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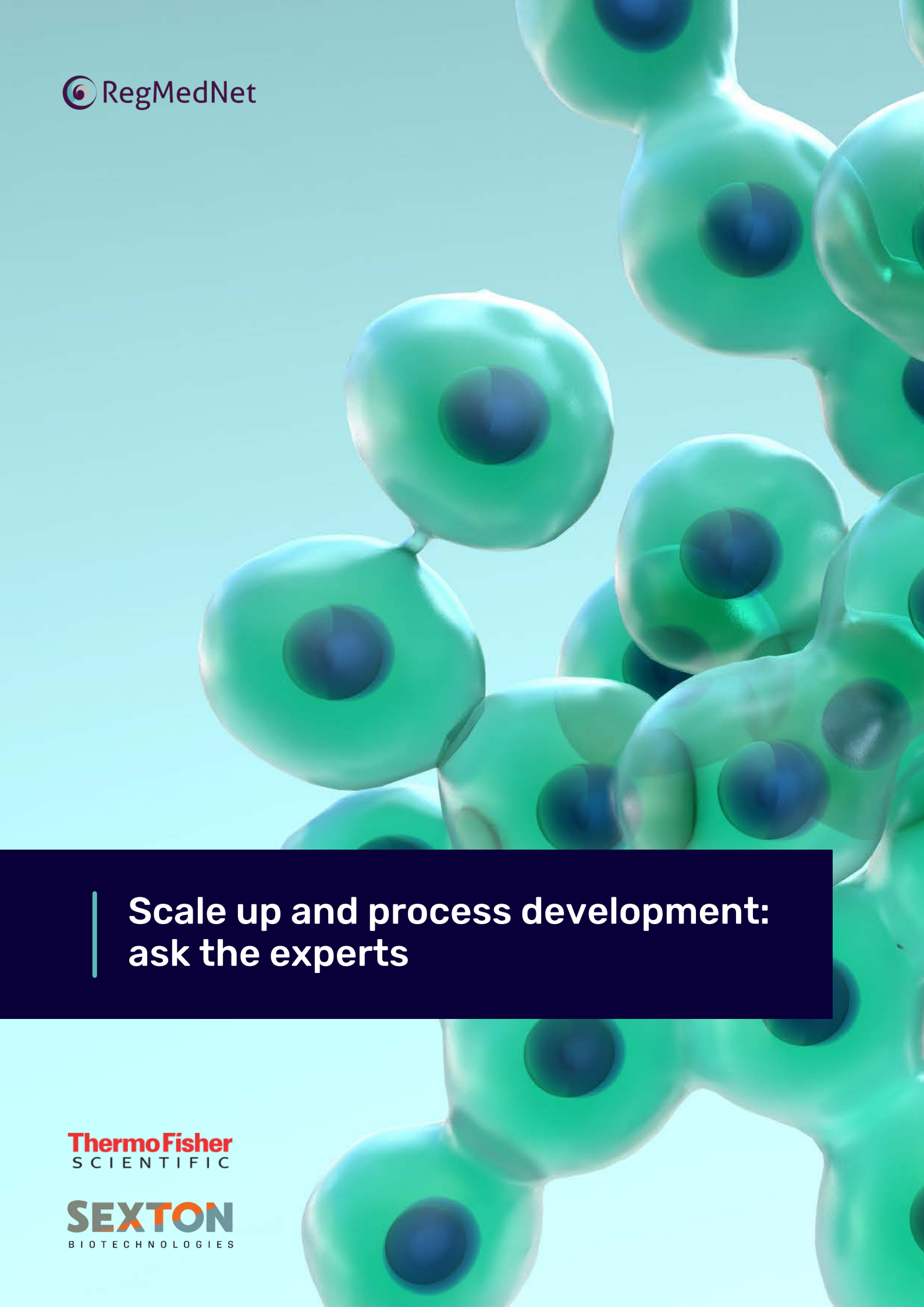

Scale up and process development: ask the experts

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Scale up and process development: ask the experts

Meet the experts:

Hetal Brahmbhatt,
Principal Investigator, Pharma Services, Viral Vector Services
Thermo Fisher Scientific

Dr. Hetal Brahmbhatt is a Principal Investigator in the Science and Technology Team, Pharma Services, Viral Vector Services at Thermo Fisher Scientific. She has a PhD in Biochemistry and Biomedical Sciences from McMaster University where she characterized small molecule modulators of apoptotic proteins. In her previous role at Thermo Fisher Scientific (former Brammer Bio), she developed and established downstream purification processes for clinical manufacturing of several gene therapy viral vectors. In her present role, she contributes to the development of platform processes and technologies for the manufacturing of viral gene transfer vectors.

