SEPARATION AND EXPANSION TECHNOLOGIES

SPOTLIGHT

INTERVIEW

Fueling a Commercial Reality: Optimization of Separation & Expansion Across the Manufacturing Pathway

DAVID DIGIUSTO Dr David DiGiusto is the Executive Director of Stem Cell and Cellular Therapeutic Operations for Stanford Hospital and Clinics and a Senior Academic Researcher in the Division of Stem Cell Transplantation and Regenerative Medicine at Stanford University. He has over 25 years of experience in the scientific, clinical and regulatory aspects of cells as therapeutic agents including the isolation, characterization and genetic modification of hematopoietic stem cells and T-cells for clinical applications. He has been instrumental in the creation of 6 GMP compliant biologics manufacturing facilities and associated quality systems, production and QC testing programs. Under his direction, plasmid DNA, CAR-T-cells, regulatory T-cells, engineered stem cell grafts and gene modified hematopoietic stem cell products have been manufactured and released for use in Phase I/II clinical trials. Dr DiGiusto is a major contributor to first in human (and other ongoing) studies for Cancer and HIV Gene Therapy and has developed methods for assessing ex-vivo stem cell manipulations using in vitro and in vivo models. His laboratory (The Laboratory for Cell and Gene Medicine) specializes in the development of manufacturing processes and QC assays and provides cGMP compliant clinical materials production and regulatory support activities for investigational cell products.

Q Your extensive experience in the field includes utilizing HSPCs for potential clinical applications. What do you see as the stand out developments in the utility of HSPCs over the last decade?

DD There are a number of areas in which hematopoietic stem and progenitor cells (HSPCs) have been investigated and developed

as potential therapeutics, with some of the most notable early successes being seen in gene therapy for monogenic diseases, with the development of Strimvelis for ADA-SCID as an example of a stem cell therapy that has gone all the way to approval, at least in Europe, as a therapeutic entity. This approval was a strong step towards validating the idea that there can be therapeutic entities beyond just transplantation for hematopoietic recovery. Another great example is the promising advances in the development of stem cell-based commercial therapies for Wiskott-Aldrich Sydrome.

More recently the demonstrable ability to engineer the genome in hematopoietic stem and progenitor cells offers the possibility of correcting other genetic diseases, or possibly infectious diseases, based on the fact they can very precisely edit the genome not only through gene disruption but also corrective recombination or gene insertion into known sites. This new technology really opens the door to novel, potentially curative treatment of many monogenic and infectious diseases which is very exciting for the field and of course patients.

Over the last decade there's been a shift in the way we use stem cells. We started to understand that T-cell depletion could lead to very successful transplantation across allogeneic barriers, as evidenced by haplo-transplantation which has become a standard approach. Engineering these grafts by removing T-cells resulted in a clear reduction in the incidence of Graft versus Host Disease (GvHD) and would enable successful engraftment, which means patients who otherwise wouldn't have a matched donor can now be transplanted with a parent or sibling's cells. This represented a huge change in the transplantation field and an important advancement for the cell therapy sector.

These are some of the most exciting developments I've seen with HSCs over the last decade, and I'm sure we'll see many more coming from those areas over the next few years.

Q Optimizing cell processing and selection for scale-up is considered critical for the successful commercialisation of cell and gene therapies. What are the major challenges in these critical steps in the manufacturing pathway?

DD Critical areas for development are the move to automated closed systems, and the ability to efficiently process small- and large-scale products. These processing steps, whether at small or large scale, include cell washing, concentration, formulation and separation. For example,

DOI: 10.18609/cgti.2018.001

processing of a master cell bank for viral vector production, or master cell bank for allogeneic mesenchymal stem cells (MSCs), might require not only an upfront processing step of the starting material, but also the downstream processing of the cultured cells that comprise the final drug product. With the current processing methods, these require large-scale processing with very high quantities of cells. Whereas at the other end of the spectrum if we look at HSPC-based gene therapies for example, the number of required cells drops by a couple of orders of magnitude, so you then require the equivalent processing systems but at a much smaller scale.

The challenge arises when you want to maximize the efficiencies of larger-scale processing but with much smaller number of cells and that's where I think the move to automated closed systems can play a crucial role.

