EXPERT INSIGHT

Overcoming the Key Challenges in Delivering Cell and Gene Therapies to Patients: A View from the Front Line

Dr Rivière received her PhD in Cellular and Molecular Biology from the University of Paris. She initiated her graduate studies at the Institut Curie in Paris and completed her thesis in the laboratory of Dr.Mulligan at the Whitehead Institute in Cambridge, MA. During this time, she developed novel retroviral vectors for in vivo long-term expression of transgenes in hematopoietic cells using MFG/SFG-based retroviral vectors that are widely used in clinical studies for the treatment of genetic and acquired disorders. She is currently the Director of the Michael G. Harris Cell Therapy and Cell Engineering Facility where she investigates the genetic modification of hematopoietic cells to increase or retarget the immune response against tumors. Her laboratory has developed cell manufacturing platforms under cGMP conditions for several Phase I/II clinical trials and currently supports 8 CAR-T cell based clinical trials under 5 INDs at MSK. She actively participated in the National Cell Manufacturing Consortium Workshop that has led to the establishment of the Technology Roadmap to 2025 for Achieving Large Scale, Cost effective, Reproducible Manufacturing of High-Quality Cells.

Q The clinical data emerging from genetically modified, patient-specific therapies are driving the field forward; however, these therapies are largely reliant upon the patient's own cells as the starting material – what are some of the challenges associated with this initial step in the manufacture of autologous therapies?

The fact that these are autologous therapies poses a number of challenges that need to be addressed. We are starting with apheresis material collected from the patient in a donor room and really we have very little control over this step as there are a number of different apheresis devices being used across clinical institutions. Therefore, some variability is already being introduced within the process.

In addition, previous treatments received by the patient also greatly impact the composition of the apheresis material – however there is presently very little we can do to reduce the variability in the composition of the apheresis product itself.

We can have some level of control over the next step in the process – in particular, for CAR-T cell therapies – we can influence the characteristics of the product and subset ratios through the selection of the initial cell populations such as CD4, CD8, CD62L or depletion of CD14 or CD25. Various groups have been looking at a number of T-cell subtypes and different proportions and combinations thereof, to determine if a particular combination would be clinically more potent. A recent industry poll asked just this question and the outcome was that there just isn't a consensus in the field right now. I think from the experimental animal models we know that probably a mixture of CD8 and 4 is required but it's not clear as to what the composition should be for example, naïve or central memory/effector memory cells. Further research is still required to understand what the 'ideal' composition of these products should be.

Q What are the key considerations when looking at getting these cells back into the patients within the healthcare facility?

One of the critical issues to consider is how the cells need to be handled from the point of storage to the point-of-care site/infusion into the patient. If the cells aren't manufactured on site, the staff in that institution might not be used to handling these products. Hence, the need for training. This training is either done by the product developers or the point-of-care staff and the approach will be dependent on the facility and their level of comfort with these new types of products.

These products are cryopreserved and have a limited shelf-life once they are thawed. The preservation method is likely to impact the point-of-care administration and handling of the product. Unfortunately, the use of fresh cell therapy products is just not an option because of a number of logistical parameters – you need flexibility in the timing and delivery of these therapies, say for example if a patient requires conditioning prior to administration; or if they fall ill on the day they were scheduled to receive the therapy. Without flexibility in delivery timing, you are going to have extensive product loss and waste. The experience for example of Dendreon demonstrated that – if you are delivering fresh product, you need to be running your facilities 24/7 with shifts of workforce, delivery networks logistics etc. and this becomes unfeasibly expensive and logistically extremely complex.

Therefore, a point-of-care facility is typically going to be working with cryopreserved cell products and therefore thawing is required prior to administration to the patient. The clinical teams at the point of care need to be trained properly and have a dedicated space where this process will be carried out. They need to be confident that they will be able to infuse the cells in the timeframe that the product is stable and viable.

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Ideally you don't want to take the cells out of the bag so as to avoid contamination opportunities; at our institution we take the bag of cells out of the freezer, thaw it and take it to the bed side. We then have a window of stability and acceptable viability of only a few hours. The nurse subsequently hangs the bag and proceeds to the infusion as is, meaning the patient receives the cells mixed with DMSO (the cryopreservation agent). It's by no means a perfect process, but until we find alternative preservation methods with a really robust process for delivery, then it's our only option.