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Steven Thompson,
Vice President of Sales and Product Management,
Sexton Biotechnologies

Steven Thompson is Vice President of Sales and Product Management at Sexton Biotechnologies. He has worked in the Cell and Gene Therapy space for 16 years in both academic and commercial settings, gaining extensive knowledge in Research and Development through to scaled GMP manufacturing. Currently, he leads the business development team to engage with therapeutic developers to fully understand challenges they face during scale up and manufacture, interpreting these problems and working with the internal development team to devise novel technologies for commercialization. He has worked within the launch of several commercial technologies which are now actively used in clinical studies around the globe, and possesses a deep understanding of the technical, regulatory and business hurdles therapy developers must address in order to successfully bring a novel therapy to market.

SEXTON BIOTECHNOLOGIES

Paul Carter
Head of Vector Processing
Quell Therapeutics Limited

Paul Carter is the Head of Vector Processing at Quell Therapeutics Limited and has over 30 years' experience in the pharmaceutical industry, working across cell and gene therapy, biopharmaceutical and small molecule R&D. Prior to joining Quell, he was leading GSK's Downstream Process Development Group in Cell and Gene Therapy.

Carl A. Gregory
Associate Professor in the Department
of Molecular and Cellular Medicine
Texas A&M University

Graduated with first class honors degree in biochemistry from the University of York, UK and a PhD in molecular and cell biology from the University of Manchester, UK. Headed an academic research program at Tulane University between 2005-2008, then Texas A&M from 2008-present day. Specializes in developmental biology, stem cell biology, regenerative medicine, bone repair and metabolism and bone malignancy. Currently working on methods for large scale production of stem cells and their therapeutic products.

Roland Kaunas
Associate Professor and Director of
the Biofabrication Laboratory in the
Department of Biomedical Engineering
Texas A&M University

Roland Kaunas is an associate professor in the department of biomedical engineering at Texas A&M University. He has over 20 years of experience in academic research investigating interactions between cells and their microenvironment. His laboratory currently focuses on the fabrication of engineered scaffolds containing mesenchymal stem cells as vehicles for scalable cell expansion, regeneration of musculoskeletal tissues and cell-based models for studying bone tumor biology. This work employs sophisticated microfluidic platforms, engineered microenvironments, custom bioreactors, and novel scaffolding strategies involving composites of natural and synthetic polymers. Roland has also served as the associate director of the National Center for Therapeutics Manufacturing, where he established capabilities for expansion of human cells for cell therapies. Current projects include expansion of mesenchymal stem cells on digestible hydrogel microcarriers in PBS vertical-wheel bioreactors, as well as production of secreted products.

Scale up and process development: ask the experts

In this 'Ask the experts' feature, a panel of key thought leaders share their perspectives on current obstacles and future developments in scale up and process development For example, at what point should you start to consider scaling your process, and what questions do you need to have answered? Would it be better to scale-up or scale-out your process, and how can external partners support you? Discover more about this from our expert panelists, Steven Thompson (Sexton Biotechnologies, IN, USA), Hetal Brahmbhatt (Thermo Fisher Scientific, MD, USA), Paul Carter (Quell Therapeutics Ltd, London, UK) and Carl A. Gregory and Roland Kaunas (Texas A&M University, TX, USA).

- Please introduce yourself and your institution
- What are some of the differences between research/investigation-scale manufacture and commercial/clinical-scale?
- What are some common mistakes developers make during process development for expansion to commercial-scale manufacture?
- How could turnkey or closed system approaches address these mistakes or challenges?
- How can fill/finish considerations change depending on the product and anticipated delivery chain?
- What will scale-up and process development look like in the future?