Another key factor that can greatly impact the efficiency of your manufacturing process is yield. Certainly, we are seeing improvements in this area with the development and refinement of specific equipment and media, but some cell types are just more difficult to grow. T-cells are routinely easier to culture than HSCs or neural stem cells and therefore the issue of yield is greatly impacted by your cell starting material selection.

And of course, what this comes down to is that these issues ultimately impact your cost of goods – and we hear this a great deal in the sector: cell-based therapies are not cheap to manufacture. As we look to develop more automated solutions the expectation is that this will help drive down the cost of manufacturing, but it's an iterative process and remains a critical challenge for the sector.

Q How have the tools and technology evolved over recent years to improve cell processing efficiency?

DD Following the positive CAR-T clinical data and recent approvals for gene modified cell-based therapies, the industry is starting to view the field as a commercial reality and manufacturers are incentivised and committed to developing tools, platforms and devices that can help address some of these critical challenges across the manufacturing pathway.

An example would be Fresenius Kabi's Lovo instrument for the automation of cell washing, concentration and formulation in a functionally closed system. We've employed this device in my laboratory for a couple of years and have seen a significant reduction in labor and

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total time required for cell processing – removing up to as much as a third of the processing time requirement. In addition, it improved our cell selection process by automating the essential step of platelet removal from apheresis products and enabled us to completely close the upstream part of cell selection including bead labelling and washing. The fact that it's automated ensures reproducibility of the process despite the highly variable source apheresis material, reduces manual input and thus reduces potential risk of product contamination. Fresenius has been very responsive in developing software routines to optimize our processes and save protocols once established for quick retrieval on subsequent products.

This is a good example of how an equipment manufacturer has addressed a specific need: replacing a labor-intensive process with a completely closed and automated alternative. This approach is going to be a key factor in the successful commercialization of cell-based therapies.

If you can take an apheresed or any heterogeneous suspension cell product, concentrate, formulate, and separate it into the components you want in a completely closed and automated system, that's a dramatic improvement upon the existing manual process. It ensures the reproducibility and robustness of the process, and can be replicated without a need for operator-specific skill sets which will be a key consideration when it comes to the commercialization of cell-based therapies.

Q There's discussion within the sector concerning the merits of upstream (pre-culture) purification of cell populations versus downstream (post-culture) purification – can you share your thoughts and experience on this?

DD The decision regarding the optimal stage at which to carry out the purification step completely depends on your overall manufacturing process. Upstream purification might be dictated by the need for efficiency within your process, for example if you want to genetically modify CD34+ cells or HSPCs it makes sense to enrich for that cell upstream because it will greatly reduce the culture volume, the amount of virus needed to transfect those cells and cut the amount of reagent required.

DOI: 10.18609/cgti.2018.001

Therefore, it's clear that if you go from 1 to 99% purity of your target cell population through enrichment then the potential time and cost savings across subsequent processing steps can be significant.

Another example of when upfront purification is beneficial is for the critical T-cell depletion step I mentioned earlier in the context of minimizing the risk of GvHD from a HSC allograft whilst preserving the Graft versus Leukemia effect. These are just two examples of meritorious upstream purification.

The rationale for downstream purification pertains to certain situations where you require a supportive cell layer for the culture of your target cell population that then needs to be removed from the final drug product. Many groups for example use irradiated peripheral blood mononuclear cells (PBMCs) to support the growth of T-cells, and whilst these supporting cells tend to die off during the process, you still require a washing/purification step to remove the debris and obtain a clean cell population.

Another scenario in which downstream purification is merited is the development induced pluripotent stem cell (iPSC) products. With these cells, differentiation towards a specific lineage is never 100% and therefore you need to have some mechanism of removing the unwanted cells or cells that have not achieved full differentiation so as to remove safety concerns of tumorigenicity.

That's just two examples of where you'd want to do purification upstream or downstream for different processes and end products.

Q How do you envisage the manufacturing pathway evolving over the coming years as we see more cell and gene therapies move towards the clinic?

DD Automation is going to play a large role: minimizing labor dependence and reducing skill set requirements will make the manufacturing processes robust, reproducible and more cost-effective.

Defining unit operations will lead to discrete processing steps and devices to support activities with broad applicability. Take those upfront cell processing steps we just discussed – that's a unit step that can move to a closed-system, automated platform. Another unit step amenable to automation could be methods for culturing cells – whether it's MSCs, HSCs, neural stem cells – the core platform technology is developed and then culture systems or media can be specifically designed for those different cell types. A great example is the evolution

of culture bags. 10-15 years ago, culture bags were seen as a way of culturing cells without having to use a flask. As technology and our understanding of the biology of the different cells types has evolved, these culture bags are now being used to optimize the cell culture step for example by customising the internal surfaces to support adherent cell culture requirements.