This thawing step introduces additional variability between sites and this is an important issue that needs to be addressed. Ideally you would want to use a thawing kit that enables you to thaw these cells in a standardized way. Standardized cryobags and thawing devices for example that could be used across multiple applications would go a long way towards helping to standardize this step in the cold chain. Some level of oversight by either a consortia or at State level would also be beneficial as we see for for stem cell transplantation and blood supplies where this oversight is already in place. Eventually we will need that level of standardization and oversight for adoptive cell and gene therapies.

Autologous therapies in particular present some unique challenges in terms of tracking the products, namely chain of custody monitoring whilst remaining compliant with confidentiality of medical information protocols. We recently took part in a workshop run by the National Institute of Standards and Technology (NIST) where there was a great deal of discussion around this issue of tracking and the requirement for suitable IT systems to facilitate this.

Q As Director of the Cell Therapy and Cell Engineering Facility as MSKK, which specializes in the manufacture of clinical-grade vectors, what do you see as the main hurdles in this manufacturing step?

The lack of a 'perfect' reagent to genetically modify cells is critical. In terms of vector production, the current focus is on either lentiviral or retroviral vectors, although we know that some others are coming down the pipeline such as sleeping beauty transposon, foamy vectors and other nucleases and gene editing platforms such as CRISPR. For the CAR-T cell and autologous cell therapies we will certainly be tied to using lentiviral or retroviral vectors for some time, until we can go for targeted integration with nucleases; but for now the efficiencies need to be increased and safety profiles need to be established. It will also depend on what subpopulations of cells we need to use and if these are amenable to being targeted by these nucleases. More research is needed to address these questions; in the context of the allogeneic setting, there will likely be more scope to use nucleases, e.g., Cellectis's approach.

The limitations at present with lentiviral vectors are that there are only a handful of stable packaging cell lines, and their usage is restricted to a

small number of investigators. The fact that the VSV-G envelope – which is currently most commonly used – is fusogenic necessitates the use of transient or inducible systems. This is very impractical and investigators are looking at developing stable packaging cell lines. We are collaborating with teams who are working on stable packaging cell lines and are following their progress closely. We hope that they will be able to deliver such reagents very soon.

This challenge is combined with the difficulty of having to adapt the cell lines in serum-free or chemically defined media, and these viruses are addicted to serum! It's proven complicated and lengthy to define the parameters of serum-free and chemically defined media, but progress is being made – we are seeing an increasing number of manufacturers who are working to overcome this issue and I am confident we will be able to find solutions in the near future, which will hugely benefit the research community and patients.

Downstream purification is yet another important factor to consider since we want to limit the contaminant opportunity here. For the gamma-retroviral vectors, the purification is a slightly more complicated process because the envelopes are not as stable; however, we are confident that we can work on changing or modifying the envelope so that we can perform better downstream purification and also produce these vectors in clinically defined media. As I mentioned previously, we are working with collaborators in trying to establish packaging cell lines that are able to expand in serum-free media, and on developing procedures to produce these vectors that can be scaled up in bioreactors at volumes of potentially 500L to 1000L, which could help treat thousands of patients.

In terms of the advantages of one vector system over another, so far the gamma-retroviral vectors are safe for terminally differentiated T-cells. These vectors are currently much cheaper and easier to produce; therefore, there is really no rationale not to use them. Some investigators are focused on demonstrating that gamma-retroviruses are not as potent but I haven't seen the scientific proof yet and further studies in animal models and patients follow-up are required

Q In terms of quality control are there different issues to consider when developing assays for different vector types?

Right now we face timing issues with quality control assays that pertain specifically to lentiviral and retroviral vectors. As manufacturers, we have been trying to come up with new assays to confirm the absence of replication-competent retro- and lenti-viruses. With the current assays available to us, it's not possible to obtain results in real time as we have to perform cellular assays which can take 4–6 weeks, plus a further 2 weeks for quality assurance review. It is also very costly because they involve a great deal of human resource. It is therefore critical that we develop new assays, likely molecular in nature, that will also be cheaper. We need to establish working groups within industry and academia to push this forward. In an ideal world, the development of these assays should remain in the pre-competitive space so that the whole field and specifically the patients can benefit from lower treatment costs.