Hetal Brahmbhatt,
Principal Investigator,
Pharma Services, Viral Vector Services
Thermo Fisher Scientific

1 Please introduce yourself and your institution.

My name is Hetal Brahmbhatt. I am a Principal Investigator in the Science and Technology Team in the Pharma Services group in Thermo Fisher Scientific. In my current role, I oversee the development and establishment of upstream and downstream platform processes for the clinical manufacturing of viral vectors. I have over 4 years of experience in the scaling up of viral vector manufacturing processes to cGMP for Phase I-III clients.

2 What are some of the differences between research/investigation-scale manufacture and commercial/clinical-scale?

In viral vector manufacturing, the commercial or clinical scale is dictated by the vector type, indication and mode of delivery, for example e.g. the amount of recombinant adeno associated-virus (rAAV) required for direct injection of a retinal gene therapy may be less than for a systemic delivery for diseases such as hemophilia. On the other hand, lentiviral vectors are mostly used ex vivo and the production scale requirements will vary accordingly.

Viral vector manufacturing processes are adherent or suspension-

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based cellular systems. Most commonly, the research or investigation studies for suspension processes are conducted in shake flasks or ≤ 10 L bioreactor scales, using non-single -use equipment, whereas the commercial or clinical scales range in the 50-2000 L scales that often rely on single-use equipment. The research or investigation studies with adherent cells are often conducted in culture systems like T- flasks, cell factories and HYPERstacksTM. Depending on dosage requirements, scaling for a commercial or clinical process may involve a scaling out approach in which the culture systems are multiplied to achieve a final volume or include a scale- up process in adherent bioreactor systems like Pall's iCELLisTM system.

3 What are some common mistakes developers make during process development for expansion to commercial-scale manufacture?

The gene therapy field is rapidly growing with potential cures for debilitating diseases, which makes speed to successful clinic/market launch more critical for biopharma. With that, gene therapy companies are having to make strategic business decisions between speed and robust CMC packages. In the former situation, gene therapy products may be approved using manufacturing processes that are not appropriate for a commercial scale. Development of commercially scalable processes occur either in parallel or sequentially and will require appropriate comparison studies requiring additional process development time. In the latter situation, the investment is made up upfront in ensuring the manufacturing process meets the commercial process requirements,; however, the compromise here is in the speed to market.

Additional areas that should be addressed during process development stages are automation approaches for commercial-scale manufacturing, compatibility with single single-use systems, incorporation of adventitious agent clearance and inactivation steps, use of cGMP suitable raw materials and ensuring supply of critical raw materials or alternatives to avoid supply chain issues.

4 How could turnkey or closed system approaches address these mistakes or challenges?

Turnkey approaches can prove to be very beneficial if designed to target the current gene therapy manufacturing challenges and address many common issues in process development. Turnkey solutions, as seen with monoclonal antibodies (mAb) processes today, can streamline the workforce training demand, increase process robustness and reproducibility, reduce tech transfer and process validation time, reduce manufacturing time, and ensure raw material supply availability. Ultimately, patients can receive their treatments faster.

5 How can fill/finish considerations change depending on the product and anticipated delivery chain?

Important consideration areas for the fill/finish of viral vectors include filter material type, filter size, vial type and product stability. Optimization of fill/finish conditions at the target product concentration in the formulation of choice is recommended to ensure minimal product loss from filtration or adsorption. Stability of viral vectors is key when planning the fill/finish process and customer delivery. Storage durations and storage temperature of intermediates and the final product must be established when planning the fill/ finish and delivery process. Notably, duration of testing required for drug product release will also impact the delivery to the customer.

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6 What will scale-up and process development look like in the future?