I think the manufacturing pathway will continue to evolve around platform technologies such as cell selection, washing, culture and separation and that those types of modular components of manufacturing process steps will start to emerge to address manufacturing challenges. While approaches to combine whole processes into one device are attractive, modular, unit operations approaches may provide more flexibility. For example, a single device may work well for upfront processing (cell washing) and then culture of an autologous T-cell product having been designed for that specific application. However, the use of the same device to manufacture a large scale allogenic T-cell product preparation would benefit from the same upfront processing but may not be able to support the scale of product culture required to produce a bank of cells to be used on 10's to 100's of patients. Similarly, upfront processing for cell enrichment followed by culture may be suitable for cells that can be grownup afterwards (CAR-T cells) but perhaps not as suitable for cells that are difficult to expand without changing their properties and thus require high yield of a limited number of starting cells (HSPC). The use of custom unit operation devices will allow the users to incorporate the best process at each functional step based on product characteristics.

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Another aspect is the actual facilities size – I'm on my sixth GMP facility and have seen them developed both as purpose-built facilities and as modular systems, and there are pros and cons to both.

A fixed, purpose-built facility for a specific commercial product is always going to be an approach some companies take to manufacturing. But I think modular facilities can be beneficial in allowing people to grow incrementally and not having to nail down your manufacturing processes upfront when you are in early stage development. This approach also provides flexibility in the availability of clean rooms and clean room support services and equipment, which can make it cost-effective option for pilot studies without the cost of a CMO or a large dedicated manufacturing facility.

DOI: 10.18609/cgti.2018.001

Q In your opinion, when is the right time to evaluate and adopt automation?

DD The decision on when to evaluate the inclusion of automation depends on product history. Many products have production procedure updates after Phase I studies in order to improve outcome. For this reason, automation may not come into play until between Phase I and Phase II clinical trials when patient number increases and the scale or nature of production may dictate a more reliable and robust process is developed. Other products may be a variation on previously tested themes and can have proven automation included in the process early on. An example of the latter is automated processing of apheresis products.

Q What was the rationale behind the development of the first dedicated cell and gene therapy manufacturing facility – the Laboratory for Cell and Gene Medicine for which you are Executive Director?

DD Stanford School of Medicine is widely known for its stem cell and medical research; we have hundreds of investigators and a very productive pipeline of candidate cell and gene therapy products. The rationale for developing a GMP facility was to support the movement of this pipeline of candidates through clinical evaluation to determine their efficacy and merit for further development by Stanford. Our approach is not just to comply with federal regulations on the identity, purity, potency and safety of these products, but also to de-risk the assets. Having been in the field for many years, I know how difficult it can be when a pharmaceutical or biotech company wants to out-licence technologies developed in an academic institution. Many of the processes are not automated, they are labor intensive, assays are not qualified or ill defined, reagents might not be suitable for clinical use or commercial-scale manufacture for example.

The facility's role is to try and de-risk the assets by addressing all these factors. When a new asset is brought to our facility for development we will assess it from all angles: raw and source materials, process development – removing non-commercializable methodologies, clinical application, target patient population and regulatory considerations. We then prioritise an asset based on the likelihood of success and where we think we can support its development through Phase 1 and Phase 2, to demonstrate the safety and efficacy, thus ensuring it is a much more attractive product for potential out-licensing.

In some ways we are operating as a small biotech, with a product pipeline of 10 assets currently in play, with 8 open clinical trials and 2 preclinical development studies going on right now and another 8 or 10 waiting in the wings as soon as we free up some resource.

Q As the cell and gene therapy industry matures, how do you see the role of academic institutions evolving and what potential impact will they have on the sector's development?

DD The model of translating a product from academic centers to a commercial partner has been shown to be possible and successful in certain places such as UPenn and City of Hope; but there are also a lot of institutions that struggle with this approach. To make this model work requires effective collaboration between often complex hospital, research and translation infrastructures, and that can be achieved in many but not all places.

That model will continue to develop over the coming years, fuelled by the success of commercial entities who have out-licenced products from academic institutions.

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DOI: 10.18609/cgti.2018.001