about large-scale distribution of our products, working with water baths as an end user may be challenging. Doctors are not expected to be cell therapy scientists, and we want to have a robust process. We developed a dry thawing device, which is a fully automated step. We can now implement this thawing device in the clinic, and the doctors don't need to make any special preparations. The device learns from the bar code which product and which process to use; the doctor just needs to press the play, and in a few minutes the thawing will be completed. The device will also alert the user if anything went wrong.

Therefore, we don't see any challenges in automation. Where there are gaps in the available technology, we see it as an opportunity to develop the technology we need.

Q What are the keys to successfully integrating automated steps in order to streamline processes?

LR: Through the years we have continuously improved the process of integrating new technologies – I would sketch out the key steps as follows:

- Everything starts from understanding your product. Close collaboration with research and clinical teams in order to understand the product's main characteristics is crucial. This gives a map of what is needed to do in order to preserve product quality during development.

- The next step is understanding the critical process parameters for each unit of operations you want to change. If you do not understand the critical process parameters, you will not learn how they are affecting the quality attributes of the product. You can end up with changes to the product that you don't understand, or possibly changes you don't even spot.

- Work on many parameters in parallel, not just one, and understand what happens when you change multiple parameters together, because that will reflect real-life situations. Build the design space of critical process parameters and understand how they affect the product.

- By understanding the critical process parameters, you understand the process that you want to automate. Then, you can change the manual process into automated steps, and do a failure mode test for the device in order to ensure it doesn't create new changes to the product.

- Finally, once you have the knowledge of the process, you can go to full implementation for the device or the process into production and measure it, which will direct your next steps.

We have learned that if you change one step, you won't see all of the effects of the change in that specific unit of operations – you will also see changes in different parts of the process. Performing fully integrated runs for all changes, and learning all of the effects, is truly a must. And as I mentioned before, in order to streamline your approach, you need to build a very good team of representatives from different disciplines in the product lifecycle. This allows your teams to collaborate and implement new designs as quickly as possible.

Q Cost of goods (COG) control remains a critical point for the entire cell therapy field. In your view, where is the field in terms of cost-saving strategies and innovations? Where would you like to see future efforts targeted?

LR: This is indeed a hot topic in cell therapy. I think it came to the forefront a few years ago when we started seeing approval for cell therapy products in the CAR T field, and other fields also.

As an industry, we got to what I call the 'day after'. We worked through the development stages of the company and the product, with Phase III and getting approval in mind. But as companies, we will be measured not on the approval side, but on the day after, where we need to deliver actual products to patients. If we are not able to supply the product, or the product is too expensive, this will affect our success.

It has become apparent in the last few years that efforts need to be made to reduce the cost of processing and manufacturing, in order to make our products viable in the real world. As a company, we have been working towards reducing COG for the last couple of years and we are starting to see the effect.

Choosing to work on allogeneic products pushes you to work at large scales in order to exploit their potential. Once working with large scales, closed systems, and automation, the overhead costs involved in manufacturing and plant size are reduced. This is because you are now working with bioreactors that have low volume but that can manufacture large quantities

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of cells. It reduces the amount of personnel needed to manufacture the same number of cells compared to what would be needed for other manual processes.

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This first decision that we took was a crucial one for reducing COG. We now work with a relatively small manufacturing clean room that produces very large quantities of cells. We also learned that working with controlled systems and automation, and understanding the design specifications of the product, can allow you to discover 'sweet spots' in the critical process parameters design space. These are points where you can increase yield but preserve product quality based on preserving critical process parameters. This allows us to produce even larger quantities of cells under the same conditions. And of course, automation and control reduce failure rates on batches - that will have an effect on the overall COG, too.

Other steps we are implementing in order to reduce cost relate to what we call increasing our ability to have operational independence. As I mentioned, we do our manufacturing in-house but additionally, in the raw materials area, we have done many development studies and have a lot of information and knowledge about what critical material attributes are needed. Therefore, if we have a specific component that we believe we need for the manufacturing process and it has only one distributor, increasing our understanding allows us to potentially work with alternative suppliers and materials. By creating the ability to work with alternatives you can reduce the cost of specific raw materials.

We have also worked on manufacturing our own solutions. We realized that working with fetal bovine serum has a crucial impact on our ability to manufacture and our product cost, so we implemented a project for switching our products to serum-free media. Once we started working with off-the-shelf serum-free media, we saw that our COG significantly increased. But then as we implemented our method of understanding the critical material attributes, we realized that we had the ability to design our own formulation of serum-free media. By doing this, we have full control of our sourcing material, costs, and the capabilities of the media to support the process. By taking control of the formulation and media development, we ended up both increasing yield and reducing the cost of the media.

The next thing we implemented in order to reduce COG was a switch from custom containers. Because we are working in a closed environment and everything needs to be sterilized before entering the clean room, the standard approach is to work with the manufacturer in order to have custom designed packaging suitable for the process. This increases the overall cost. In parallel to the development of serum-free media, we have started building a team that can filter each material solution we buy and adapt it for our process needs, so the container for our process needs is created internally. This gives us the ability to buy any packaging for the raw materials that we need off-the-shelf, and we can then do the container design in-house. This increases the availability of the specific raw material, which increases our independence. The risk of not having the raw materials that you need at the time that you need them is also reduced.

Q Can you sum up your chief priorities for the next 1-2 years ahead?

LR: My chief priority is preparing and readying all of Pluristem's operations for potential commercial market entry, and for worldwide distribution of our product.

On the distribution side, we are maximizing scale of manufacturing and developing new ways of approaching cold chain logistics in order to support our products around the world. It is very interesting, because we are working in both chronic and acute indications. Standard models of distribution for cell therapy that are aimed to support chronic indications will not apply – in acute indications you have just a few hours to get to the patient. We are working very fast and very hard to develop solutions that will get products to patients in under four hours from admission, which we are now implementing in our clinical trials.

On the development side, we are continuing our process of building the next larger scale of technologies that will support our ability to increase production capacities based on the same footprint of manufacturing, in order to preserve and even reduce COG.

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AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Pluristem's process development was partially funded by the Israel Innovation authority. Pluristem is a public traded company (NASDAQ: PSTI). Since establishment Pluristem has been awarded with several R&D grants including but not limited to grants obtained from the Israel Innovation authority, the European Horizon 2020 program and NIH. The process is protected by several patents and patent applications.*

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

Article source: *Invited.*

Interview conducted: *Sep 10 2020;* ***Publication date:*** *Nov 20 2020.*

 DOI: 10.18609/cgti.2020.160