In the future, it is expected that the manufacturing processes for gene therapy will be standardized like the mAb processes today. As the demand for viral vectors and scalable manufacturing processes increases, it is likely that the cell lines used will be suspension- -based. The field is seeing a shift towards the use of producer cell lines as ideal solutions. As seen with mAb processes, it is expected that the manufacturing processes will be conducted in closed systems in a semi- continuous mode with automated feedback PAT loops. All offline analytics will be conducted in a high-throughput manner with capabilities for absolute quantification. With this, it is expected that the process development stage will be focused solely on the establishment and fine- tuning of process steps rather than the development of process steps. Additionally, it is also expected the additional analytical technologies, which will provide orthogonal readouts for safety, purity, and identity of the product, will be incorporated earlier on during scale-up and process development.

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Steven Thompson,
Vice President of Sales and Product Management,
Sexton Biotechnologies

1

Please introduce yourself and your institution.

My name is Dr Steven Thompson, Vice President of Sales and Product Management at Sexton Biotechnologies, now part of BioLife Solutions. We are focused on developing tools specifically for cell and gene therapy manufacturing, with a view to improving process efficiencies and reducing failures. Our approach is to actively work with therapy developers and other tools providers to truly understand current issues within the space, thereby allowing us to develop technologies that address an overarching industry challenge as opposed to a specific niche problem.

Sexton's Cell Performance portfolio is focused on the need to carefully consider the effect of ancillary materials on a final drug product, with safety, quality, regulatory coverage, and performance the foundation of our range of industry leading human platelet lysates. Our Processing and Handling platform has focused around CellSeal closed-system cryogenic storage vial, which is used as the final drug product container for commercial CAR-T products. The CellSeal platform is used in both autologous and allogeneic applications, with early small-scale use requiring no significant capital investment, and late scale manufacturing is supported by high-throughput automation.

More recently, recognizing a need for true flexible automation, we launched our Signata CT-5 fluid management system, enabling therapy developers to introduce automation at an early stage of process development (PD) and transition through to commercial manufacture. The design allows users to perform specific unit operations such as formulation/fill of upstream products or final drug substance into CellSeal vials or bags, as well as implementing task automation to close out other interstitial steps within the manufacturing process, thereby truly realizing the concept of flexible automation.

Scale up and process development: ask the experts

2 What are some of the differences between research/investigation-scale manufacture and commercial/clinical-scale?

Traditional thinking of mature biopharma process development dictates that research scale operations are open, manual processes, carried out at the bench with disposable consumables. The linear relationship of Critical Process Parameters (CPPs) at scale, combined with the ability to test specific activity of the final product, enables these open processes to be readily scaled to commercial scale bioreactor systems complete with defined operating and cleaning protocols. Unfortunately, whilst these approaches are highly tested for the manufacture and purification of recombinant proteins, the non-linear relationship between research and commercial scale processes in cell therapy applications prevents such a defined progression.

Firstly, particularly in the autologous space, every starting material is different. Age, sex, diet, number of rounds of chemotherapy or radiotherapy, all affect the overall health of the starting material. So, where to begin defining the starting point? How many days should the cells be expanded? How long will it take to achieve sufficient transduction of any construct? What are the effects of cryopreservation of the final product? The answer is that every process will have some level of variability, predominantly due to the uncontrollable nature of the starting material. It is therefore key that therapy developers look to limit all other potential variable inputs during manufacture, a process which must therefore begin at the research scale.

Human interaction represents the highest cause of variability in cell therapy manufacturing, thus taking the biopharma approach of manual, bench top development is difficult. Implementing automated processing at the research scale can enable defined ranges of CPPs to be established, which can then be transitioned to commercial scale. However, whilst the concept of introducing automation at the investigational scale seems an evident solution, the cost and rigid processing of many automated devices are hindering this approach. It is also key to remember that an overwhelming number of therapeutic concepts stem from academic institutes operating at the investigational scale, thus it is essential to allow freedom to realize these novel therapeutic concepts within budget constraints. An attractive midway can therefore be to introduce flexible automation tools, such as the Signata CT-5, which can be implemented at the investigational stage and scaled in line with therapeutic development.

3 What are some common mistakes developers make during process development for expansion to commercial-scale manufacture?