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These vectors have proven quite safe and thousands of patients have now been treated with CAR-T cells genetically modified with retroviral or lentiviral vectors. Assays that are sensitive and fast enough need to be developed and monitoring needs to be continued until the scientific community is comfortable with the risk profile. It is also important to collate the data we've created thus far to demonstrate that these vectors are safe; not only to link this to how the vectors have been manufactured, but also to use this information to continue manufacturing vectors in a safe way and find new approaches to accelerate their production and reduce costs. Right now we spend between $7,000–15,000 per patient on assays which just isn't viable if we are going to move towards adoptive cell therapies being a facet of routine healthcare.

Q What do you see as the critical steps in the manufacturing process that offer the most opportunity to transform the industry?

From my perspective, I do not think it's a viable option to manufacture these cells in GMP facilities – it is far too expensive, too labor-intensive and prone to too much risk of human error. The processes need to be streamlined, but there are only a few options at the moment which is why it's so important that consortia representing industry, academia, manufacturers and regulators are being formed to tackle this. We want to achieve a level of automation that not only enables you to move seamlessly from the collection of the cells to their genetic modification, but also that will enable us to perform sampling and quality control in an automated fashion. This would also include characterization of the cells during expansion and transduction. Cell counting, which you would think is a very simple process is not. It poses unique challenges that require instrument standardization. NIST has recently created an initiative to address standardization such as cell counting which is performed at various steps of the manufacturing process; characterization of cells using flow cytometry measurements also needs to be standardized if it is going to be used to determine the cell dosing for infusion. There are differences in the clones of antibodies that are currently being used, the types of flow cytometers – and that's before you even introduce inter-operator variability; you can see that there is a huge need for standardization of the analytical methods used to control the manufacturing process.

In terms of the facility requirements and the equipment, there have been some discussions about automating the processes and the need for a continuum between academia and industry. In the academic setting, automating the entire manufacturing process might not be possible due to cost and manpower but that's not to say that there aren't ways in which the processes could be improved. It is essential that academia be involved in developing and validating automation processes; there are a few initiatives in place to support this in terms of grants and consortia for example the Cell & Gene Therapy Catapult in the UK

and CCRM in Canada. The US also need to establish consortia to focus on bringing all the stakeholders together to think critically about the path to commercialization and establish the structure and research necessary to achieve that goal. NIST have recently put forward this type of RFA and we are part of the group working on outlining the roadmap for 2025 for high-quality cell manufacturing gearing towards automation. Krishnendu Roy at Georgia Institute of Technology research center is heading up this particular initiative to develop processes and techniques for ensuring the consistent, low-cost, large-scale manufacture of high-quality living cells used in cell-based therapies. This will hopefully serve as the blueprint to establish the consortia that will be competing for the NIST application which will be in the order of $70 million and should bring together all the key stakeholders including manufacturers, academia, biotech, suppliers, government organizations and regulatory bodies.

Whilst automation is going to be an important component in being able to bring these adoptive cell therapies to the commercial space, so too is the issue of 'closing up' the processes. The industry is trying to achieve this aim by selecting subsets of T cells from leukopheresis products through column purification (with beads or other means), followed by transduction in a chamber that is linked to that purification column and growing the cells within the same device. Using software, we would want to be able perform sampling, checking parameters of the cell growth, characterization and counting without taking the sample out. Perhaps this is not achievable within the next 5 years, but we are certainly working towards that goal.

Q What's your vision for how vector and cell therapy production will be carried out in the next 5 years?

In terms of vector production, I think the focus will be on site-specific integration of vectors. In the packaging of cell lines, there are already some groups producing cell lines in which the vectors integrate into a specific site so that there aren't multiple integrations, which will greatly reduce risks. The utilization of nucleases will probably prove to be useful and whilst the level of non-specific integration will need to be controlled and mapped before we can move them towards the clinic, I think these issues can be overcome.

Automation and closing-up the manufacturing processes will be critical. I think it is equally important for the IT systems to monitor the chain of custody of the product from collection in the donor room through to infusion back into the patient. This includes production records detailing the parameters and how these parameters compared to other patients etc., so that this information can be collected and analyzed in real time and could in turn inform the outcome in the patient.

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