Navigating through process development to lockdown a robust manufacturing process is a minefield for any therapy developer, with a huge number of considerations needing to be made. Certainly, failing to undertake a Quality by Design approach as early as possible in development can present challenges as the product moves towards commercial manufacture, with CPPs needing to be defined to limit variability of the overall process. However, the stringent locking down of CPPs with defined values can prove troublesome, particularly in an autologous scale out setting, whereby variability in patient starting material requires that the process can be adjusted to meet Critical Quality Attributes (CQAs) of the final product. Indeed, it is imperative that flexibility is embedded throughout process development, enabling commercial scale manufacture of autologous products to have CPPs within a defined range, as opposed to a rigid value, which consistently results in a product that meets specifications.

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However, one of the major challenges comes from the sheer competitive nature of the industry, with VC investors often looking for quick returns in a crowded and fast-paced industry. There is often therefore a resistance to implement such approaches if there has already been previous development work which has taken a group so far. Indeed, many manual and open processes devised at the research scale transition through to commercial manufacturing, with the concept of 'if it isn't broken, why fix it'. However, often the damage cannot be seen until later in development, but it is imperative to recognize potential pitfalls as early as possible, and work to address them at a lesser stage and scale. The impact of manufacturing shortfalls is akin to building a wall – a few millimeters error at the start can result in a several meter error at the end.

4 How could turnkey or closed system approaches address these mistakes or challenges?

'The process is the product' – this is one of the major ways of thinking that the industry needs to change. The concept of being able to manufacture a handful of cell products at a single facility, using a non-defined process and a dash of knowhow, will not allow the industry to reach the scale required to meet patient demand. Processes need to be defined, transferrable and reproducible at scale.

Limiting variability by incorporating automated closed-system approaches at the investigational scale can allow CPP ranges to be defined and translated through to commercial scale. Ideally any automation should have the flexibility to enable process development but be able to be restricted or completely locked down when moving towards commercial scale manufacture. This continuity of automation can allow highly skilled scientists to develop and optimize processing parameters but move towards lower skilled labor once the process moves towards a turnkey type of manufacturing, thereby significantly reducing the cost of goods.

5 How can fill/finish considerations change depending on the product and anticipated delivery chain?

Fill/finish represents a challenging aspect of the manufacturing process for almost all therapy developers, with the variable upstream processing leading to different downstream requirements. Firstly, there is the consideration of autologous versus allogeneic products. Autologous products typically necessitate a need for accurate and repeatable fill of a lower number of containers, which likely comprises both final drug packaging as well as QC vials. Therefore, when considering automation, the flexibility to fill into both larger containers such as bags but maintaining the ability to retain lower volume QC samples can reduce the need to waste precious therapeutic material.

Allogeneic therapies look more towards higher throughput to meet demand for scale, although the industry has yet to move towards true high-throughput automation, with very few developers requiring throughput associated with traditional drug vial filling. Automated approaches at the allogeneic scale have thus far focused upon filling hundreds of vials per hour, with Sexton's AF-500 device capable of filling 560 CellSeal vials in 90 minutes. However, one of the challenges of allogeneic processing is final product volumes applicable for bags, with high-throughput automation technologies for these containers still an unmet need.

Regardless of required scale, careful consideration should be given to the effect of the fill/finish process on cell viability. Extended processing time may negatively impact cells prior to freeze, and hence it is important to fully validate this effect per

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given cell type and implement risk mitigation strategies such as passive or active cooling. Homogeneity of the formulated product is also variable depending on cell type, with appropriate mixing required to ensure consistent cell concentrations.

6 What will scale-up and process development look like in the future?

Potentially, it will resolve into two distinct pathways depending on the allogeneic or autologous nature of the final product. We've seen (contrary to a lot of writing early on), that autologous products can be manufactured at a scale meaningful to large pharma but, it's not yet clear that this is sustainable or can meet the need of an expanded market such therapies for solid tumors.

Allogeneic products will likely be manufactured in purpose-built facilities with appropriate bioreactors, analytics, and controls. Once the clinical and biological hurdles around allogeneic treatments are solved, the industry will be able to develop a strong enough understanding of how perform culture where the cells are the product. Very likely, it falls into the same kind of pattern as large molecule pharma, where there are a few workhorse cell-types (lines or primary) with relatively standardized transduction and growth conditions. Real innovation will be focused on the specific genetic modifications, particularly for immune based therapies, with the manufacturing/scale-up process fitting into well-developed paradigms. That's not to say it will be easy, just understood.

Reverting to autologous products, this is where innovation and unique, distributed manufacturing solutions will continue to have a place. The promise of cell and gene therapy is that we can utilize biology to develop true disease modifying treatments for a broad range of conditions. However, whether that reality can be realized in the context of financial and product risks as modeled by pharma remains to be seen. Certainly, regulatory expectations will continue to evolve to support high-quality, targeted therapies developed by experts using flexible manufacturing systems at-, or near-bedside. This is where the GMP-in-a box systems will be deployed. For that to be realistic, these systems must be developed to fit the needs of this type of user, thereby defining the goal of Sexton Biotechnologies.

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Roland Kaunas
Associate Professor and Director of the Biofabrication Laboratory in the Department of Biomedical Engineering
Texas A&M University

Carl A. Gregory
Associate Professor in the Department of Molecular and Cellular Medicine
Texas A&M University

1

Please introduce yourself and your institution.

My name is Carl A. Gregory and I am an Associate Professor in the Department of Molecular and Cellular Medicine at Texas A&M University (TX, USA) and faculty of the Institute for Regenerative Medicine. My laboratory studies the general biology of human mesenchymal stem cells (hMSCs), including hMSCs derived from induced pluripotent stem cells (ihMSCs). We have developed decellularized matrices deposited by hMSCs and ihMSCs for acceleration of bone repair.

My name is Roland Kaunas and I am an Associate Professor and Director of the Biofabrication Laboratory in the Department of Biomedical Engineering at Texas A&M University. Together with Carl Gregory's lab, my laboratory develops methods for fabricating hydrogel scaffolds for hMSC expansion and skeletal tissue regeneration. We are now using dissolvable hydrogel microcarriers to expand undifferentiated hMSCs in up to 3L PBS vertical wheel bioreactors, as well as generate decellularized osteogenic extracellular matrix from osteogenically differentiated hMSCs.

2

What are some of the differences between research/ investigation-scale manufacture and commercial/clinical-scale?

The focus of much stem cell and biomanufacturing research in academic laboratories is on cutting edge technologies that are attractive to high-impact journals. These technologies are rarely developed with manufacturing practicalities in mind. Typically, cell manipulations are performed by hand in a biosafety cabinet using plasticware since this approach provides flexibility when performing a range of one-off studies. Results may appear quite promising, but when a technology is selected for potential commercialization, the methods will likely need to be modified and this in turn can lead to unfavorable results. At the commercial scale, it is important to use methods that are economical, reliable, scalable and amenable to process monitoring.

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3 What are some common mistakes developers make during process development for expansion to commercial-scale manufacture?

Since hMSCs perform well in media containing fetal bovine serum, this is the culture system of choice in academic research. It is becoming very difficult to justify the use of xenogeneic materials when asking for regulatory approval, however. Xenogen-free media is relatively expensive and their utility for hMSC growth and differentiation often require expensive optimization studies. Nonetheless, it is worthwhile to establish xenogen-free culture conditions when establishing a new technology with commercialization potential.

Expansions of hMSCs for clinical trials are currently performed using monolayer cultures at academic centers. Midwest Stem Cell Therapy Center recently published their Phase I trial for treating acute graft-vs-host-disease with hMSCs. The cells were expanded in CellSTACKs to generate 12 billion viable cells for a ten-patient trial. They are now facing Phase II and III trials that will require 5 and 60 times as many cells, which are no longer practical using the same monolayer culture approach.

4 How could turnkey or closed system approaches address these mistakes or challenges?

Replacing traditional procedures with closed, automated bioreactor-based culture can lower costs by saving on operator expenses, media usage and cleanroom requirements. Recognizing the limitations of monolayer culture, the Midwest Stem Cell Therapy Center has begun studies using 2L stirred-tank bioreactors for their Phase II trials with success in terms of cell-based critical quality attributes. Notably, they are able to generate on the order of one billion cells per bioreactor. Their next steps are to further expand in larger stirred-tank bioreactors.

5 How can fill/finish considerations change depending on the product and anticipated delivery chain?

Cell therapies are unique from biopharmaceuticals in that the cells themselves are the product. Harvesting and isolating cells are a major challenge, especially for allogeneic culture in which microcarrier culture represents an attractive strategy for scale-up. The materials used for current commercial microcarriers require that cells be enzymatically removed and then separated, which can potentially reduce the number of viable cells. Further, clear guidance is lacking regarding the requirements for microcarrier removal and acceptable amount of contamination by either small or broken microcarriers.

6 What will scale-up and process development look like in the future?

Large-scale cell therapy manufacturing has yet to be realized. Stem cells are much more sensitive to the conditions used for suspension culture than engineered cells currently used in the biopharmaceutical industry for producing secreted therapeutics. A concern we have is that the impellers used in large stirred-tank bioreactors will generate high fluid shear stresses that hMSCs on microcarriers are quite sensitive to. For this reason, we have chosen to use PBS vertical-wheel bioreactors, which disperse the energy used to suspend microcarrier cultures more widely so as to reduce the maximum shear stresses that are generated. Currently, the largest PBS vertical-wheel bioreactors are 80L and to my knowledge are only used at a single facility. Allogeneic cell therapies will benefit from scale, so even larger bioreactors are very attractive.

Scale up and process development: ask the experts

Paul Carter
Head of Vector Processing
Quell Therapeutics Limited

1

Please introduce yourself and your institution.

My name is Paul Carter and I am Head of Vector Processing at Quell Therapeutics, which is a London-based biopharmaceutical company working to bring engineered Treg cell therapies to patients across a range of autoimmune and inflammatory diseases, as well as solid organ transplantation.

2

What are some of the differences between research/ investigation-scale manufacture and commercial/clinical-scale?

At the research phase the questions being dealt with are: How to make the product? Can the desired efficacy be achieved? Is it of sufficient quality?

Once it is possible to produce the product the questions then become: How do I make more of the product? Is it possible to scale up or is scaling out a better option? Does the scale of operation effect the efficacy or quality of the product? What are the Critical Process Parameters and how do they interact with the Critical Quality Attributes? What are the costs of goods?

When you have a process to produce your product and are moving to the clinical /commercial scale, the questions change again: How robust is my process? What is the best way to control the process? Is it possible to make the process more efficient? Can I reduce complexity and minimize costs?

Raw material variation has a profound effect on all stages of the lifecycle involving human pluripotent stem cells. We can see the effects of these variations through morphological changes during cell line maintenance and via the propensity of the cells to differentiate robustly.

3

What are some common mistakes developers make during process development for expansion to commercial-scale manufacture?

The greater process understanding you have, for example by using design of experiments methodologies to define safe operating ranges for process parameters, the earlier you can look to include

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automation in your process development. Automation of the process allows for better monitoring and control of the process, leading to better reproducibility and greater process robustness.

The inclusion of single use technologies early in process development removes the requirement for time-consuming cleaning steps making the process easier to operate. Single use technologies do have their downsides in that you need to pay attention to chemical compatibilities and interactions between materials to avoid any extractable and leachable issues.

4 How could turnkey or closed system approaches address these mistakes or challenges?

Turnkey or closed systems can accelerate process development as they avoid the need for custom system or manifold designs. If the systems are flexible in the way they operate there is potential for them to be used at multiple steps in the process.

5 How can fill/finish considerations change depending on the product and anticipated delivery chain?

The major considerations for fill finish are the compatibility of the product with the container chosen and how that container interacts with the next step in the process or the delivery method for the product. Flexibility to fill the product into bags is especially important in the advanced therapies space. Being able to have representative samples in representative containers is an important consideration for autologous therapies, as this allows the patient to receive as much of the manufactured dose as possible.

6 What will scale-up and process development look like in the future?

The key tools for scale-up and process development in the future will be to use statistical methods to build a greater understanding of the experimental and process space where processes can be operated optimally. The development of robust scale-down models really help to build process understanding; they also give insights to the process steps that are sensitive to changes in scale, be that scaling up or down.